

Chapter 9

Use of Stem Cells in Acute and Complex Wounds

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9.1 Introduction

Millions of people in the United States and around the world will suffer from acute and chronic wounds. It is estimated that 300,000 people are hospitalized each year in the United States due to acute wounds along with 11 million people affected (Hostetler et al. 2006). Chronic wounds affect approximately 6.5 million people, and it costs an excess of 25 billion annually to treat these wounds, a number that represents 2% of annual health care spending (Sen et al. 2009). These alarming numbers are mainly the result of an aging population and a rise in the incidence of diabetes. For example, roughly five million people suffering from diabetes will develop chronic wounds that will fail to heal (National Diabetes Statistical Report 2014). In recent decades, clinicians and scientists have expanded upon their knowledge of the mechanisms of wound repair and wound care (Dreifke et al. 2015). As a result, the goal of wound therapy has

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shifted from managing symptoms to a more practical approach that will ultimately promote optimal wound healing and improve patients' quality of life. Despite these advancements wound healing remains a challenge. Stem cells are essential for tissue homeostasis and repair, and their versatility holds tremendous potential for tissue regeneration in a number of different clinical applications, including acute and chronic wounds. The unique self-renewal and differentiation capacity of stem cells make them attractive alternative to traditional wound treatments (Cha and Falanga 2007). Stem cell therapy aims to enhance cutaneous regeneration by completely restoring the structure and function of tissue so it is indistinguishable from its native state. In this chapter, we will focus on current and emerging stem cell-based treatments in the management of acute and chronic skin wounds. We will address what type of stem cells are used, how these stem cells are administered to damaged tissue, and finally the challenges and future directions that stem cell therapy faces.

9.2 Wound Healing

The skin is composed of two main layers: the epidermis which forms a barrier to the external environment and the dermis which is made of connective tissue that provides the skin with its mechanical properties. The mammalian epidermis is made up of stratified keratinized epithelium with hair follicles and glands scattered throughout (Heng 2011). In contrast, the dermis is subdivided into the papillary region and reticular dermis. The papillary region is superficial to the dermis, while the reticular dermis is a deeper and thicker layer; both regions consist of collagen, elastic fibers, and extrafibrillar matrix (Watt and Fujiwara 2011). Following injury to the skin and if operative, there is a coordinated and complex response that aims to achieve tissue integrity and homeostasis. There are a number of intracellular and extracellular events that become activated, and the intensity of the response depends on the severity of the injury, the size of wound, and the type of wound (Gurtner et al. 2008; Bielefeld et al. 2013).

9.2.1 Stages of Wound Healing

The healing process can be summarized in three phases that coincide: hemostasis/inflammation, proliferation, and tissue maturation and remodeling. The hemostasis phase is responsible for preventing blood loss and infection at the wound site. There is vascular constriction and platelet aggregation that form a fibrin clot to temporarily seal the wound (Gurtner et al. 2008; Schultz et al. 2011; Guo and Dipietro 2010). The formation of the fibrin clot is crucial to the infiltration of inflammatory cells such as neutrophils, macrophages, and lymphocytes (Gurtner et al. 2008; Schultz et al. 2011; Guo and Dipietro 2010). Neutrophils remove foreign objects and bacteria, while macrophages phagocytose foreign particles and dead neutrophils

(Mahdavian Delavary et al. 2011). The infiltration of these cells also results in the release of cytokines and growth factors such as transforming growth factor (TGF- β), platelet-derived growth factor (PDGF), tumor necrosis factor (TNF)- α , interleukin (IL), and fibroblast growth factor (FGF) (Gurtner et al. 2008). The release of these cytokines and growth factors stimulates the migration and proliferation of fibroblasts and keratinocytes into the wound, a process that is crucial to initiating the subsequent healing process (Singer and Clark 1999).

The proliferative phase occurs approximately 3 days after injury in humans and is marked by angiogenesis, formation of granulation tissue, and reepithelization of the epidermis (Singer and Clark 1999). The dermis is repaired by fibroblasts that produce fibronectin, promote collagen deposition, and secrete growth factors (Bielefeld et al. 2013). Matrix metalloproteinases (MMPs) cleave the ECM allowing for the passage of fibroblasts and keratinocytes into the wound bed (Singer and Clark 1999). As a consequence, a temporary matrix is formed that epithelial cells can migrate on and proliferate leading to the restoration of a functional epidermal layer (Clark 1990). A number of different growth factors such as keratinocyte growth factor (KGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF- β , heparin-binding EGF, FGF-2, and vascular endothelial growth factor (VEGF) help facilitate the proliferative phase (Gurtner et al. 2008; Bielefeld et al. 2013; Werner and Grose 2003). This includes regulating the proliferation and mobility of keratinocytes (Gurtner et al. 2008) and supporting the formation of new capillaries and blood vessels (Werner and Grose 2003). Lastly, the proliferative stage is augmented by stem cell populations within the hair follicles and epidermis that contribute to the reepithelization of the epidermis in a wound environment (Vagnozzi et al. 2015). Stem cells from the hair follicle and interfollicular epidermis migrate to the wound site in mouse full-thickness wounds (Mascre et al. 2012; Tumber et al. 2004). Interestingly, lineage tracing studies have demonstrated that hair follicle stem cells (marked by K15) transiently contribute to wound reepithelization and are subsequently lost from the epidermis weeks later (Ito et al. 2005). These stem cells produce transit amplifying cells which can later differentiate into the stratified epithelial layer. This suggests that stem cells from the hair follicle are not necessary for the long-term maintenance of the epidermis after injury.

Once there is adequate deposition of collagen and closure of the epithelial gap, the maturation and remodeling phase begins. The properties of the tissue begin to change as type III collagen and fibronectin are slowly replaced by more organized type I collagen (Gurtner et al. 2008). Fibroblasts also transition to a more contractile myofibroblast phenotype (Darby et al. 1990). This reorganization dramatically improves the tensile strength in the new scar tissue, although the original strength of the uninjured skin is never recuperated (Singer and Clark 1999). Recently, skeletal muscle progenitors (Pax7⁺) have been shown to contribute to the repair of the skin by adopting a myofibroblastic phenotype through a process mediated by β -catenin (Amini-Nik et al. 2011). These skeletal muscle stem cells may potentially be utilized to increase tensile strength of scar tissue. Overall, this phase can last from weeks to years and results in the contraction of the wound and formation of scar tissue. The coordinated and timely response of these three phases is required for efficient skin

healing, and any complications may result in the development of acute or chronic wounds that fail to heal.

9.2.2 Signaling Pathways

There are several key signaling pathways that are activated during cutaneous wound repair that are also active during embryonic skin development. These include, but are not limited to, Wnt/ β -catenin, TGF- β , Notch, and Sonic hedgehog signaling pathways. Despite this similarity, there are a number of critical differences between the molecular mechanisms that control embryonic skin development and postnatal skin repair. Consequently, these differences may be at fault for the inability of injured skin to recapitulate the structure and function of uninjured skin. A number of studies have shown that these signaling pathways regulate the contribution and the fate of stem cells during the process of healing (Huelsenken et al. 2001; Athar et al. 2006; St-Jacques et al. 1998; Karlsson et al. 1999; Niemann et al. 2003; Chiang et al. 1999); therefore these pathways may have significant role in understanding the mechanisms of stem cells in wound repair. We will briefly discuss these developmental pathways as they may have implications in the evolution of stem cell therapies for skin repair/regeneration.

The Wnt/ β -catenin pathway plays a prominent role in cutaneous wound repair and development. Numerous Wnt = proteins are highly expressed in cutaneous wounds during the first 7 days of healing (Okuse et al. 2005), and Wnt is primarily responsible for the regeneration of hair follicles (Ito et al. 2007). On the other hand, β -catenin regulates fibroblasts during the proliferative phase of wound repair by altering fibroblast numbers, cellularity, and matrix production (Cheon et al. 2002, 2005, 2006; Sato 2006). The proliferative phase is also marked by an increase in the expression of β -catenin and its target genes fibronectin and MMP7 (Cheon et al. 2005). Upregulating β -catenin results in a fibrotic phenotype characterized by scarring, increased collagen deposition, and the formation of myofibroblasts (Cheon et al. 2006). At cellular level, an essential role for β -catenin in macrophages has been shown during wound repair. Moreover, as mentioned previously, β -catenin regulates dermal repair through regulation of Pax7⁺ cells; knocking out β -catenin in Pax7-expressing cells in mice leads to fewer dermal fibroblast cells and a smaller scar size (Bielefeld et al. 2013; Amini-Nik et al. 2011).

TGF- β is one of numerous growth factors involved in cutaneous wound healing, and it is comprised of three isoforms: TGF- β 1, TGF- β 2, and TGF- β 3. TGF- β s primarily exert their effects by activating (through phosphorylation) Smads 2 and 3 which subsequently allows them to translocate to the nucleus and alter gene expression (Schultz and Wysocki 2009; Owens et al. 2008; Biernacka et al. 2011). Similarly to the Wnt/ β -catenin, the TGF- β pathways exert its healing effects by regulating fibroblast proliferation and behavior and matrix production (Bielefeld et al. 2013). TGF- β 1 in particular has fibrosis and scar healing properties. In aged rats, treatment with topical TGF- β 1 improves ECM deposition and fibroblast proliferation and

migration (Puolakkainen et al. 1995). Moreover, research has demonstrated TGF- β 1's role in the synthesis of collagen I and fibronectin (Varga et al. 1987; Hocevar et al. 1999), proliferation of fibroblasts (Schreier et al. 1993), alteration of fibroblasts to a myofibroblast phenotype (Desmouliere et al. 1993), and finally bolster wound contraction (Martinez-Ferrer et al. 2010). Unlike Wnt/ β -catenin signaling, however, TGF- β has been shown to inhibit reepithelization. For example, treatment of porcine burn wounds with TGF- β 1 antagonist increased the rate of complete reepithelization, and deletion of Smad3 in mice accelerates proliferation (Ashcroft et al. 1999). However, in double mutant TGF- β ^{-/-} Scid^{-/-} mice, there is a significant delay in reepithelization (Crowe et al. 2000). These data suggest that TGF- β exerts some inhibitory effects on keratinocyte proliferation. Despite its positive effects in stimulating cutaneous wound healing, excessive TGF- β 1 activity may lead to the formation of hypertrophic scars through a β -catenin-mediated mechanisms (Cheon et al. 2004). In contrast, higher TGF- β 3 activity is associated with reduced scar formation as evidenced by scarless embryonic wounds in rats (Soo et al. 2003). A clinical trial which administered TGF- β 3 prophylactically to prevent dermal scarring showed accelerated healing in phase I/II studies testing (Ferguson et al. 2009); however phase III trials were unsuccessful later.

The Notch signaling pathway regulates epidermal differentiation during skin homeostasis and development, and it is vital for vascular maintenance (Moriyama et al. 2008). In terms of wound healing, blocking the expression of Notch causes delayed healing, whereas the Notch ligand, Jagged, accelerates wound closure (Chigurupati et al. 2007). Furthermore, Notch interacts with Wnt/ β -catenin and Sonic hedgehog, two other signaling pathways involved in cutaneous wound healing (Okuyama et al. 2008). The exact mechanisms by which Notch promotes healing is not as well defined as Wnt/ β -catenin, but there is data indicating that Notch regulates macrophage behavior and matrix formation. The Notch signaling pathway is necessary during the inflammatory phase of wound healing. Outtz et al. (2010) observed reduced TNF- α expression and macrophage recruitment in Notch1^{+/-} mice (Outtz et al. 2010). Furthermore, they found that Notch1 regulates vascular endothelial growth factor 1 (VEGF1) expression and inflammatory cytokine expression in macrophages in vitro (Outtz et al. 2010). With regard to matrix formation, an in vitro study has shown that Notch signaling mediates the adhesion of bone marrow-derived vascular precursor cells to the ECM (Caiado et al. 2008), an essential step during wound healing.

The Sonic hedgehog (Shh) signaling pathway plays an important role in skin development (Athar et al. 2006), but like Notch its role in wound healing is not fully understood. Shh is primarily involved in dermal reconstruction. Treating diabetic mice with Shh increases wound vascularity and cellularity and even increases VEGF expression and recruitment of endothelial progenitors (Asai et al. 2006). Interestingly, the Shh inhibitor cyclopamine hinders wound vascularity and cell proliferation leading to delayed wound closure in mice (Le et al. 2008). Further research is needed to elucidate the mechanisms that allow Shh to influence dermal wound repair.

9.3 Acute and Complex Wounds

Acute wounds will generally heal within a few weeks in healthy individuals, and the healing process strictly follows the aforementioned wound healing phases. In contrast, complex wounds do not conform to the cellular and molecular events that lead to healthy wound healing. Instead, there may be difficulties in the phases of repair that result in the wound failing to heal. As such, complex wounds are defined as wounds that have failed to proceed through an orderly and timely reparative process to produce anatomic and functional integrity over a period of 3 months (Mustoe et al. 2006). A common theory is that complex wounds remain in the inflammatory stage for too long resulting in a wound that is unable to progress to the proliferative and remodeling phases of healing. Additionally, there may be increased free radical production, ischemia, impaired growth factor and cytokine production, infection, and diminished cellular infiltration that disrupt the healing process (Agren et al. 2000; Mast and Schultz 1996). A robust understanding of the mechanisms involved in chronic wound healing is necessary to develop effective stem cell-based therapies that go beyond the treatments currently available. The following section will discuss wound healing in diabetic patients and the elderly, two groups of people that may suffer from complex wounds that result in deficient healing.

9.3.1 Deficient Healing

The prolonged inflammatory response in chronic wounds causes reepithelization to stall as granulation tissue is defective and does not foster healing (Martin and Nunan 2015). In diabetic ulcer wounds, fibroblasts become defective as they are unresponsive to growth factor signals, and their ability to migrate to the wound bed is reduced (Brem et al. 2007; Lerman et al. 2003; Seibold et al. 1985). Ulcers also display a significant decrease in the number of TGF- β receptors and the TGF- β signaling cascade (Pastar et al. 2010). These aberrations combined with elevated levels of degrading MMPs reduce collagen deposition and thus affecting ECM integrity and tissue remodeling in diabetic patients (Tregrove et al. 1999). Moreover, the effectiveness of immune cells such as neutrophils and macrophages to phagocytose debris is hindered resulting in a buildup of necrotic debris at the wound edge. There is an enrichment of pro-inflammatory cytokines such as TNF- α and IL-1 β in the wound bed which can inhibit the growth and phenotype of fibroblasts (Zykova et al. 2000). There is also a decrease in IL-6, a cytokine that is responsible for mobilizing fibroblasts and keratinocytes to the wound bed (Werner and Grose 2003). Accordingly, this exaggerated and unbalanced production of pro-inflammatory cytokines adversely affects wound closure. In elderly patients, wound healing follows a similar trajectory to diabetic wound healing. There are several factors that lead to deficient healing in the elderly; however there are two that are well described in the literature. First, there is impaired formation of new blood vessels (neovascularization) (Chang et al. 2007). Second, aged stem cells may be dysfunctional

compared to their younger counterparts, and this dysfunction may possibly be due to the microenvironment aged stem cells that are exposed and/or cell intrinsic alterations (Blau et al. 2015). Research has shown that aged bone marrow-derived stem cells (BM-MSCs) have reduced wound healing, angiogenesis, and proliferation capabilities compared to younger BM-MSCs (Choudhery et al. 2012). Moreover, it has been shown that mesenchymal stem cells (MSC) isolated from the skin of elderly burn patients have diminished cell proliferation and deficient migration (Jeschke et al. 2015).

9.3.2 *Excessive Healing*

In contrast to deficient healing, excessive healing leads to the formation of a keloid or hypertrophic scar. As mentioned previously, there is a delicate balance between ECM deposition and degradation in normal wound healing. In both keloids and hypertrophic scars, there is increased collagen deposition that is oriented in thick bundles rather than basket weave-like fibrils in normal dermis (Martin and Nunan 2015). In human hypertrophic scars, the number of macrophage cells is positively correlated with scar size and the level of β -catenin, suggesting that macrophages play a role in the development of excessive healing (Amini-Nik et al. 2014). Furthermore, deletion of β -catenin in macrophage-specific cells resulted in impaired migration, cell adhesion, and inability to produce TGF- β 1 leading to inadequate wound healing (Amini-Nik et al. 2014). Moreover, there is a positive correlation between the number of inflammatory cells and expression of β -catenin (Cheon et al. 2002). Considering that β -catenin plays a role during the proliferative phase of wound healing and that increased β -catenin activity associates hypertrophic scars, it is plausible that β -catenin is partly responsible for the excessive healing. TGF- β signaling may also play a role in excessive healing as blocking the activation of TGF- β 1/2 receptors prevents scarring (Ferguson and O’Kane 2004). The difference between keloids and hypertrophic scars arises primarily from the arrangement of the collagen fibers and the composition of the wound (Martin and Nunan 2015). Keloids feature collagen fibers that spill beyond the wound margin, while hypertrophic scars do not (Martin and Nunan 2015). Keloids are also characterized by occluded blood vessels and fewer fibroblasts when compared with hypertrophic scars (Martin and Nunan 2015).

9.4 Current Treatments of Acute and Complex Wounds

As mentioned previously, diabetes mellitus is a condition in which insufficient wound healing can result in foot ulcers, hence the term “diabetic foot” (Singh et al. 2005). Foot ulcers are a common problem in diabetic patients with some experiencing significant epidermis damage that can expose the dermis and even deeper layers

like the muscle and bone (Singh et al. 2005). The International Wound Bed Preparation Advisory Board provides a systemic approach to the management of chronic wounds such as diabetic ulcers: debridement, management of exudate, and management of infection (Schultz et al. 2003; Falanga 2000). Following a severe cutaneous wound, there may be an accumulation of necrotic tissue which can lead to infection. Debridement and drainage of wound fluid is done to remove infected tissue, clean the wound, ensure adequate blood flow, and provide a moist wound environment for optimal wound healing (Tsourdi et al. 2013). After the wound is contained, frequent dressing changes and use of topical antibiotics help maintain a clean environment and curtail infection. In some cases the necrosis and infection may be uncontrollable, and as such an amputation of the leg is necessary to prevent system infection (Margolis et al. 2005). Debridement strategies can be a strenuous task and can be painful for the patient. Some diabetic patients and sufferers of chronic wounds require negative-pressure wound therapy (NPWT). This is a treatment option that uses a vacuum to reduce swelling in the wound area which allows for blood and nutrients to reach the wound site resulting in improved blood flow and removed bacterial fluid (Guffanti 2014).

Chronic wounds are often lacking in growth factors necessary for wound healing. Growth factors control many key cellular processes in normal tissue repair such as cell migration, proliferation, angiogenesis, and production of ECM components (Branski et al. 2007). Therefore, increasing growth factor concentrations in chronic wounds may enhance wound healing. Applying growth factors directly to the wound bed is a common method of delivery. For instance, human recombinant platelet-derived growth factor BB (PDGF-BB) was the first cytokine growth factor approved by the FDA (Snyder 2005). PDGF-BB is topically applied to the wound where it promotes proliferation of cells involved in cutaneous wound repair. When PDGF-BB is used in conjunction with the wound healing practices described above, there is a reduction in healing time in patients with diabetic ulcers (Signorini and Clementoni 2007). An alternative to growth factors is applying or transplanting cultured keratinocytes and/or fibroblasts to the wound in order to stimulate repair. In fact, implanting cultured keratinocytes into the wound bed has been shown to promote reepithelization in diabetic pigs (Karagoz et al. 2009).

In some cases, grafting skin from cadavers onto the wound bed may be necessary to prevent infection and promote healing; the transplanted skin provides a scaffold for epithelial cells that boost cell proliferation (Snyder 2005). The skin transplanted from a member of the same species is called an allograft, whereas an autograft is a technique in which a patient's own skin is used for transplantation significantly reducing the risk of rejection and may be required if allografts are unsuccessful (Snyder 2005). Over the decades, bioengineering has produced dermal substitutes that can facilitate wound healing when traditional methods cannot be used or have failed. In severe burns, for example, a dermal substitute named Integra (Integra Limit Uncertainty, Plainsboro, New Jersey, USA) serves as temporary epidermis and is often used to assist wound closure (Branski et al. 2007; Jeschke et al. 2004). Integra is an acellular bilaminar structure made of silicone that provides a provisional dermal replacement which prevents fluid loss and acts as barricade against

pathogens. Integra is composed of two layers: the bottom layer is made of a cross-linked matrix of bovine collagen and chondroitin-6-sulfate that is vital to preventing hypertrophic scarring, while the top layer is the silicon layer described above (Branski et al. 2007; Jeschke et al. 2004). The matrix in the bottom layer is vascularized and populated by host cells as the wound heals which forms a new dermis layer. Clinical trials have demonstrated the efficacy of Integra in promoting wound closure in addition to its ability to lessen hypertrophic scarring (Branski et al. 2007; Jeschke et al. 2004). Despite its advantages and success in the clinic, Integra is unable to recapitulate the structural properties of intact skin due to its lack of cellularity. Furthermore, it is expensive to produce, especially in the case of large total surface area burns (TBSA).

In excessive healing like hypertrophic scars and keloids, nonsurgical techniques are the most common methods of reducing scar size. The most accepted treatment of hypertrophic scars currently is the topical application of silicone gels or sheets onto the scar. The use of silicone has become standard practice among plastic surgeons, and it is effective in treating hypertrophic scars (Signorini and Clementoni 2007). Silicone decreases scar size mainly through wound hydration; silicone decreases water vapor transmission at the wound site resulting in a build of moisture in the wound (Signorini and Clementoni 2007). While it is a simple and effective treatment, silicone is difficult to apply, requires multiple applications per day, and may cause adverse skin reactions (Karagoz et al. 2009). An alternative method of reducing scar size is the use of mechanical force exerted by pressure garments. The theory behind this treatment is that the pressure limits collagen synthesis by creating a hypoxic environment that prevents the delivery of blood, oxygen, and nutrients (Puzey 2002). Pressure garments remain a prevalent treatment despite there being no conclusive data illustrating a reduction in scar size (Macintyre and Baird 2006). Lastly, an interesting and unusual treatment has emerged in the use of topical onion extract gel. The healing properties of onion extract are derived from its ability to inhibit fibroblast proliferation and hinder ECM production by increasing expression of MMP-1 (Cho et al. 2010). The efficacy of onion extract in decreasing scar size, however, is lacking in literature. Two studies observed no significant difference in scar appearance and size in patients treated with a 10% onion extract gel (Willital and Heine 1994; Chanprapaph et al. 2012). Stem cells hold great potential in producing treatments that are simple, safe, prevent complications, and go beyond typical treatments of acute and chronic wounds by fully regenerating skin. We will now describe the characteristics of different types of stem cells and how they have used in generating exciting new therapies for skin regeneration.

9.5 Stem Cells in Wound Healing

The goal of stem cell therapy is to restore skin back to its functional state after cutaneous wound injury. Unfortunately, dermal substitutes lack the biological activity necessary to regenerate the skin despite the outstanding physical support they

provide to the wound bed. Incorporating stem cells and growth factors into these dermal substitutes would add the cellularity needed to accelerate skin regeneration. It is believed that the addition of these stem cells will enhance wound healing through trophic and paracrine activity that will regenerate the skin by neutralizing the damaging effects of the healing process and repairing skin by replacing lost or damaged cells (Falanga 2012).

Stem cells are groups of undifferentiated cells capable of transforming all types of cells in the human body. Using these cells as a renewable source for new tissue is the basis of regenerative medicine. The ability of these cells to self-renew and differentiate into different cell types is termed “stemness,” and it is these unique features that make stem cell-based therapies an attractive alternative to traditional treatments (Cha and Falanga 2007). Not all stem cells demonstrate true “stemness” however as only embryonic stem cells are capable of differentiating into all cell lineages. This is a serious problem for regenerative medicine as the most commonly used stem cells, adult stem cells, only partially fulfill the requirements of stemness since their differentiation potential is limited to a few cell lineages. In the following section, we will discuss recent progress in the application of different types of stem cells for cutaneous wound healing.

9.5.1 Embryonic Stem Cells

Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst of a human embryo (Cha and Falanga 2007). ESCs are the standard to which all stem cells are held due to their ability to differentiate into all three germ layers (ectoderm, mesoderm, and endoderm) given the correct stimulus, an ability termed pluripotency (Cha and Falanga 2007). ESCs can be maintained in culture in an undifferentiated or be differentiated into specialized cell types. With regard to wound healing, ESCs have been differentiated into basal keratinocytes which were used to construct a stratified epidermis. Nevertheless, there are no stem cell therapies in wound healing that utilize ESCs despite their unlimited potential for self-renewal and plasticity. This is due to ethical and legal restrictions that limit the application of ESCs in regenerative medicine. Moreover, ESCs are restricted by their allogenic nature and safety concerns regarding the risk of developing tumors (Cha and Falanga 2007). As a result, regenerative medicine has primarily focused on adult stem cells since they are able to provide some of advantages of ESCs without the rigorous ethical and legal constraints.

9.5.2 Bone Marrow-Derived Stem Cells

The bone marrow (BM) is comprised of a heterogeneous cell population which includes hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), two groups of cells that are highly plastic progenitor cells (Salem and Thiernemann

2010). HSCs are responsible for the bone marrow and blood, while MSCs are non-hematopoietic stem cells that are self-renewing and differentiate into various cell types such as osteoblasts, adipocytes, chondrocytes, and even dermis (Wu et al. 2007a). MSCs are marked by their ability to adhere to a plastic surface, and cultured MSCs are strongly positive for CD105, CD90, and CD73 but negative for CD34 and CD45 (Boxall and Jones 2012). During wound healing, HSCs and MSCs mobilize to the wound site where they modulate the inflammatory response so that the wound does not progress to a chronic state. BM-MSCs attenuate the inflammatory response by decreasing the secretion of pro-inflammatory cytokines while amplifying the production of anti-inflammatory cytokines in the wound bed (Aggarwal and Pittenger 2005). In addition to diminishing the inflammatory response, BM-MSCs demonstrate antimicrobial activity (Maxson et al. 2012) and secrete growth factors such as FGF and VEGF that promote fibroblast proliferation, collagen deposition, and angiogenesis (Gnecchi et al. 2008). An alternative explanation for the wound healing properties of BM-MSCs is that these stem cells migrate to the wound site and differentiate into cells necessary for the renewal and repair of damaged tissue (Wu et al. 2007a). BM-MSCs are well characterized and most widely studied stem cell population; however the isolation procedure is painful for patients, and there are few stem cells present in bone marrow aspirate.

Stem cell therapies in cutaneous wound healing have relied heavily on BM-MSCs, and numerous studies have demonstrated the usefulness of BM-MSCs in treating deficient and excessive healing in animals and humans (Table 9.1). In humans, autologous BM-MSCs delivered via a fibrin spray reduced wound size in patients with acute surgical excisional wounds (Falanga et al. 2007). In another study, direct injection of BM followed by topical application of cultured MSCs directly onto the wound bed led to complete wound closure, dermal rebuilding, and less scarring in three patients with chronic ulcer wounds (Badiavas and Falanga 2003). Moreover, examining biopsies from the healed tissue revealed significant vascularization and dermal thickness (Badiavas and Falanga 2003). The clinical benefits of BM-MSCs have also been illustrated in several trials involving diabetic ulcers where traditional therapies have failed. In one study, intramuscular injection of autologous BM-MSCs in patients with diabetic ulcers resulted in improved blood flow to the wound area (Lu et al. 2011). The injected was well tolerated, and patients experienced a significant reduction in pain and a marked improvement in healing rate of the ulcer at 24 weeks postinjection (Lu et al. 2011). Another study observed improved circulation and complete wound closure after transplantation of BM-MSCs in 18 out of 22 patients with diabetic ulcers (Kirana et al. 2012). Two other studies show that injection of BM-MSCs in patients with diabetic ulcers improves recovery time (Liu et al. 2014; Dash et al. 2009), decreases wound size (Dash et al. 2009), and enhances development of fibroblasts and inflammatory cells (Dash et al. 2009).

Animal studies involving BM-MSCs provide a more comprehensive and detailed view of the effect of these cells on cutaneous wound healing. In mouse acute excisional wounds, local injection of BM-MSCs accelerated wound closure and increased epithelization and angiogenesis (Wu et al. 2007b). Similar beneficial effects of BM-MSCs have been observed in other animals as transplantation of BM-MSCs improved tensile strength and healing in albino rat excisional wounds

Table 9.1 Published clinical studies utilizing stem cell therapies for the treatment of chronic wounds

Wound category	Type of stem cell	Delivery method	Patient outcome	Reference
Diabetic limb ischemia ($n = 41$)	BM-MSC and BM-MNC	Intramuscular injection	BM-MSC treated had improved healing rate compared to BM-MNC. Pain-free walking time was higher as well	Lu et al. (2011)
Chronic wound ($n = 3$)	BM-MSC	Topical application	Complete wound closure in all three patients. Improved inflammatory response and angiogenesis	Badiavas and Falanga (2003)
Chronic limb ischemia ($n = 6$) and acute surgical wound ($n = 4$)	BM-MSC	Topical application via fibrin spray	40% reduction in wound size in four CLI patients. Direct correlation with number of cells sprayed and decrease in wound size	Falanga et al. (2007)
Diabetic nonhealing ulcer ($n = 24$)	BM-MSC	Intramuscular injection and topical application	Ulcer size decreased by 73% in treated group. Improved reepithelization and pain-free walking distance	Dash et al. (2009)
Intractable dermatopathies ($n = 20$)	BM-MSC	BM-MSCs on collagen sponges applied to wound	Improved wound healing in 18 patients. Two patients died	Yoshikawa et al. (2008)
Radiation burn ($n = 1$)	BM-MSC	Local injection	Complete healing in one patient	Lataillade et al. (2007)
Critical limb ischemia ($n = 7$)	BM-MSC	Intramuscular injection	Six of seven patients experienced complete wound healing	Gupta et al. (2013)
Diabetic foot ($n = 1$)	BM-MSC	Direct application + injection	Wound size decreased and thickness of epidermis increased after 1 month in this single patient	Vojtassak et al. (2006)
Critical limb ischemia ($n = 4$)	BM-MSC	Intra-arterial administration	Significant pain relief and improved wound healing	Das et al. (2013)
Critical limb ischemia ($n = 9$)	ASC	Intramuscular injection	Improved wound healing in six of nine patients	Lee et al. (2012)
Ischemic ulcer ($n = 6$)	ASC	Intramuscular injection	Ulcer size was decreased	Bura et al. (2014)
Chronic ulcer lower limb ($n = 10$)	ASC	Direct injection	Six of ten patients experienced complete wound healing	Marino et al. (2013)

(Basiouny et al. 2013). As mentioned earlier, the dermis is remodeled in part through contribution of fibroblasts and deposition of collagen, and BM-MSCs may impact this process. In acute mouse wounds, BM-MSCs transcribe both collagen I and III and contributed to the production of 15–20% of the dermal fibroblast population (Fathke et al. 2004). Similar beneficial effects of BM-MSCs have been shown in diabetic wound healing in animals. Treatment with BM-MSCs has led to enhanced wound closure (Falanga et al. 2007), accelerated wound healing (Ha et al. 2010), and increased reepithelization (Fiorina et al. 2010) in various acute wound healing animal models.

The method of delivery of BM-MSCs may influence the healing effects observed. For instance, BM-MSCs seeded on a pullulan-collagen hydrogel scaffold accelerated healing and increased angiogenesis in a murine excisional wound model (Rustad et al. 2012). Interestingly, BM-MSCs seeded on this hydrogel expressed increased levels of VEGF and were able to differentiate into fibroblasts, endothelial cells, and pericytes, but not epithelial cells (Rustad et al. 2012). In contrast, injection of BM-MSCs after acute skin damage caused by irradiation speeds up wound healing and increases expression of growth factors such as TGF- β 1 and SDF-1 in rats (Zheng et al. 2015). Lastly, one study observed increased healing rate, increased collagen fibers, increased tensile strength, and infiltration of CD68+ macrophages in mice after BM-MSCs were delivered topically via fibrin glue (Mehanna et al. 2015). Treatment of the BM-MSCs also has an effect on wound healing as BM-MSCs that undergo hypoxia pretreatment are more effective at healing in rats with diabetic hindlimb ischemia (Tong et al. 2015). Optimizing the method of delivery and the way BM-MSCs are prepared is crucial to developing effective stem cell therapies in cutaneous wound healing.

There are no clinical studies that examine the effect of BM-MSCs in treating hypertrophic scarring and keloids; however there are a couple animal studies that demonstrate the potential of BM-MSCs in reducing fibrosis. Infusion of human MSCs significantly reduced fibrosis in a dermal fibrosis mouse model through suppression of fibroblast proliferation (Wu et al. 2015). Another study showed that local injection of human MSCs prevented hypertrophic scar formation in rabbits through regulation of the anti-inflammatory protein, TSG-6, rather than modulating fibroblast activity like in the previous study. The efficacy of BM-MSCs in treating acute and complex wounds and the associated mechanisms need to be further explored.

9.5.3 Adipose-Derived Stem Cells

As mentioned earlier, harvesting BM-MSCs from patients is invasive and painful and yields relatively few cells. Adipose-derived stem cells (ASCs) have been identified as a new source of adult stem cells that appear to be promising in the field of regenerative medicine. Harvesting ASCs from adipose tissue is less invasive, less painful, and avoids the ethical and immunological concerns plaguing other stem

cells (Zuk et al. 2002). Adipose tissue is also remarkable source of ASCs and can easily be isolated from a section of whole fat (biopsies) or lipoaspirate following surgery in patients (Zuk et al. 2002). For example, adipose tissue yields a 500-fold greater number of stem cells when compared to an equivalent amount of the bone marrow (Strioga et al. 2012). ASCs can differentiate into adipocytes, chondrocytes, and myogenic lineages; however one drawback is that ASCs do not offer as much plasticity as BM-MSCs (Gimble et al. 2012). Similarly to BM-MSCs, ASCs secrete a number of different cytokines and growth factors that promote wound healing (Kilroy et al. 2007). There are a couple drawbacks to ASCs however. First, the metabolic activity of ASCs (i.e., proliferation and differentiation capacity) depends on the anatomical location of the fat, age, and gender of the patient (Bailey et al. 2010). Second, it has been reported that ASCs undergo spontaneous malignant transformation after 4 months of culturing (Rubio et al. 2005); however researchers argue that this results from contamination rather than some inherent characteristic of ASCs (Garcia et al. 2010; Torsvik et al. 2010). Nevertheless, the above characteristics make ASCs an exciting alternative in the field of stem cell therapy, and a number of studies in various conditions have shown the therapeutic potential of ASCs in wound healing.

In 2012, Lee et al. (2012) showed that multiple intramuscular injections of ASCs resulted in clinical improvement in 66.7% of patients with critical limb ischemia. These patients exhibited decreased pain and improved claudication walking distance suggesting that multiple intramuscular injections of ASCs may be a viable therapeutic method of increasing blood flow in patients with critical limb ischemia (Lee et al. 2012). An interesting technique that may be used in the future to prevent the development of chronic wounds may be attaching ASCs (or other stem cells) to surgical sutures. Biodegradable surgical sutures filled with ASCs create a pro-regenerative environment *in vitro* with a steady release of cytokines and endothelial growth factors (Reckhenrich et al. 2014). Moreover, conditioned media from the supernatant of these cell-filled sutures significantly decreased wound area compared to controls in a wound healing assay (Reckhenrich et al. 2014).

Animal studies have demonstrated the wide variety of positive effects ASCs can have on cutaneous wound healing. Following acute wounds, injection of ASCs increases epithelization, decreases healing time, and reduces inflammation in fisher rats and rabbits (Pelizzo et al. 2015; Uysal et al. 2014). A recent study by Mendez et al. (2015) has shown that subcutaneous injection of ASCs decreases wound size and increases granulation and collagen synthesis in mice with full-thickness wounds. Interestingly, these researchers were able to develop a fibrin formulation that transforms ASCs into vascular tubes that express various endothelial markers (Mendez et al. 2015). It is thought that the growth of new blood vessels via the release of endothelial growth factors such as VEGF and HGF may be one mechanism by which ASCs exert their healing effects. In fact, preclinical studies have reported improved tissue hydration and angiogenesis after treatment with ASCs in patients with radiation-induced skin damage (Rigotti et al. 2007; Akita et al. 2010). Two animal studies also show increased vascularization and improved healing after acute wounds in mice and rats, respectively (Garg et al. 2014; Meruane et al. 2012).

One study has shown that ASCs may be more effective than BM-MSCs in treating acute wounds (find reference) and found that local injection of ASCs in rabbit excisional wounds was more effective than BM-MSCs in improving tensile strength and attenuating scar formation. With regard to deficient healing, allogenic transplantation of ASCs accelerated wound healing and vascularization in a diabetic rat wound model (Kato et al. 2015). Recently, injection of ASCs had positive effects in young and old mice with pressure ulcers (Strong et al. 2015): observed accelerated wound closure, increased adipogenesis, and improved epidermal structure in these mice.

There are no human studies examining the effect of ASCs in excessive healing; however there are couple of animal studies which are promising. In fibrosis rabbit ear model, it was shown that intralesional injection of ASCs decreased expression of α -SMA and collagen type I leading to diminished collagen deposition and hence reduced hypertrophic scarring (Zhang et al. 2015). Another study revealed that ASCs delivered through an ECM patch derived from porcine small intestine submucosa (SIS) significantly reduce fibrotic scar size in mice (Lam et al. 2013). In the future, more animal studies are needed to justify the use of ASCs in treating hypertrophic scars and keloids in humans. The unique trophic functions of ASCs combined with the studies described above make them an exciting alternative in stem cell-based therapies and support their applicability in cutaneous wound healing.

9.5.4 Umbilical and Placental Derived Stem Cells

It is believed that umbilical cord blood is the largest potential source of hematopoietic and non-hematopoietic stem cells with naïve immune status (Knudtzon 1974). These cord blood stem cells have been shown to differentiate into hepatic, pancreatic, and neural precursor cells (McGuckin et al. 2006; Denner et al. 2007). In fact, hematopoietic stem cells from cord blood have been used to treat many pediatric and adult diseases (Branski et al. 2009). Moreover, MSCs have been isolated from umbilical cord blood and successfully differentiated into epithelial cells in vitro (Sanmano et al. 2005; Kamolz et al. 2006). In addition to the blood, different layers of the umbilical cord such as Wharton's jelly (the gelatinous supporting matrix) and the outer lining are a source of stem cells. Stem cells isolated from the cord lining can be divided into two groups: epithelial cells and mesenchymal cells. These cells express typical stem cell markers such as Oct-4 and Nanog (In 't Anker et al. 2003) and are capable of differentiating into epithelial cells, hepatocytes, neural cells, and endothelium. These characteristics suggest that cord lining stem cells can potentially be used in the clinic to regenerate epithelial cells in cutaneous wound healing. While the umbilical cord is large reservoir of stem cells, isolating these cells is difficult as the umbilical cord provides 30% less cells when compared with MSCs isolated from the bone marrow (Wilson et al. 2011). Umbilical cord stem cells are a promising new source of new tissue in skin regeneration, and coadministration with other stem cells like BM-MSCs or ASCs may offer synergistic benefits in wound healing. Many clinical trials are ongoing to test the efficacy of umbilical

cord-derived stem cells in treating diabetic wounds and burns. These studies have not reported any immunological rejection or tumor formation indicating that these cells pose no risk to patients.

Transplantation of Wharton's jelly MSCs increases expression of genes in reepithelization and promoted wound healing in mouse excisional wounds (Shi et al. 2015). Other studies have reported improved wound healing after administration of Wharton's jelly MSCs in addition to increased keratinocyte differentiation (Luo et al. 2010), reduced scar formation, and improved hair growth (Sabapathy et al. 2014). Delivering umbilical cord MSCs via a collagen-fibrin double-layered reduced healing time in a mouse full-thickness wound model further illustrating that cell delivery is an important aspect of developing stem cell therapies (Nan et al. 2015). The mechanism of action of umbilical cord-derived stem cells may be modulation of growth factors involved in the TGF- β signaling pathway. Interestingly, intraperitoneal injection of placenta-derived MSCs accelerates wound healing by increasing the expression of pro-angiogenic factors such as VEGF (Arno et al. 2014). In deficient healing, cord-derived MSCs delivered via topical application accelerated wound healing in db/db mice (Ha et al. 2010). Interestingly, TGF- β expression was significantly increased after the first week of treatment. Lastly, Wharton's jelly MSCs increase proliferation rate of keloid fibroblasts in vitro through regulation of TGF- β 2 further supporting the notion that umbilical cord-derived MSCs modulate TGF- β activity (Arno et al. 2014).

9.5.5 Epidermal Stem Cells

The skin has become a promising source of adult stem cells that could potentially be seeded onto dermal substitutes to create a scaffold with biological activity. In contrast to ESCs and iPSCs, epidermal stem cells are non-oncogenic, have no ethical concerns, and are easily accessible (Fuchs and Nowak 2008). As a result, these cells may solve many problems in the field of skin engineering such as immune rejection and lack of skin appendages. Epidermal stem cells are located in the basal layer of the epidermis and are unipotent and able to regenerate the epidermis in adults (Morasso and Tomic-Canic 2005). In contrast, stem cells located within the bulge region of the hair follicle are multipotent cells capable of repopulating the epidermis, sebaceous glands, and hair follicles (Blanpain et al. 2004). Additionally, these cells are K15-positive and can differentiate into smooth muscle cells, neurons, and glial cells (Amoh et al. 2010). During injury, hair follicle stem cells rapidly mobilize and migrate to repair the epidermis in vivo by contributing to reepithelization (Ito et al. 2005). This response is tightly regulated and stops once the wound has healed. Lineage tracing studies have confirmed that hair follicle stem cells are normally unipotent as they maintain their niche compartment, the hair follicle, during homeostasis (Tumbar et al. 2004).

In the treatment of deep burn wounds, transplantation of cultured epithelial cells containing epidermal stem cells and keratinocytes via a fibrin matrix is well estab-

lished (Fang et al. 2014). There are also reports of cultured epidermal cells being effective in treating chronic skin ulcers and deep dermal wounds (Oshima et al. 2002). Likewise, animal studies have illustrated the potential of epidermal stem cells in treating acute and complex wounds. Transplantation of epidermal stem cells via a collagen-chitin biomimetic membrane has been shown to regenerate whole skin in mice with full-thickness skin defects (Shen et al. 2014). Comparable healing effects were also found in diabetic mice treated with stem cells isolated from the hair follicle dermal sheath (Ma et al. 2015). Furthermore, in vitro examination revealed that these stem cells secreted paracrine factors such as IL-6 and enhanced wound healing. Recently, a novel epidermal stem cell population marked by Lgr6 was found in the isthmus region of the hair follicle (Snippert et al. 2010). Similar to other hair follicle stem cells, transplantation of Lgr6-positive cells gives rise to all epidermal lineages and also migrate to and repair the interfollicular epidermis during injury (Snippert et al. 2010). Unlike other hair follicle stem cells, however, Lgr6-positive cells contribute to the maintenance of the isthmus region and the sebaceous glands. Lastly, in mice with full-thickness wounds, local injection of Lgr6-expressing epithelial cells onto the wound bed promotes hair growth, reepithelization, and angiogenesis demonstrating the potential of these cells to facilitate cutaneous wound healing (Lough et al. 2014).

9.5.6 Induced Pluripotent Stem Cells

The ethical and legal concerns surrounding ESCs were quickly forgotten in 2007 when a new class of stem cells was discovered which combines the advantages of embryonic and adult stem cells. Induced pluripotent stem cells (iPSCs) were generated by exposing fibroblasts cultures to a cocktail of genes such as Oct3/Oct4, Sox2, and Klf4 by retroviral delivery (Takahashi et al. 2007) and Oct4, Sox2, Nanog, and Lin278 by lentiviral vectors (Yu et al. 2007). These iPSCs were immature, pluripotent, and nonimmunogenic cells generated from adult-differentiated tissue that exhibited similar plasticity to human ESCs. Their pluripotency was confirmed by their capability of differentiating into various cell types in vitro, expression of pluripotent marker genes, and their ability to produce all three germ layers (Takahashi and Yamanaka 2006). There are, however, safety concerns regarding iPSCs as they are cancerous in an undifferentiated state, and 20% of chimeric mice develop tumors due to expression of the well-known oncogene, c-Myc (Cartwright et al. 2005). Despite these concerns, iPSCs hold great promise in regenerative medicine. Human-derived iPSCs have been differentiated into epithelial cells resulting in the development of all hair follicle lineages (Yang et al. 2014). To take this concept further, Itoh et al. (2011) generated a 3-D skin equivalent in vitro that was comprised mostly of iPSC-derived keratinocytes and fibroblasts. Lastly, iPSC-derived keratinocytes have been used to create a stratified epithelium that was subsequently used to treat recessive dystopic epidermolysis bullosa, a type of deficient healing (Sebastiano et al. 2014). Overall, iPSCs will play an important role in stem cell therapies, but further research is needed to improve safety and refine the methods of reprogramming these cells.

9.6 Methods of Delivering Stem Cells

The therapeutic benefit of stem cells is limited by the method of delivery, and this is a unique challenge that regenerative medicine faces. There are three main techniques that are utilized in stem cell therapy: injection-based delivery, topical application via spray, and scaffold-based delivery.

9.6.1 *Injection-Based Delivery*

Injection-based delivery is a commonly used technique to deliver stem cells, and it is well described in preclinical and early clinical studies (Falanga et al. 2007). This method involves directly injecting stem cells within a suspension or hydrogel matrix into the site of injury. While local administration of stem cells via injection is the simplest method available, it may not be the optimal method to deliver cells to a cutaneous wound. Cell viability is a major issue as the immense pressure from the injection process results in massive cell death (Garg et al. 2014). Moreover, the wound environment increases cell death and impairs cell attachment resulting in stem cells becoming disorganized and delocalized (Zhang et al. 2008). This is problematic because these stem cells may migrate and integrate with tissue away from the wound site, thus creating unwanted harmful effects in other parts of the body such as cancer.

9.6.2 *Spray-Based Delivery*

Another local administration technique is spray-based delivery of stem cells. This technique involves isolating stem cells and mixing them with a fibrinogen that forms fibrin upon administration of thrombin (Zimmerlin et al. 2013). The fibrin spray prevents the degradation of the stem cells and also facilitates the adherence of the stem cells to the wound (Falanga et al. 2007). Fibrin spray can also distribute cells across a large area unlike injection-based therapies; however this can also be a potential weakness as there might be poor control of cell density and spacing (Duscher et al. 2015). Furthermore, because the stem cells are topically applied to a non-protective and harsh wound environment, cell viability may be problematic. Regardless, many studies have proven spray-based delivery as a safe and effective technique at promoting wound healing (Falanga et al. 2007; Wu et al. 2011).

9.6.3 *Scaffold-Based Stem Cell Therapy*

In the past decade, alternative techniques have been developed to overcome the weaknesses of spray- and injection-based delivery methods. Scaffold-based therapy has been the foundation of new stem cell delivery techniques that hope to improve

therapeutic benefit. The goal of scaffold-based therapy is to seed stem cells on bioscaffolds in the hope that stem cell viability, differentiation, and engraftment into the wound are enhanced. Bioscaffolds are typically composed of collagen, hyaluronic acid, pullulan, or chitosan that mimic the native tissue environment (Jayakumar et al. 2010; Landsman et al. 2009). An important characteristic of these scaffolds is sufficient porosity to support adequate transport of nutrients and waste (Celiz et al. 2014). Moreover, these scaffolds provide protection for the stem cells and important spatial cues that mimic a natural environment, thus increasing the likelihood that these cells become functional parts of the wound. Indeed, seeding stem cells onto a pullulan-collagen matrix preserves the “stemness” of the cells and accelerate wound healing (Rustad et al. 2012).

While bioscaffolds have tremendous potential, they are still limited by poor nutrient transport and vascularization after engraftment (Meinel et al. 2004). Novel approaches need to be developed that will improve cell seeding efficiency, cell viability, cell uniformity, and cell integration. For instance, ASCs have been seeded more effectively on a hydrogel scaffold via capillary force than other methods such as injection and centrifuge and were effective at improving wound healing (Garg et al. 2014). As mentioned earlier, growth factors and cytokines play an important role in the wound healing process. Integrating these molecules, or even plasmids and vectors that modulate stem cell activity, within a scaffold could potentially help facilitate healing. Another novel approach to improving scaffold-based cell delivery is modifying the structure and composition of these scaffolds. An example would be altering the intermolecular bonds within the matrix to improve porosity, and interestingly one study has demonstrated that this can modulate cell proliferation and differentiation (Jeon and Alsberg 2013). These examples illustrate the importance of optimizing the delivery method so that stem cell-based therapies in wound healing can be readied for clinical trials. Unfortunately, bioscaffolds are limited by manufacturing costs and safety concerns (Duscher et al. 2015), but future advancements in biology and engineering may eventually produce a viable stem cell-based treatment option for acute and complex wounds.

9.7 Challenges and Future Directions in Stem Cell Therapy

Most stem cell-based therapies discussed in this chapter have not been proven clinically. While stem cell therapy in cutaneous wound healing has shown tremendous promise, there are still significant challenges that need to be addressed before these therapies are clinically relevant. Firstly, there is no evidence that skin and appendages regenerated by stem cell therapies are functional – flawless regeneration without scarring has yet to be achieved. There needs to be studies demonstrating confirming whether regenerated skin recapitulates the function of intact skin. Second, harvesting enough adult stem cells with high purity is obstructing the progress of developing new therapies. Lastly, the differentiation potential of stem cells may give rise to malignant cells as the niche microenvironment is crucial in directing tumor growth. Optimizing cell growth to replace damaged tissue while at the

same time preventing disproportionate cell proliferation and differentiation remains a challenge for researchers. To accomplish this a robust understanding of the cellular and molecular mechanisms underlying stem cell action is necessary. Therefore, innovative approaches are needed to refine the manipulation of stem cells, improve the methods of delivering these cells, and finally validate these therapies through clinical trials so that patients can reap the benefits of regenerative medicine.

9.8 Conclusion

In this chapter we addressed the potential of stem cells in the treatment of acute and complex cutaneous wounds. There are various aspects to consider when developing stem cell therapies such as the signaling pathways involved in wound healing, the source of stem cells, and the method of delivery. While there may be significant barriers limiting the clinical translation of stem cell-based therapies, the clinical and animal studies discussed illustrate the therapeutic potential of stem cell therapy in regenerative medicine. More clinical studies are needed so that the plethora of research in basic science can be translated into clinical wound care.

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