

The potential role of stem cells in wound healing

KEY WORDS

- ▶ Bone-marrow derived
- ▶ Mesenchymal
- ▶ Scaffold
- ▶ Stem cell

Wound healing consists of an intricate and highly coordinated process during which the skin repairs itself following injury. Classically, wound healing proceeds through the sequential but overlapping phases of inflammation, proliferation and remodelling. Conventional wound healing strategies have shown only limited success in the reduction of hypertrophic scars and, despite advances in treatment, chronic wounds only achieve a 50% healing rate (Margolis et al, 2003; Kurd et al, 2009). Regenerative medicine mainly focuses on the therapeutic strategy of using stem cells to aid wound healing. In this article, the authors will review the use of exogenous stem cells and their role in wound healing.

Within minutes following injury to the skin, platelets aggregate at the injury site, forming a fibrin clot that prevents bleeding. Subsequently, bacteria and other debris are enveloped and destroyed by certain types of blood cells and removed from the wound sites, and factors that promote cell migration and proliferation are released. This leads to:

- ▶ The formation of new blood vessels.
- ▶ Deposition of collagen and the formation of provisional extracellular matrix (ECM) by fibroblasts.
- ▶ Contraction of the wounds by myofibroblasts, which finally results in wound closure.
- ▶ Re-epithelialisation of the epidermis by keratinocytes (skin cells).

Wound healing proceeds through the sequential but overlapping phases of inflammation, proliferation and remodelling/maturation (Figure 1). During the last phase the newly formed ECM is remodelled and cells that are no longer needed are removed by apoptosis (the process of programmed cell death). Wound healing is not only very complex, but also fragile, and interruption in any of these phases can lead to excessive healing, as observed in the case of hypertrophic scars and keloids, or deficient healing, as observed in nonhealing chronic wounds.

STEM CELLS

Stem cells are specialised, undifferentiated cells that are potent and can differentiate into multiple cell types. They are also capable of self-renewal and can go through several cycles of cell division

while remaining undifferentiated. Broadly, stem cells can be classified into embryonic stem cells and adult stem cells.

Embryonic stem cells are isolated from the inner cell mass of pre-embryonic structures called blastocysts, while adult stem cells are found in almost all tissues.

The main sources of autologous (when donor and recipient are the same person) adult stem cells that could be used for wound repair and regeneration are: bone marrow, adipose tissue (body fat), and blood.

Stem cells from the bone marrow are known as mesenchymal stem cells and are typically extracted from the iliac crest of the femur, while adipose stem cells are isolated from adipose tissue, which can be easily obtained through liposuction. Other sources of stem cells that could be used for wound healing are:

- ▶ Umbilical cord stem cells.
- ▶ Stem cells resident in the dermis.
- ▶ Induced pluripotent stem cells (adult cells that have been genetically reprogrammed to an embryonic stem cell-like state by being forced to express genes essential for maintaining the defining properties of embryonic stem cells).

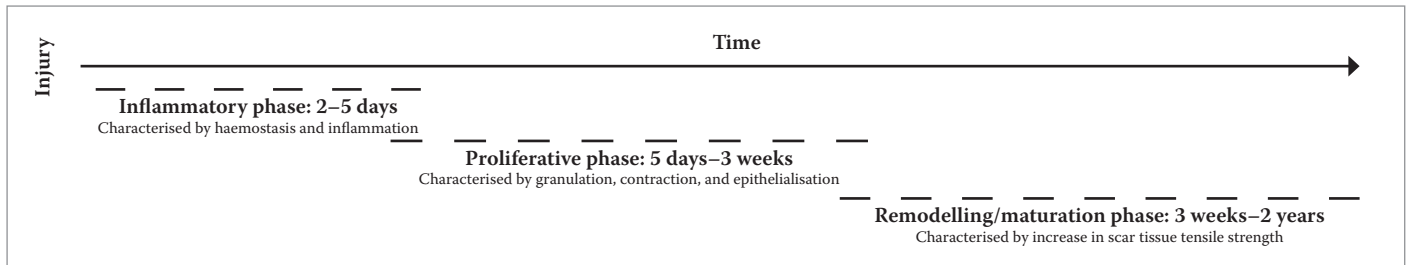
Stem cells in the dermis and bulge region of hair follicles are believed to contribute to wound repair and re-epithelialisation in the case of partial thickness skin burn wounds. However, when the loss of skin is extensive, as in third-degree burns, stem cells in the bone marrow — known as bone marrow-derived mesenchymal stem cells — are believed to contribute to wound healing. The

MATHEW VARKEY
Post-Doctoral Fellow,
Wound Healing Research
Group, University of Alberta,
Edmonton, AB, Canada

JIE DING
Research Associate, Wound
Healing Research Group,
University of Alberta,
Edmonton, AB, Canada

EDWARD TREDGET
Professor, Department of
Surgery, Director of Surgical
Research, Director of Wound
Healing Research Group;
Director of Firefighters' Burn
Treatment Unit, University of
Alberta, Edmonton,
AB, Canada

Figure 1. The overlapping stages of wound healing.



advantages and disadvantages (Table 1) of the exogenous use of these different sources of stem cells to aid wound healing are described in detail later in this article.

Embryonic stem cells

Embryonic stem cells (ESC) have the ability to divide without undergoing differentiation (Thomson et al, 1998); they are obtained from the innermost layers of blastocysts (early-stage embryo).

Studies have shown that long-term pluripotency (the ability to differentiate into different lineages) of these cells can be maintained in culture and they

can be directed to differentiate into endodermal, mesodermal or ectodermal lineages (Beddington and Robertson, 1989).

However, isolation of the inner cell mass from blastocysts results in the destruction of these early-stage embryos, which gives rise to ethical concerns. Also, there are concerns about potential immune rejection and neoplastic conversion of these cells (Yao et al, 2006). Hence, more research efforts are being directed towards:

- ▶▶ Bone marrow mesenchymal stem cells (BM- MSC).
- ▶▶ Adipose stem cells (ASC).
- ▶▶ Dermal stem cells (DSC).

Table 1. Advantages and disadvantages of the different sources of stem cells.

Source	Advantages	Disadvantages	References
Embryonic stem cells	Pluripotent Long-term propagation without differentiation	Immune rejection Neoplastic conversion Ethical concerns	Beddington et al (1989) Thomson et al (1998) Yao et al (2006)
Adult stem cells			
Bone marrow-derived mesenchymal stem cells	Multipotent Can be autologous Easily harvested	Careful control of differentiation	Pereira et al (1995) Azizi et al (1998) Badiavas and Falanga (2003) Devine et al (2003) Rasulov et al (2005) McNiece (2007) Ladak et al (2011) Wang et al (2012)
Adipose-derived stem cells	Multipotent Can be autologous Easily harvested	Careful control of differentiation	Lee et al (2004) Falanga et al (2007)
Tissue resident stem cells			
Dermal stem cells	Multipotent	Malignant transformation into melanoma	Amos et al (2010) Li et al (2010)
Induced pluripotent stem cells	Autologous pluripotent cells can be generated	Use of viral vectors Tumor formation Long-term safety not clear	Takahashi and Yamanaka (2006) Wernig et al (2007) Park et al (2008) Zabierowski et al (2011)

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Mesenchymal stem cells

Most of the stem cells found in the skin originate from the bone marrow (Fathke et al, 2004). The bone marrow contains two main types of stem cell populations, hematopoietic stem cells (HSC) and BM-MSC.

HSC are capable of differentiating into all blood cell types, including erythrocytes, platelets and white blood cells, in addition to fibrocytes and endothelial progenitor cells (Urbich and Dimmeler, 2004; Bellini and Mattoli, 2007).

Stem cells are normally identified and classified based on the cell surface molecules known as the cluster of differentiation factors (CD) present on them. Cells of hematopoietic lineage, except for mature red blood cells and their progenitors, typically express the cell-surface molecule CD45. Alternatively, BM-MSC are precursors to non-hematopoietic tissues and are significantly less abundant than HSC, about 0.001–0.01% of nucleated cells (Prockop, 1997).

They are expandable in culture and can differentiate into several cell types (Pereira et al, 1995; Azizi et al, 1998; Devine et al, 2003), including osteoblasts, adipocytes, chondrocytes, astrocytes, pneumocytes, neurons, hepatocytes, and cardiac myocytes.

The minimum criteria, according to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, used to classify cells as BM-MSC are:

- ▶▶ The cells should be plastic-adherent under standard culture conditions.
- ▶▶ They must express CD105, CD73 and CD90.
- ▶▶ They must be able to differentiate into

osteoblasts, adipocytes and chondroblasts *in vitro* (Figure 2; McNiece, 2007; Ladak et al, 2009).

Studies using animal models reveal that BM-MSC contribute to healing and regeneration of a variety of tissues including, myocardium, cardiac valves, bone, tendon, cartilage, meniscus and hair follicles (Wu et al, 2010; Wang et al, 2012). Our group recently demonstrated that the treatment of wounds with BM-MSC facilitated enhanced wound closure in an excisional wound-splinting model in both normal and diabetic mice (Wu et al, 2007). Clinically, transplantation of BM-MSC onto the debrided wounds of a patient with deep thermal burns resulted in enhanced wound regeneration and neoangiogenesis (Rasulov et al, 2005). Generally, the use of BM-MSC to treat cutaneous wounds has contributed to better clinical outcomes compared with conventional wound treatments (Badiavas and Falanga, 2003; Flaanga et al, 2007).

Adipose-derived stem cells

ASC can be easily isolated from lipoaspirates, the waste product of liposuction surgery. ASC are multipotent *in vitro* (Guilak et al, 2006), and express cell-surface receptors similar to BM-MSC (Lee et al, 2004). Animal wound models using ASC have provided promising results, but ASC are yet to be used in the clinic for treatment of cutaneous injuries (Amos et al, 2010).

Dermal stem cells

Dermis, which predominantly contains fibroblasts, also has DSC, most frequently

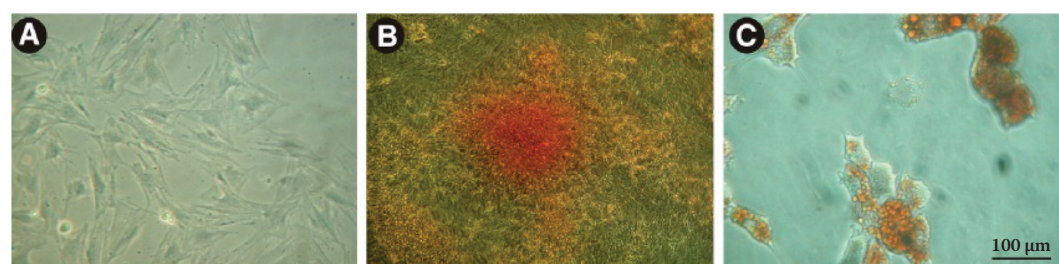


Figure 2. Bone-marrow mesenchymal stem cells (BM-MSC) obtained from rats and cultured using standard medium. Cells in culture had one of two distinct morphologies – elongated, fibroblast like, or large, flat. (a) BM-MSC were differentiated along osteogenic and adipogenic phenotypes resulting in positive staining for alizarin red (b) and oil red O (c), respectively. (From Ladak et al [2011]; with permission.)

“Currently, tissue-engineered skin available for clinical use does not fully simulate native skin in that it cannot form differentiated structures, such as hair follicles, sebaceous and sweat glands.”

around fibroblasts associated with hair follicles. DSC have been isolated and propagated from the dermal region of neonatal foreskin (Li et al, 2010). They can differentiate into neurons, smooth muscle cells, chondrocytes, adipocytes and melanocytes. These cells were identified recently and require further assessment for the possibility of ultraviolet light-induced malignant transformation into melanoma (Zabierowski et al, 2011).

Induced pluripotent stem cells

Induced pluripotent stem cells (iPS) can be obtained by reprogramming adult somatic cells such as dermal fibroblasts into an ESC-like state. iPS cells were originally generated by the incorporation of the transcription factors, Oct4, Sox2, c-Myc and Klf4, into adult somatic cells before culturing them under ESC-conditions (Takahashi and Yamanaka, 2006).

Since then, other groups have confirmed and improved this technique; the c-Myc gene has been shown to be dispensable for reprogramming, which has led to a reduction in malignant transformation observed in iPS cells (Wernig et al, 2007; Park et al, 2008; Müller et al, 2009).

This technique could enable the custom development of stem cells from the patient's own somatic cells. However, before iPS technology can transition into the clinic, there are issues which need to be resolved, including the hazards posed by using viral vectors for cell reprogramming and potential for tumour development (Müller et al, 2009).

BM-MSC in healing: mechanisms of action

BM-MSC take part in wound healing either by differentiation into different cell types to regenerate damaged tissue directly, or by regulation of local cell response to tissue injury through paracrine production of growth factors. Although BM-MSC are understood to differentiate into keratinocytes, endothelial cells and pericytes (Wu et al, 2007; Sasaki et al, 2008), the contribution of BM-MSC to wound repair through differentiation is believed to be limited due to low engraftment of BM-MSC at injury sites (Wu et al, 2007).

Paracrine signalling through the release of growth factors, such as insulin growth factor-1, angiopoietin, VEGF and macrophage inflammatory protein are considered the predominant mechanism mediating the wound healing effects attributed to BM-MSC (Chen et al, 2008; Lee et al, 2009).

This idea is supported by the fact that conditioned-media from BM-MSC has been observed to contribute to accelerated wound repair (Chen et al, 2008). Additionally, allogenic BM-MSC appear to cause similar effects on wound healing and persist in excisional wounds *in vivo* similar to autogenous cells, suggesting a unique immunologic tolerance.

Bone marrow-derived fibrocytes

Fibrocytes are a distinct type of bone marrow-derived cell that constitutes 0.1%–0.5% of peripheral blood cells; they exhibit monocyte and fibroblast-like characteristics and express stromal cell molecules such as type I-collagen, fibronectin and hematopoietic stem cell markers, such as CD11b, CD34 and CD45 (Chesney et al, 1997; Quan et al, 2004).

Fibrocytes were discovered in 1994 due to their rapid recruitment from blood into wound chambers implanted in mice (Bucala et al, 1994). Herzog and Bucala (2010) found fibrocytes to be associated with the following:

- ▶▶ Fibrosis of the kidney.
- ▶▶ Fibrosis of the liver.
- ▶▶ Pulmonary fibrosis caused by bleomycin.
- ▶▶ Nephrogenic systemic fibrosis.
- ▶▶ Scleroderma.
- ▶▶ Cardiac fibrosis.

Interestingly, the blood of burn patients has been found to have significantly more fibrocytes compared with normal individuals (Yang et al, 2002) and, subsequently, more fibrocytes were found in hypertrophic scar tissues after thermal injury (Yang et al, 2005). Fibrocytes secrete the pro-fibrotic cytokines, TGF- β 1 and its downstream mediator connective tissue growth factor (CTGF), as well as collagen type I and III and fibronectin (Bucala et al, 1994; Abe et al, 2001; Quan et al, 2004). Also, fibrocytes from burn patients have been shown to regulate dermal fibroblasts via production of TGF- β and CTGF, but their

production of collagen protein is relatively small compared with dermal fibroblasts (Wang et al, 2007). Thus, our data indicate that fibrocytes have an important role in abnormal wound healing and the formation of hypertrophic scar, as well as other fibroproliferative disorders.

Tissue engineering strategies using BM-MSC and biomaterial scaffolds

Clinically, extensive skin loss or damage due to burns, trauma, giant congenital hairy nevi or other causes, needs reconstruction of the skin for coverage of the injury and to facilitate repair and regeneration. Currently, autologous 'split-thickness' skin autografts are applied to such wounds. However, in large total body surface area second and third-degree burns, the use of skin substitutes are an important treatment option due to the lack of suitable undamaged donor skin.

Skin substitutes can be limited to epidermal cells delivered to the surface of the wound bed on their own or cells delivered within biomaterials, which is also known as tissue-engineered skin. Biomaterials maybe used for replacement of dermis only, or for tissue engineered skin that can serve as a replacement for both epidermis and dermis. The biomaterials used for the preparation of scaffolds for tissue-engineered skin can be organic polymers, inorganic materials or synthetic polymers.

Currently, tissue-engineered skin available for clinical use does not fully simulate native skin in that it cannot form differentiated structures, such as hair follicles, sebaceous and sweat glands and also lack melanocytes (skin cells that produce melanin), Langerhans cells, adipose tissue and a nerve supply. Since BM-MSC can differentiate into a variety of cells, depending on growth and differentiation conditions, and release a variety of growth factors and cytokines essential for tissue repair and regeneration, it will be advantageous to seed BM-MSC on scaffolds to be applied to wounds and so exploit their regenerative potential. Many scaffolds can be designed with appropriate biomechanical characteristics (stiffness, pore size and density) to regulate differentiation of the stem cells seeded on them (Engler et al, 2006).

Alternatively, instead of seeding stem cells on scaffolds, 'homing signals' or binding sites can be included on scaffolds, which aid mobilisation of endogenous stem cells to wound sites. In mice, wound site injections of the chemokine SLC/CCL21 resulted in the increased homing of systemically administered BM-MSCs to wound sites and their subsequent transdifferentiation into multiple skin cell lineages, leading to accelerated wound repair (Sasaki et al, 2008). Similarly, BM-MSC accelerated wound healing and tissue organisation by differentiating into wound myofibroblasts, thereby enhancing wound contraction and collagen deposition (Fathke et al, 2004; Yamaguchi et al, 2005).

CONCLUSION

Stem cells have great potential for use in accelerating wound healing. Stem cells from different sources could be used for wound repair and regeneration, such as ESC, adult stem cells in the form of BM-MSC, ASC or DSC and iPS cells. Stem cells enhance wound healing by regenerating lost tissue or through paracrine signalling and the release of growth factors. Although some bone marrow-derived cells including fibrocytes may also play a role in abnormal wound healing and fibrosis, stem cells offer great potential for skin healing after damage or loss through the combined use of novel scaffolds to enhance the regeneration of the skin.

“Bone marrow mesenchymal stem cells accelerated wound healing and tissue organisation by differentiating into wound myofibroblasts, thereby enhancing wound contraction and collagen deposition.”

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