

## Adipose-Derived Stem Cells, their Secretome, and Wound Healing

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Rec date: Apr 22, 2014; Acc date: Jun 12, 2014; Pub date: Jun 16, 2014

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### Abstract

Adipose tissue is an abundant source of progenitor cells with multidifferentiation potential and these cells, termed adipose-derived stem cells (ASC), have become an important proposed treatment for use in wound healing. The ability of these cells to increase angiogenesis (vessels arise from other vessels) and vasculogenesis (vessels arise from progenitor cells), produce growth factors and cytokines, produce cell matrix proteins, and increase cell proliferation place them in a unique position to improve wound healing.

**Keywords:** Adipose-derived stem cells; Mesenchymal stem cells; Wound healing; Mesenchymal stem cell secretome

### Introduction

Wound healing consists of 4 phases: hemostasis, inflammation, proliferation, and remodeling. The hemostasis phase involves the formation of a clot, and this phase starts within seconds of tissue injury. In the inflammatory phase, neutrophils, macrophages, monocytes, and other defensive cells migrate to the site of injury. Proliferation is when collagen secretion, re-epithelialization, and scar formation occur. And finally, in remodeling, the scar undergoes changes that may go on for as long as one year. It should be noted that even though these 4 phases are distinct, they do overlap in time. Any therapy that would increase the rate of one or more of these phases would theoretically be effective at reducing complications, such as infection, by decreasing the time a wound is exposed to the outside environment.

These wound healing events involve chemical signaling, cellular migration, extracellular deposition, proteolysis, and angiogenesis. Examples of these chemical signals include fibroblast growth factor receptor [1,2], Wnt [3], heparin binding EGF-like growth factor [4], and transforming growth factor-Beta [5]. Some of the signaling pathways activated during wound repair are Hedgehog, Notch, Wnt/b-catenin and the growth factor and cytokine pathways [6].

Adipose-Derived Stem Cells (ASCs) have been studied for their effects on the wound healing process, and, thus far, the results have been encouraging. Many cytokines and growth factors play a vital role in the process of healing. Specifically, in the mouse model of Ebrahimi et al. [7], ASCs can produce keratinocyte growth factor and vascular endothelial growth factor, both of which accelerate the proliferation phase of healing in addition to keratinocyte migration [7]. Huang et al. studied the effects of ASC therapy in rats with radiation ulcers, and they found that in wounds treated with ASC therapy, wound size decreased faster, and VEGF and hepatocyte growth factor were found at higher concentrations [8]. In 2013, ASCs' effect on skin grafts in diabetic rats were studied, and the ASC-treated skin grafts had increased amounts VEGF, TGF- $\beta$  3, capillary density and collagen intensity [9]. In animal studies by Tamari et al.

[10] MSC (mesenchymal stem cell) and MSC-CM (conditioned media) treatments accelerated wound healing compared to controls in an excisional wound mouse model [10].

In 3-Dimensional cultures adipose-derived stromal cells have been shown to influence wound healing. Because the exact mechanism of wound acceleration and the fraction of cells involved are not known, the cells were termed "stromal" rather than "stem" cells. Adipose-derived stromal cells (ADSC) were shown to accelerate wound healing in CO<sub>2</sub> laser injured raft cultures containing human skin explants or primary human keratinocytes [11].

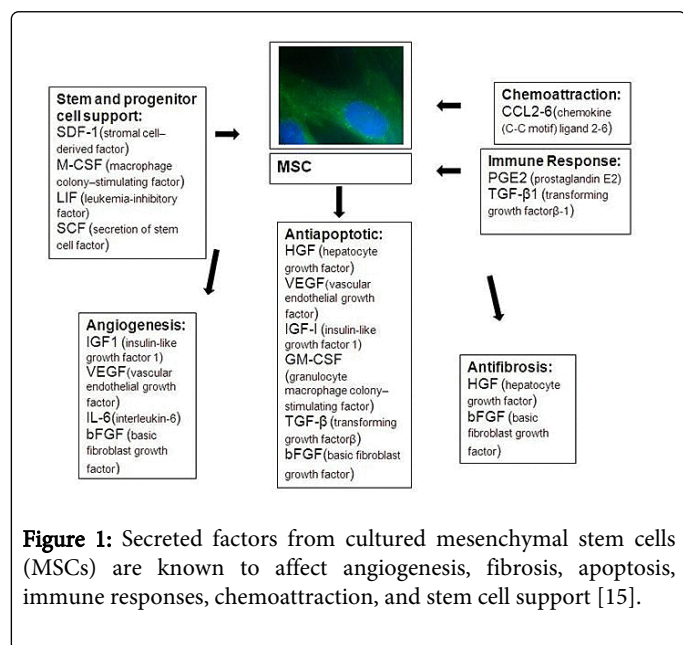
In human studies, a phase 1 trial involving ASCs for the treatment of patients with critical limb ischemia showed ulcer improvement and an increase in transcutaneous oxygen [12]. In 2013, ASCs were used for skin defects without skin grafts, and observation showed rapid coverage of wounds with the patient's own regenerated tissue [13]. Furthermore, Reckhenrich et al. [14] did a study on the use of an innovative surgical suture filled with ASCs. Their results showed a decrease in open wound area compared to control methods, but this advantage came with a suture with less stiffness and less maximum force [14]. During this study, VEGF was released by the sutures for about 16 days in vitro.

The paracrine effects are an important role of MSC (mesenchymal stem cells). Their secreted factors can alter cell division and differentiation, cell homing, apoptosis, immune status, and influence other processes (Figure 1) [15,16]. The secretome consists of secreted proteins and other factors, and also membrane ectodomains that are released by sheddases [17]. Oki et al. [18] have found that transplanted human induced pluripotent stem cells (iPSCs) into the stroke-damaged mouse and rat striatum or cortex promoted recovery [18] of a clinical motor deficit. Improvement was thought to be due to VEGF (vascular endothelial growth factor) levels rather than neuronal replacement. Sart et al. [16] found that human bone marrow MSC-conditioned media stimulated endogenous secretion of extracellular matrices (ECMs) from NPC (Neural progenitor cell) aggregates and enhanced cell adhesion and proliferation [16]. Their results showed that the MSC secretome enhanced proliferation, migration, and neurite extension. Inhibition of specific multiple growth factors reduced cell migration suggesting the involvement of many MSC derived factors [16]. Also in studies by Wang et al. [19] co-culture of

neural stem cells (NSC) with murine bone marrow-derived stem cells promotes proliferation of the NSC [19]. The fact that the cells were physically separated implies the proliferative effect was due to secreted factors. A Notch signal-blocking agent, a secretase inhibitor, DAPT, decreased the proliferation of NSC and decreased the expression of Notch-1 [19]. Therefore, this interaction between MSC and NSC is thought to involve Notch signaling.

In human trials, Zhou et al. [20] studied the effect of adipose-derived stem cell media on wound healing after carbon dioxide laser resurfacing of the inner arms of patients [20]. Their study showed that this conditioned media enhanced wound healing and reduced side effects associated with carbon dioxide laser therapy, such as erythema [20].

Human mesenchymal stem cells appear to be of importance for improving situations that involve an inflammatory response such as injury and autoimmune disease [15]. Singer and others have shown that adult human MSCs and their secretome can inhibit T-lymphocyte proliferation and activation induced by alloantigens [15]. Aggarwal et al. [21] found that co-culture of human mesenchymal stem cells with immune cells altered the secretions of dendritic cells, T cells and natural killer cells to increase interleukin-10 (IL-10) and decrease tumor necrosis  $\alpha$  (TNF- $\alpha$ ) secretion which therefore produced a more anti-inflammatory phenotype [21]. In an important recent study by Lee et al. [22] it was shown that injecting adipose tissue-derived stem cells (ASC) or ASC-conditioned media intravenously improved allograft skin graft take in a mouse model [22]. The immunosuppressive properties of these ASC were thought to be mediated through the paracrine effects of ASC.



In summary, mesenchymal stem cells have been able to accelerate wound healing in many studies and clinical studies are ongoing to document their usefulness in the treatment of wounds and grafts. Currently, the many signaling pathways that are triggered by the secreted factors are being examined. In the future, identification of the signaling pathways for specific processes that occur during wound healing will lead to the development of drug therapies that assist or hinder specific processes. Such discoveries will lead to the

development of wound healing protocols that may not require the addition of cells.

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