

# The Role of Mesenchymal Stem Cells in Skin Wound Healing

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## Abstract

Mesenchymal stem cells (MSCs) could differentiate into various types of tissues. These cells serve as a backup for the regeneration and repair of tissues or cells after injury. A skin wound is defined as an injury to the skin that needs to be restored. All types of cells in skin especially mesenchymal stem cells play important role in wound healing process. In particular, paracrine signaling of MSC regulates the cellular responses at the wound site leading to reduction of inflammation, stimulation of angiogenesis and induction of cell migration and proliferation. Because of these abilities, MSCs are one of the most common stem cells for cell therapy in wound healing. This review focuses on the role of MSCs on wound healing process. In addition, major phases of wound repair and challenges of cell therapy are discussed.

**Keywords:** Mesenchymal Stem Cells, Wound healing, Inflammation, Angiogenesis

## Introduction

Mesenchymal stem cells have been characterized to be cells mainly responsible for the repair of damaged tissues (Ai et al., 2002). MSCs are multipotent stem cells that play crucial roles in the maintenance and repair of tissues; these cells are essential for wound healing (Nuschke, 2014). In addition, MSCs promote regulation of immune response and inflammation as well as induction of cell migration and epithelial changes (Maxson et al., 2012). These abilities of MSCs and the development of tissue engineering and production of cellular scaffolds, have been introduced as a new strategies in the treatment of chronic wounds (Nuschke, 2014).

The skin is the largest organ of the body and contains a large number of MSCs (Li et al., 2006). Since most important function of skin to disease control is the formation of a physical barrier against pathogen factors, any factor that would break the barrier can produce lesion and will ultimately weaken the body, so wound healing emerges as an alternative mechanism to the treatment of injured tissues (Singer and Clark, 1999). Recent studies indicate that skin stem cells participate broadly in dermal repair during healing of skin legions particularly chronic diabetic foot ulcer (Kato et al., 2014). Although wound is generally defined as cut and severe damage, a wound by true definition is an

injury or disruption in normal skin structure and function. It can be included a wide range of damage, ranging from a simple epithelium injury to deep damage involving tissues below the level of the skin and even tendon, muscle, vessels, nerves and bones (Velnar et al., 2009). Skin legion treatment is a diverse part of the health care system, including surgical and accidental lacerations, burns, pressure ulcers, diabetic and venous ulcers (Chen et al., 2009).

After the injury, the body organizes programmed repair process called wound healing. Wound healing is a complex and multistage process in which damaged skin is repaired (Velnar et al., 2009). This review considers function of MSCs on wound healing as well as main phases of the process and ongoing challenges in cell therapy.

## Wound Healing Phases

Wound healing can be divided into several phases which must occur in a specific sequence at a specific time and each phase must continue for a specific duration at an optimal intensity (Guo and DiPietro, 2010). Thus, wound healing process involves four phases: hemostasis, inflammation, proliferation and remodeling (Bielefeld et al., 2013).

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## Hemostasis

When tissue is injured, the small blood vessels suffer from damage. This condition can lead to bleeding and body momentarily stops loss of blood, a process called hemostasis (Young and McNaught, 2011). In this phase, vasoconstriction occurs before activation of platelets and coagulation. The endothelium of damaged vessels produces a vasoconstrictor, endothelin, which leads to vasoconstriction. Other mediators for vasoconstriction are derived from sympathetic nervous system (norepinephrine), the circulating catecholamine (epinephrine), and the release of prostaglandins from injured cells (Teller and White, 2011). Vasoconstriction of damaged blood vessels will cause a hypoxic microenvironment within wound area that leads to production of reactive oxygen species (ROS). It can enhance expression of antioxidant enzymes to detoxification of excess ROS (Behm et al., 2012). At this stage, blood loss is also prevented through the formation of a clot (Young and McNaught, 2011).

The coagulation cascade is made up of two converging pathways: extrinsic and intrinsic. Although both pathways start in different ways, each of them leads to the activation of factor X and the production of thrombin. Thrombin plays two important roles in clot formation: a catalyst for the conversion of fibrinogen to fibrin and an originator for platelet activation (Teller and White, 2011). Platelets have also important role in this phase. Platelets contact with collagen of the damaged vessels and thrombin which leads to their activation (Olczyk et al., 2014). Activated platelets adhere at site of exposed collagen to form a platelet plug and temporarily stop bleeding (Young and McNaught, 2011). Moreover, the blood clot contains fibrin, fibronectin, vitronectin and thrombospondins that create a temporary matrix, which serve as scaffold to migration and adhesion of fibroblasts, keratinocytes, and endothelial cells (Olczyk et al., 2014; Reinke and Sorg, 2012).

The aggregated platelets, trapped in the temporary matrix, release various growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF- $\beta$ ), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and basic fibroblast growth factor (bFGF) from  $\alpha$  granules. These mediators influence neutrophils, monocytes, macrophages, smooth muscle cells, and fibroblasts (Olczyk et al., 2014).

## Inflammation

This stage is accompanied by specific

inflammatory symptoms, such as redness, body heat, swelling, and pain around the wounded place. In the early inflammation phase, with subsiding of the initial vessel contraction, vascular permeability of walls increases by factors such as histamine, kinases, prostaglandins, leukotrienes, hyaluronic acid and ROS (Olczyk et al., 2014).

Neutrophils are the first subset of leukocytes to enter the wound and within 24 hours will become the dominant neutrophils in wound area. The presence of neutrophils stimulated by prostaglandins, complement, TGF- $\beta$ , tumor necrosis factor alpha (TNF- $\alpha$ ), Interleukin-1 (IL-1), and bacterial products at the wound site. Neutrophils release various types of proteolytic enzymes, which break down bacteria and extracellular matrix (ECM) within the injury area as well as produce reactive oxygen free radicals to prevent microorganism's penetration (Teller and White, 2011; Werner and Grose, 2003).

When neutrophils carried out their tasks, these specific cells must be eliminated from the wound by either apoptosis or macrophage phagocytosis (Olczyk et al., 2014; Teller and White, 2011; Young and McNaught, 2011).

After 48 to 96 hours, the predominant leukocyte within a wound is the macrophage (Teller and White, 2011). Macrophage play several function within wound site including: host defense, promote inflammation, removal of dead cells, supporting cell proliferation and tissue repair. Along with the immunological function, macrophages play crucial roles in cell proliferation and synthesis of extracellular matrix component of skin cells by production of TGF- $\alpha$ , TGF- $\beta$ , bFGF, PDGF, and VEGF (Reinke and Sorg, 2012).

## Proliferation

In the proliferative phase, angiogenesis, collagen deposition, granulation tissue formation, and epithelialization occur (Behm et al., 2012). Fibroblasts are the key cells involved in production of extracellular matrix (Harding et al., 2002). The fibroblasts are stimulated by growth factors released from the platelets, including TGF- $\beta$ , IGF-1 and PDGF (Teller and White, 2011). In the third day, the wound will become rich in fibroblasts which cause to precipitate extracellular matrix proteins (hyaluronan, fibronectins and proteoglycans) and then produce collagen and fibronectin. This results in fibrous tissue formation which along with vessels and macrophages replaces the clot at the site of the wound that is called granulation tissue. This is composed of a different types of collagen particularly type III collagen (Young and McNaught, 2011). In this phase of

wound healing, collagen type III is predominant, giving the feature of tensile strength to the newly created tissue (Olczyk et al., 2014). The matrix in and around the wound margin is degraded by different enzymes such as matrix metalloproteinase and plasminogen activators. The effect of matrix metalloproteinase is regulated by tissue inhibitors, which is important in wound healing by preventing excessive matrix degradation (Harding et al., 2002).

### Angiogenesis

A massive angiogenesis supplies oxygen and nutrients necessary for the wound healing process (Rodero and Khosrotehrani, 2010). Angiogenesis begins 1 to 2 days after vessel disruption and can be evident within about 4 days after injury (Teller and White, 2011). In response to hypoxia, VEGF is released which in combination with the other cytokines, begin angiogenesis and repair the damaged vessels (Young and McNaught, 2011).

### Epithelialization

Within several hours after injury, the process of epithelialization is stimulated by growth factors such as EGF and TGF- $\alpha$  that are produced by activated wound macrophages, platelets and keratinocytes (Diegelmann and Evans, 2004). This process begins with epidermal thickening along wound edges (Teller and White, 2011). Keratinocytes from the wound edges migrate to the wound bed, between the wound dermis and the fibrin clot. This migration is facilitated by the production of specific proteases such as the collagenase produced by the epidermal cells to degrade the extracellular matrix (Rodero and Khosrotehrani, 2010). Epithelial cells continue to migrate and proliferate until they contact with epithelial cells coming from other directions (Teller and White, 2011).

### Remodeling

The final phase of wound healing is remodeling and it may last for 1-2 years or more. In remodeling stage, fibroblasts differentiate into myofibroblasts. The first function of myofibroblasts is granulation tissue regeneration by producing new extracellular matrix, contraction and remodeling of wound and formation of scar tissue. Myofibroblasts differ from normal fibroblasts by the expression of alpha smooth muscle actin ( $\alpha$ -SMA), which is most commonly used as marker for myofibroblasts characterization and it also convert them to contractile cells. They are also characterized by expression of the ED-A fibronectin and the enhanced synthesis of several ECM proteins and growth factors. Conversion of fibroblast to myofibroblast and expression of  $\alpha$ -SMA depend on

combination of mechanical tension and TGF- $\beta$ 1 (Eckes et al., 2010).

During wound maturation period, the content of the ECM undergoes specific changes. Produced type III collagen in proliferative phase are replaced by stronger type I collagen (Reinke and Sorg, 2012). Since type III collagen have no specific structure and doesn't provide enough strength, its destruction depends on matrix metalloproteinases and inhibitors, which they are produced by macrophages, keratinocytes and fibroblasts in response to cytokines, growth factors or cell contact with ECM. Type I collagen are in a parallel orientation and do not interlace like an intact dermis (Ocleston et al., 2010; Reinke and Sorg, 2012). With the progress of the remodeling phase, the amount of fibroblasts decreases, the vascular density is lowered, and disabled cells are destroyed by apoptosis (Olczyk et al., 2014).

In chronic wounds, overexpression of proteolytic enzymes leads to destruction of ECM and essential growth factors. Moreover, neutrophils infiltration and proteinase-antiproteinase imbalance results to enhanced matrix destruction and chronic wounds with chronic inflammation (Behm et al., 2012).

### Role of Mesenchymal Stem Cells in Wound Healing Process

Stem cells have the potential to differentiate into other cell lineages and the capacity of self-renewing. The presence of stem cells ensures tissue damage regeneration in different tissues and functional disorders improvement. These cells can be divided into two categories: embryonic stem cells and adult stem cells such as MSCs. The MSCs can play effective roles in injury improvement including the wound healing process (Wu et al., 2007). The development of therapies using stem cells in the wound healing has mainly relied on adult stem cells, especially mesenchymal stromal cells or mesenchymal stem cells (MSCs). These stem cells have self-renewing potential and capable of differentiating into various cells lineages (Chen et al., 2009). MSCs can migrate to sites of injury in response to chemotactic signals modulating inflammation, repairing damaged tissue, and facilitating tissue regeneration. These cells have pivotal roles in inflammation, proliferation and remodeling phases (Maxson et al., 2012).

MSCs are a category of stem cells originated from mesodermal germinal layer and they are formed only a very small percentage of cells in the bone marrow about 0.01-0.0001% of mononuclear cells (Yolanda et al., 2014). MSCs can also be isolated from different tissues, such as umbilical cord, endometrial polyps, bone marrow, and

adipose tissue (Ding et al., 2011). MSCs can differentiate into osteoblasts, chondrocytes, adipocytes and bone marrow stromal fibroblasts under the proper conditions. The stem cells retain high degree of flexibility and are capable of regeneration of skin progenitor cells including the keratinocyte stem cell (KSCs) (Fathke et al., 2004). When tissue undergoes damage, bone marrow-derived stem cell migrate towards the wound site, because a small number of mesenchymal stem cells is always present in peripheral blood. Thus, in severe injury, the number of circulating stem cells increases to accomplish reconstruction properly (Chen et al., 2009).

### Immunomodulation

Another mechanism of action of MSCs is that they directly weaken immune response in order to decrease inflammation. Indeed, they decrease secretion of the proinflammatory cytokines while increasing the production of anti-inflammatory cytokines. These anti-inflammatory property make them particularly useful to chronic wounds healing so that these cells promote the inflammatory phase into the next stage of healing (Yolanda et al., 2014). In the inflammation phase, pre-inflammatory mediators, such as IFN- $\delta$ , TNF- $\alpha$  and IL-1 $\beta$  can activate regulatory functions in MSCs that enable them to modulate the immune response. In this phase, the MSCs can inhibit the employment, proliferation, and biological activity of mast cells, T cells, B cells, and natural killer cells (NK), thus, they weaken the severe immune response to injury (Jackson et al., 2012a). The inflammatory wound environment also stimulates cyclooxygenase 2 (COX2) activity in MSCs, which leads to upregulation of prostaglandin E2 (PGE2) and a change in wound function for desired regeneration of dermis (Jackson et al., 2012b). In addition to weakening T-cell proliferation, PGE2 can modify the behavior of leukocytes resident in the wound, corresponding to decreased expression of IL2 and INF- $\delta$ , and increased expression of IL4 and IL10 (Jackson et al., 2012a).

Indole-amine-2,3-dioxygenase (IDO) produced by MSCs suppresses many immune cells, such as T cells and NK cells. IDO is the first rate-limiting enzyme in the degradation of tryptophan through the kynurenine pathway leading to the tryptophan depletion. The reduction in local tryptophan concentration and the production of tryptophan metabolites, which cause immunomodulatory as well as the immunosuppressive effects of IDO-expressing cells (Hass and Otte, 2012; Shi et al., 2012).

These finding suggest that the immunomodulatory

function of MSCs is very important for prevention of excess inflammation and wound healing (Murphy et al., 2013).

In addition to anti-inflammatory activity, MSCs have also antimicrobial effect. This mechanism is based on secretion of LL-37, a peptide with a broad array of antimicrobial properties including suppressing wide spectrum microbial defense via disruption of bacterial cell membranes (Nuschke, 2014).

Due to the activity of immune cells at the wound site, ROS produced by neutrophils, including superoxide, hydrogen peroxide and alkyl peroxides, are highly cytotoxic compounds that prepare the sterile environment for wound, but these ROS increase collagen deposition. ROS exposure for long time during wound healing results in enhanced fibrogenesis and accumulation of fibrotic tissues through a mechanism involving membrane lipid oxidation and induction of TGF- $\beta$ 1. Nitric oxide produced by MSCs in the wound can remove ROS and produce reactive nitrogen species such as peroxynitrite. Although products of these reaction can also be cytotoxic, they react more slowly than ROS and prevent oxidative damage to DNA and membrane lipids (Jackson et al., 2012b). MSCs also increase the nitric oxide synthesis significantly in response to the interaction with T-cells in the proinflammatory environment. Nitric oxide is complementary to prostaglandin E2 for the inhibition of T-cell through suppression of signal transducer and activator of transcription (STAT5) phosphorylation in T cells and induction of immune cell apoptosis (Jackson et al., 2012b; Shi et al., 2012).

### Angiogenesis Promotion

Cell growth, proliferation, migration that mediate injured tissue healing require energy, which provide by blood capillary. Thus, blood vessel formation is the essential step in wound healing to protect granulation tissue and survive keratinocytes. Secretion of various factors involved in angiogenesis, such as VEGF, IGF-1, and angiopoietin-1 from MSCs is the powerful cues to promote proliferation, migration and differentiation of endothelial cells leading to increased angiogenesis. MSCs also express paracrine factors such as adrenomedullin to promote vascular stability and vasoprotection, which protect the healing process (Jackson et al., 2012b).

MSCs express stromal-derived factor-1 (SDF-1), VEGF and other important cytokines for angiogenesis, including PDGF-BB, FGF, Ang-1, IGF-1, MMP, IL-8 and IL-6. VEGF is a homodimer glycoprotein that stimulates the

recruitment and migration of endothelial cells, increasing angiogenesis. VEGF enhances Ang-1 expression, which acts via phosphorylation of Tie2 receptor. The Ang-1/Tie2 interaction could mediate maturation of neovessels into more complex and functional vasculature (Li et al., 2013; Zou et al., 2012). The SDF-1 activity is essential for endothelial cells survival and recruitment of stem cells to sites of injury. SDF-1 $\alpha$  not only acts as signal to the progenitor cells with chemokine receptor CXCR4 recruitment to hypoxic tissue, but also it is a signal for retention of angiogenesis of the bone marrow-derived stem cells (Bollag and Hill, 2013).

### Paracrine Signals

Paracrine signals can effect on adjacent cells; for example, VEGF not only supports neovascularization but also increases keratinocyte proliferation, which suggests a paracrine property for MSCs in wound re-epithelialization. MSCs produce various secretory factors, which play a role crucial in stimulation of skin fibroblast proliferation, angiogenesis, and collagen deposition. These cells respond to the local inflammation and hypoxic conditions of wound environment, and they protect important wound healing events, such as matrix deposition and blood vessel formation by stimulation of increasing in the rate of proliferation, differentiation, and growth factor production such as VEGF and FGF (Balaji et al., 2012). Many of these signaling mediator molecules have been studied (Table-1). MSC paracrine signaling regulates the cells responses at the wound site, which is the primary mechanism for the beneficial effects of MSCs on wounds, leading to reduce inflammation, stimulate angiogenesis, and induce cell migration and proliferation. Analyses of MSC-conditioned medium indicate that MSCs secrete many famous mediators that have role in healing process, including growth factors, cytokines, and chemokines, specifically VEGF, PDGF, bFGF, EGF, keratinocyte growth factor (KGF), and TGF- $\beta$  (Maxson et al., 2012). MSCs also secrete mitogens such as TGF- $\alpha$ , TGF- $\beta$ , HGF, EGF, FGF-2, and IGF-1, which promote proliferation of keratinocytes, dermal fibroblasts and endothelial cells (Jackson et al., 2012b; Murphy et al., 2013).

In the final stage of wound healing in adults, the unusual matrix deposition may be associated with the scar production. The growth factors regulation can control the scar formation. MSCs produce various cytokines and growth factors that have anti-fibrotic properties, including hepatocyte growth factor (HGF), IL-10 and adrenomedullin. HGF

attenuates fibrosis and scar formation through a variety of mechanisms. In response to HGF, fibroblasts reduce the expression of TGF- $\beta$ 1, collagen type I and collagen type III. HGF also enhances the expression of MMP-1, MMP-3 and MMP-13 in fibroblasts, resulting change the ECM. HGF also prevents the fibroblast differentiation to myofibroblast, thereby restricting pro-fibrotic function of these cells (Jackson et al., 2012b).

IL-10 has direct effects on fibrosis through down-regulation of the expression of TGF- $\beta$ 1 in macrophages and T-cells, and reprogramming wound fibroblasts to regeneration of ECM by up-regulation the expression of metalloproteinases and down-regulation the expression of collagen (Li and Fu, 2012). IL-10 also prevents excessive collagen deposition by decreasing the expression of proinflammatory cytokines in the wound, such as IL-6 and IL-8 (Jackson et al., 2012b; Nuschke, 2014).

### Cell Therapy and Methods of Stem Cell Transplantation to Wound Site

Cell therapy can be defined as a set of strategies to use live cells with therapeutic purposes. In this method, the cells are multiplied through *in vitro* cell proliferation in the desired volume and are provided to the target tissue. The aim of the therapy is to repair, replace or restore the biological function of a damaged tissue or organ (Yolanda et al., 2014). The use of stem cells is a new hope for chronic wound healing. Since transplant rejection is one of the problems in cell therapy, it is one of the important benefits of MSCs that allogeneic MSCs promote low immune reactions in hosts after transplantation. In addition, MSCs express the major levels of histocompatibility complex (MHC) class I but does not express MHC class II or molecules CD80, CD86, or CD40, which are involved in controlling humoral or cell-mediated immune responses. Therefore, MSCs have low inherent immunogenicity and contain an immunomodulation and immunosuppression function, which makes them appropriate candidate for autologous and allogeneic transplantation (Volk, 2010). Beside, bone marrow-derived MSCs synthesize higher amounts of various growth and angiogenic factors compared to native dermal fibroblasts, indicating a potential use in accelerating wound healing.

Today with technological advances, MSCs can be isolated from the patient's bone marrow and other tissues such as adipose tissue, nerve tissue, umbilical cord blood, and dermis (Chen et al., 2009).

**Table 1.** Growth factors released from mesenchymal stem cells during wound healing.

Abbreviation	Growth Factor	Target cell	Functions
EGF	Epidermal growth factor	Endothelial cells	Angiogenesis (Li and Fu, 2012)
PGE2	Prostaglandin E2	Leukocytes	Modulators of inflammation (Jackson et al., 2012a)
IDO	Indoleamine 2,3-dioxygenase	Leukocytes	Suppression of inflammation (Shi et al., 2012)
PDGF	Platelet-derived growth factor	Endothelial cells	Angiogenesis, Endothelial cell proliferation (Li and Fu, 2012; Volk, 2010)
VEGF	Vascular endothelial growth factor	Endothelial cells	Angiogenesis, Vascular permeability (Li and Fu, 2012; Zou et al., 2012)
HGF	Hepatocyte growth factor	Fibroblasts, Endothelial cells	Angiogenesis (Li and Fu, 2012), anti-fibrotic, inhibition of myofibroblast differentiation (Jackson et al., 2012a)
SDF-1	Stromal cell-derived factor 1	Endothelial cells	Angiogenesis (Zou et al., 2012)
IL-10	Interleukin 10	Leukocytes, Fibroblasts	Modulators inflammation, anti-fibrotic, inhibition of myofibroblast differentiation (Jackson et al., 2012a)
AM	Adrenomedullin	Fibroblasts, Endothelial cells	vascular stability, vasoprotection, anti-fibrotic (Jackson et al., 2012b)
Ang-1	Angiopoietin 1	Endothelial cells	Angiogenesis (Zou et al., 2012)

It seems that using a fibrin polymer spray that contains autologous bone marrow-derived or adipose tissue-derived MSCs is proper way for topical delivery. This procedure concentrates the cells and provides a non-toxic matrix from which cells migrate into wound beds (Shi et al., 2013). Fibrin spray supports junction, proliferation, and migration of MSCs. Fibrin in spray increases the viability of MSCs (Sorrell and Caplan, 2010). This approach is used to accelerate the rate of healing of acute and non-healing cutaneous wounds in both humans and mice. In a study, an autologous graft composed of autologous skin fibroblasts on biodegradable collagen membranes combined with autologous MSCs applied directly to the wound, which leads to a decrease in the wound size, and an increase in the vascularity of the dermis and dermal thickness of the wound (Chen et al., 2009).

### Cell Therapy Limitations for Skin Wound Healing

Delivering stem cells to the wound is a technical challenge. In order to optimize the therapeutic potential of MSCs, the delivery medium should support cell adhesion, proliferation, migration, and differentiation. The unfavorable non-healing wound environment, characterized by increased proteolytic activity and chronic inflammation, and it is another

challenges to cell viability after delivery (Falanga et al., 2007). One potential limitation to use of MSCs for treating chronic wounds is varying degrees of cell survival after implantation, which might shorten the therapeutic effects in the long-term. Another limitation to using MSCs as a standard therapy in any context is a general functional heterogeneity that turn standardization of these cells for manufacturing and quality control purposes to a severe challenge (Nuschke, 2014). Moreover, the use of MSCs as a therapeutic agent needs to *ex vivo* expansion that could remain as a problem, because heterogeneity may limit the normal lifespan (Brower et al., 2011). This treatment approach generally requires to the MSCs that must be cultured in sufficient numbers for topical application; this may not be an important issue for small chronic wounds, but is impractical for large wounds treatment. The severe burns and trauma that lead to bone marrow damage decrease the MSCs as a result of silver sulphadiazine toxicity used for treatment of burn wound infection. The bone marrow MSCs also significantly decrease with age that may decrease the applicability of using autologous MSCs for chronic wounds (Chen et al., 2009). In total, according to the potential of these cells to control of inflammation and wound reconstruction, with further development in culture and transplantation techniques we hope to use the cells as an excellent strategy for wound healing,

particularly in chronic and extensive wounds.

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