

## Review Article

# Adipose-derived stem cells as a potential weapon for diabetic foot ulcers

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**Abstract:** Diabetes mellitus is responsible for delayed or impaired wound healing, which often leads to non-healing chronic wounds. Adipose-derived stem cells can promote angiogenesis, secrete growth factors, regulate the inflammatory process, and differentiate into multiple cell types makes them a potential ideal therapy for chronic wounds. The aim of this review is to summarize the current status of ADSC-based treatments for diabetic wounds, describe their mechanism of action and several clinical trials of diabetic wound repair and introduce some published clinical applications for Diabetic Foot Ulcers (DFUs).

**Keywords:** Adipose-derived stem cells, clinical trials, diabetic foot ulcers

### Introduction

Diabetes is a chronic disease that affects approximately 350 million people (6.5%) worldwide. Currently, 15% of all diabetic patients worldwide suffer from foot ulcerations, which often become non-healing chronic wounds [1]. The annual incidence of amputation is 0.21-1.37% [2], which is 15-70 times higher in diabetic individuals than it is among the general population [3]. Many factors contribute to diabetic non-healing wounds, including prolonged inflammation, decreased synthesis of collagen, reduced growth factors, and impaired neovascularization [4]. Reduced tissue regeneration and angiogenesis are crucial for wound healing [5].

The current therapies for diabetic wounds, including frequent inspection with irrigation and debridement, protective dressings, infection and inflammation control, and plantar off-loading [6], cannot achieve satisfactory outcomes. Although new therapeutic methods have been developed, some cases remain ineffective or slowly progressive [7], and treatment strategies that are more effective must be established.

Stem cell therapy has emerged as an attractive approach for enhancing wound healing [8]. This field of regenerative medicine focuses primarily on stem cells, which are specialized cells that possess the ability to self-renew and differentiate into multiple cell types [9]. Mesenchymal stem cells (MSCs) have been isolated from various sites, including bone marrow, adipose tissue, umbilical cord blood and amniotic fluid. It has been reported that bone marrow-derived stem cells (BMSCs) accelerate the rate of healing in cutaneous wounds [10]. However, because of difficulties in obtaining sufficient BMSCs, adipose-derived stem cells (ADSCs) represent an alternative source of multipotent stem cells with characteristics that are similar to those of BMSCs [11]. ADSCs can secrete many growth factors, including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), and fibroblast growth factor (FGF) [12-14]. In addition, ADSCs are easier to isolate and are relatively abundant.

To date, preclinical studies on the use of ADSCs in diabetic wounds have been performed, and the efficacy of ADSCs has been determined in several clinical trials. Therefore, the aim of this review is to highlight the recent progress in dia-

betic wound healing by ADSCs and identify the trends in clinical studies.

### **The function of ADSCs in diabetic wound healing**

DFUs represent one of the most common long-term complications in diabetic patients. Although they are common, the treatments for these intractable conditions remain limited and largely ineffective. Recently, cell therapy using ADSCs has been a successful approach in diabetic wound healing. In brief, the wound-healing effect of ADSCs is that they can directly differentiate into various cell types, such as neurons, smooth muscle cells, and endothelial cells, and can secrete many factors that induce the proliferation and migration of endothelial cells, fibroblasts, and keratinocytes.

#### *Differentiation potential of ADSCs*

Interest in the application of ADSCs for cell-based therapies such as tissue engineering has been increasing because ADSCs possess the capacity to differentiate into various cell lineages. Several studies have demonstrated the ability of ADSCs to undergo differentiation when they are cultivated under lineage-specific conditions and along classical mesenchymal lineages, such as adipocytes [15], osteocytes [16] and chondrocytes [17]. Moreover, there is evidence for the ability of ADSCs to differentiate into other cell lines, such as neurons [18], hepatocytes [19], smooth muscle cells [20], endothelial cells [21] and cardiomyocytes [22].

Nie [23] showed that ADSCs can significantly accelerate wound closure in normal and diabetic rats, including increased epithelialization and granulation tissue deposition. Furthermore, they applied GFP-labeled ADSCs on wounds to determine whether ADSCs could differentiate along multiple lineages of tissue regeneration in the specific microenvironment. Immunofluorescent analysis indicated that GFP-expressing ADSCs were costained with pan-cytokeratin and CD31, which suggested their spontaneous site-specific differentiation into epithelial and endothelial lineages, respectively. These data suggest that ADSCs not only contribute to cutaneous regeneration but also participate in new vessels formation. In conclusion, their results demonstrate that ADSC therapy could accelerate wound healing through differentiation and vasculogenesis.

Rocco [24] used genetically modified ADSCs to overexpress SDF-1 and topical administration to the wound site to promote healing in diabetic mice and reported improved impaired wound healing. In particular, the authors monitored the biodistribution and kinetics of engraftment, survival, and proliferation of the administered cells and showed that the cells settled permanently in the regenerated tissue after healing was complete by *in vivo* bioluminescent imaging analysis. In summary, their study indicates the therapeutic potential of ADSC administration in wound healing via cell differentiation and enhanced cellular recruitment at the wound site.

#### *Secretion capacity of ADSCs on wound healing*

ADSCs secrete many different growth factors, including hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), insulin-like growth factor (IGF)-1, basic fibroblast growth factor (bFGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)- $\alpha$ , interleukins 6, 7, 8 and 11, adiponectin, angiotensin, cathepsin D, pregnancy zone protein, retinol-binding protein and CXCL-12 [25, 26].

A preclinical study showed that the topical administration of autologous ADSCs, in conjunction with a type I collagen sponge matrix, into a diabetic animal accelerated the healing of diabetic ulcers [27]. In addition, Nie et al. reported that targeted delivery of ADSCs via ADM scaffold accelerate wound healing in diabetic rats through a paracrine mechanism, with enhanced granulation tissue formation and increased re-epithelialization and neovascularization [28]. It has been speculated that the healing mechanism is attributable to the release of many growth factors, including VEGF, HGF, TGF- $\beta$  and bFGF.

Tsang et al. studied the healing effect of recombinant human epidermal growth factor (hEGF) on diabetic foot ulcers in a double-blind randomized controlled study. In their study, Kaplan-Meier survival analysis suggested that application of cream with 0.04% (wt/wt) hEGF caused more ulcers to heal by 12 weeks and increased the rate of healing compared with the other treatments [29].

## The ADSCs in DFUs

**Table 1.** Current clinical trials using ADSCs in DFUs

Clinical trials	Ref	Status	Phase
A Clinical Study Using Adipose-derived Stem Cells for Diabetic Foot	NCT02831075	Recruiting	1
Safety of ALLO-ASC-DFU in the Patients With Diabetic Foot Ulcers	NCT02394886	Completed	1
Clinical Study to Evaluate Safety and Efficacy of ALLO-ASC-DFU in Patients With Diabetic Foot Ulcers	NCT02619877	Recruiting	2
Human Adipose Derived Mesenchymal Stem Cells for Critical Limb Ischemia (CLI) in Diabetic Patients	NCT01257776	Completed	2 (Diabetes)
Assessment of the Efficacy and Tolerance of Sub-cutaneous Re-injection of Autologous Adipose-derived REGenerative Cells in the Local Treatment of Neuropathic Diabetic Foot ulcers (REGENDER)	NCT02866565	Not yet recruiting	2
Adipose-derived regenerative cellular therapy of chronic wounds	NCT02092870	Unknown	2

All clinical trials taken from clinicaltrials.gov and PubMed.

According to a new study, ADSCs significantly enhance diabetic wound healing, engrafted into the local wound tissue, and implanted into circulating blood. ADSC treatment stimulated neoangiogenesis and increased tissue regeneration through paracrine and autocrine mechanisms. Histological examination revealed that the ADSC-treated group showed a significant reduction in the proinflammatory reaction, with significantly increased levels of EGF, VEGF, prolyl-4-hydroxylase (rPH), and Ki-67 expression compared with the controls [30].

Further, becaplermin, recombinant platelet-derived growth factor, which is safe and easy to use, has been used for DFUs in clinical settings for decades [31].

### *The ability of ADSCs to promote angiogenesis*

Kim et al. reported the therapeutic effect of human ADSCs in the healing of ischemic wounds in a diabetic nude mouse model, following a local injection of human ADSCs. Earlier, abundant neovessel formation and better tissue remodeling were observed in treated mice compared with the control group. Furthermore, a higher level of VEGF was detected in plasma and tissue, which is required for local angiogenesis [32]. Gao et al. confirmed that ADSCs accelerate neovascularization in ischemic diabetic skin flap via the expression of hypoxia-inducible factor-1 $\alpha$  [33]. Kato et al. [34] transplanted ADSC sheets created using cell sheet technology into full-thickness skin defects in a rat model of type 2 diabetes and obesity. The results indicate that the transplantation of ADSC sheets combined with artificial skin accelerated wound healing and vascularization, with significant differences observed

2 weeks after treatment. The ADSCs sheets secreted large amounts of several angiogenic growth factors in vitro, and transplanted ADSCs were observed in perivascular regions and were incorporated into the newly constructed vessel structures in vivo. In conclusion, the allogeneic transplantation of an ADSC sheet combined with artificial skin accelerates wound healing in a rat wound model of type 2 diabetes and obesity.

To determine the effects of locally administered ADSCs in a full-thickness skin graft model, 20 rats were randomly divided into 2 groups (diabetic and control groups). One week later, the gross and histologic results showed significantly increased survival, angiogenesis, and epithelialization. The mean area of graft necrosis was significantly less in the diabetic group than in the control group (7.49% vs 39.67%,  $P < 0.001$ ). Statistically significant increases in capillary density, collagen intensity, VEGF, and TGF- $\beta$ 3 expression were noted in the diabetic group compared with the control group. These findings suggest that autologous ADSC transplantation can enhance skin graft survival in diabetic rats through differentiation, vasculogenesis, and secretion of growth factors such as VEGF and TGF- $\beta$ 3 [35].

### **Clinical studies**

Because of the recent achievement shown by ADSCs in preclinical models of chronic diabetic wounds, it is unsurprising that ADSCs have rapidly moved into the transformation stage. To date, five clinical trials involving ADSC therapies for DFUs have been found (**Table 1**). However, not all of their published results are available.

## The ADSCs in DFUs

A phase I study of allogeneic adipose-derived stem cell transplantation is proceeding in China in patients with DFUs for more than 6 weeks. Local injection enriched with ADSCs derived from adipocyte transplantation is being performed, and the primary outcome measure is the size of the DFUs. This study intends to establish an optimal clinical research program and attempts to break the technical bottleneck in the stem cell therapy for treating diabetes-related vascular complications.

Another phase I study in Korea is evaluating the safety of ALLO-ASC-DFU (a hydrogel sheet containing allogenic adipose-derived mesenchymal stem cells) in patients with diabetic foot ulcers and has been completed, with no results posted on ClinicalTrials.gov. The primary outcome measure is the number of participants with adverse events as a measure of safety and tolerability.

A phase II single-blinded study of the safety and efficacy of ALLO-ASC-DFU in patients with DFUs compared with standard therapy is being performed, with an estimated 44 subjects enrolled by September 2016. The primary outcome measure is the proportion of re-epithelialization.

Another phase II parallel-group controlled study examined autologous adipose-derived mesenchymal stem cells for critical limb ischemia (CLI) in diabetic patients. The primary outcome measures were angiographic assessment of neovasculogenesis at 6 months and major adverse events at 1 month, 6 months, and 12 months. This study was conducted in Seville, Spain, and has been completed. A total of 33 patients with type 1 or type 2 diabetes were enrolled, but the study results have not yet been posted on ClinicalTrials.gov.

In France, a phase II study by GEINDRE was initiated on August 5, 2016. This study aims to assess the efficacy and tolerance of injection of adipose-derived regenerative cells for the local treatment of neuropathic or neuro-ischemic DFUs. The primary outcome measure is the percentage of patients achieving 100% wound closure at 20 weeks.

In summary, to the best of our knowledge, 104 cases of patients treated with ADSCs for DFUs have been published to date. Eighty-two

patients have been enrolled in clinical trials (**Table 1**). In all of the published studies, no major adverse effects have been reported. The results were encouraging for soft-tissue augmentation and diabetic wound healing.

### Published clinical applications in DFUs

Thus far, ADSCs have been used in the treatment of diabetes and complications, but the number of patients who have been treated with ADSCs is still very limited.

In 2009, some studies showed that the intramuscular injection of ADSCs had clinical efficacy in patients with diabetic mellitus foot and arteriosclerosis obliterans. Six months after the ADSC injections, the peak walking time, rest pain score, and vascular collateral networks improved significantly without any adverse event (IFATS meeting, 2009).

In 2012, to confirm the safety and efficacy of multiple intramuscular adipose tissue-derived mesenchymal stem cells (ATMSC) injections in CLI patients, Lee [36] injected ADSCs into the muscles of the lower extremities of 15 patients. Among these patients, 12 had thrombo angitis obliterans (TAO), and 3 patients had diabetic foot. There was clinical improvement in 3 of the 3 patients with diabetic foot (100%) and in 7 of the 12 patients with TAO (58.3%), including increased ulcer healing rates, advanced walking distances, and the formation of numerous vascular collateral networks. To the best of our knowledge, this is the first report of using ATMSC therapy to treat CLI in humans.

In 2014, the first phase I trial to evaluate the feasibility and safety of intramuscular injections of autologous ADSCs in patients with non-revascularizable CLI, named A Cell DREAM, revealed significant improvements in leg pain, ulcer size and pain-free walking distance at a 2-year follow-up. In this study, sixty consecutive patients with CLI who were non-suitable for revascularization were initially screened, as recommended by the international guidelines; ultimately, seven patients were enrolled and underwent the entire procedure. Of these patients, three were diabetic [37].

In summary, these studies reported no major adverse effects after treatment with ADSCs, and the results were promising. The character-

istics of adipose-derived stem cell therapy were as follows:

1. The adipose-derived stem cells used were autologous or allogenic.
2. The administration doses were changeable, as was the number of adipose-derived stem cells per dose.
3. Adipose-derived stem cells were administered systemically (intravenous infusion) or locally (intramuscular injection).

### Conclusions and future perspectives

DFUs are a complex issue that involves a myriad of factors, and with careful research, its mysteries are slowly being illuminated. The important role of ADSCs in DFUs is now under closer scrutiny because of their rich source and easy access, and the use of ADSCs to treat DFUs has been shown in many studies. To date, relatively few clinical trials in a limited number of research areas have been conducted to assess the therapeutic potential of ADSCs compared with the large number of published preclinical studies. However, a few recent clinical trials involving ADSCs are either ongoing or recruiting patients. In preclinical studies, ADSCs and their secretory factors have shown enhanced wound healing in animal models of DFUs. Until now, their mechanisms of action have not been fully understood, and therefore, further research in this area is needed.

All published clinical applications in DFUs reported no major adverse effects after treatment with ADSCs, and the results were promising. In fact, clinical application of ADSCs in DFUs is in its early stages. Additional large-scale and long-term follow-up clinical trials are needed to determine the long-term safety of ADSC-based therapies.

In general, ADSCs have an important role in the future therapeutics for DFUs.

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### Disclosure of conflict of interest

None.

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