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WOUND CARE AND HEALING (H LEV-TOV, SECTION EDITOR)

Use of Stem Cells in Wound Healing

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Abstract

Purpose of Review This review provides an overview of the principal stages of wound healing, the populations of endogenous and therapeutic stem cells, applications of stem cells in specific types of wounds, and current approaches of stem cell delivery for tissue regeneration.

Recent Findings New uses of progenitor stem cells have been developed for the treatment of wounds. Stem cells improve wound healing through both local and paracrine effects. Stem cell populations of therapeutic utility include embryonic stem cells, induced pluripotent stem cells, adult bone marrow and adipose-derived mesenchymal stem cells, as well as stem cells from skin, cord blood, and extra fetal tissue. Induced pluripotent stem cells mitigate many of the ethical and immunogenic concerns related to use of embryonically derived stem cells.

Summary Skin, the largest organ in the human body, serves as a protective barrier for mammals. Both aging and disease contribute to loss of skin barrier function, which can result in consequences such as chronic wounds. Recent advances in many types of stem cell therapy may revolutionize treatment of difficult wounds. Optimal techniques for obtaining and delivering stem cells are still being refined.

Keywords Stem cells · Wound healing · Chronic wounds · Biologic therapies

Introduction

Structure and Development of Skin

The skin is composed of three layers, the epidermis, dermis, and hypodermis, which serve to protect the body from pathogens, irradiation, dehydration, and other physical stressors, as well as functioning as a thermoregulatory and sensory organ [1, 2]. The skin barrier may become damaged through a number of means, including burns, ischemia or hypoxia, trauma, poor nutritional or immune status, and infections or inflammatory processes. Wound healing is also impaired in many common chronic conditions, including diabetes, renal disease, vascular insufficiency, and poor mobility resulting in pressure-induced ulcers [3]. To repair damaged skin, the body utilizes progenitor stem cells for regeneration [4].

The epidermis, a multilayered epithelium, extends upward from the basement membrane, a fibrous extracellular matrix which houses progenitor stem cells that continuously self-renew and differentiate into keratinocytes. Keratinocytes, which constitute approximately 90% of epidermal cells, undergo terminal differentiation and maturation to ultimately form the outermost cornified layer of the skin, which confers its main barrier property [1, 4–6]. The dermis, connective tissue with an extensive extracellular matrix, is the thickest layer of the skin, accounting for its mechanical properties, providing strength and elasticity through fibroblasts that secrete precursors to collagen and elastin and contributing to the skin's regenerative properties by housing vascular endothelial cells and accessory organs (sweat glands, sebaceous glands, and hair follicles), which also serve as nuclei for development of progenitor stem cells. Finally, the hypodermis, composed primarily of adipose cells, functions as insulation and cushion between the skin, muscle, and bone [4, 7].

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Stages of Wound Healing

A basic understanding of the stages of normal wound healing is required to understand mechanisms by which stem cell therapies may improve wound healing; as such, stages of wound healing are reviewed here. Cutaneous tissue repair follows a complex sequential process of hemostasis, inflammation, proliferation, and maturation (remodeling), respectively [8–16]. To achieve these phases, numerous cytokines, growth factors, and cell types are required, including lymphocytes, neutrophils, macrophages, keratinocytes, fibroblasts, and endothelial cells [9, 17]. Various stem cells secrete cytokines and growth factors, accelerating or inducing phases of wound healing (Table 1). The earliest stage of wound healing, hemostasis, occurs rapidly after skin trauma via vascular constriction, fibrin clot formation, and secondary release of proinflammatory cytokines and growth factors from surrounding tissue, including platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, and epidermal growth factor (EGF). Together, these mediators and fibrin clots establish a transient wound matrix that serves as protection against fluid loss and pathogens, as well as a means for inflammatory cells to migrate (chemotaxis) to the area of injury [1, 4, 9, 10, 17].

The inflammatory (exudative) phase, which lasts approximately 4 days, is defined by localized erythema and edema due to involvement of neutrophils, macrophages, and lymphocytes. Neutrophils and lymphocytes defend against microbes, foreign or cellular debris to prevent infection, while macrophages aid in the disposal of necrotic tissue [4, 17]. These inflammatory cells also secrete growth factors (fibroblast growth factor [FGF], vascular endothelial growth factor [VEGF], TGF-β and -α), cytokines (IL-1α, IL-1β, IL-6, TNF-α), and prostaglandins to promote angiogenesis and recruitment of vascular endothelial cells, fibroblasts, and keratinocytes, ultimately forming granulation tissue [9, 18].

Granulation tissue marks transition into the proliferative stage, a 2–3-week period dedicated to re-epithelialization, marked by the differentiation of endothelial cells into mesenchymal components, production of collagen III, glycosaminoglycans, and proteoglycans, as well as continued removal of microbes and debris by macrophages [9]. To maintain successful wound healing, Kanji et al. note that keratinocytes and fibroblasts require a continuous paracrine loop of bidirectional communication [11, 13, 18]. Similarly, angiogenesis is critical for wound healing, which requires ample levels of oxygen and other nutrients. Epidermal stem cells have been shown to aid in wound healing through keratinocyte production, repair of accessory organs, and remodeling of damaged stroma [9].

The final stage is maturation, or remodeling, in which type III collagen is replaced by more permanent type I collagen. In addition, fibroblasts differentiate into myofibroblasts which then coordinate wound contraction by producing α-smooth muscle actin (α-SMA) and crosslinking it with type I collagen. Finally, granulation tissue growth terminates, and regression of capillaries ensues. Together, these processes occur over approximately 12 months and results in avascular and acellular scar [1, 4, 9, 10, 17]. Fully healed scar may only regain 70–80% of its original tensile strength. On occasion, problems with wound repair processes may lead to hypertrophic or keloidal scarring, chronic wounds, and numerous other skin pathologies.

Report

Role of Epidermal Stem Cells in Physiologic Wound Healing

Wound healing is the result of a complex cascade of cell-signaling events between immune-modulating cells through overlapping phases. Among these immune-modulating cells are populations of epidermal stem cells localized to hair follicles (HF), the interfollicular epidermis (IFE), and eccrine sweat and sebaceous glands (Fig. 1) [1, 19–22]. Dermal sheath stem cells and mesenchymal stem cells are present through all

Table 1 Growth factors at stages of wound healing

Growth factors	Hemostasis	Inflammation (exudative phase)	Proliferation	Maturation (remodeling)
Epidermal growth factor (EGF)	X		X	X
Basic fibroblast growth factor (bFGF)		X	X	
Insulin-like growth factor (IGF)			X	
Interleukin (IL)-1A, -6		X		X
Platelet-derived growth factor (PDGF)	X			X
Transforming growth factor (TGF)-B	X		X	
Transforming growth factor (TGF)-A	X		X	
Tumor necrosis factor (TNF)-a		X		
Vascular endothelial growth factor (VEGF)		X		

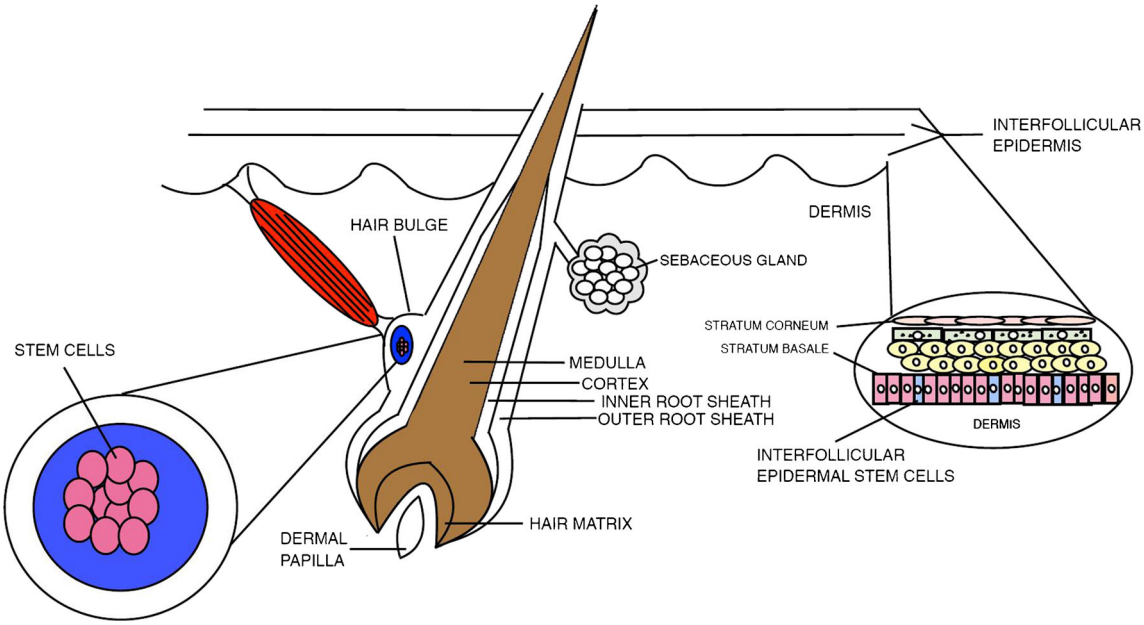

Fig. 1 Location of hair bulge stem cells. Hair follicle stem cells originate in the bulge region(s) of the follicular unit, and interfollicular epidermal stem cells arise from the stratum basale layer of the epidermis

layers of the skin. Epidermal stem cells coordinate tissue regeneration through both local and paracrine effects. Chu et al. demonstrated that epidermal stem cells transition between two life cycles: a slow phase during which they remain quiescent and an active phase, following skin injury, in which they contribute to re-epithelization [23].

Locally, cells within the basal membrane of the epidermis maintain continual mitotic activity until they detach and migrate towards the skin surface, during which process they undergo terminal differentiation to become mature keratinocytes. Multipotent mesenchymal stromal cells (MSCs) aid in type I and III collagen production, thus forming the connective tissue matrix necessary for wound healing [24, 25]. Within sebaceous glands, a thin layer of progenitor basaloid cells and sebocytes collectively maintain production of sebum, which empties into hair follicles to lubricate and waterproof the skin. Multipotent cutaneous stem cells of the hair follicle are localized to the bulge of the outer root sheath, which is contiguous with the epidermis. Research by Ito et al. demonstrated de novo production of hair follicles after wounding in genetically normal adult mice, as well as an increased production of hair follicles with Wnt pathway overexpression. This production of hair follicles was decreased with inhibition of Wnt [26]. Paralleling work by Shi et al. and Whyte et al., this suggests that sites of cutaneous injury revert to an embryonic phenotype to amplify regeneration and reduce scarring [27, 28]. Both mesenchymal stem cells and melanocyte precursors frequently localize to the bulge region of hair follicles—an ideal environment for cell proliferation due to the highly innervated, vascularized, and protected nature of follicular structures. Wong et al. also described dermally derived MSCs of two distinct populations, one localized to the dermal papilla of hair follicles and another identified as perivascular stem cells [29].

Efficient wound healing response is partially dependent on paracrine signaling, for which MSCs are key players. MSCs are defined by their ability to self-renew and produce multiple cell lineages, including osteoblasts, adipocytes, chondrocytes, tenocytes, and myocytes. In skin healing, epidermally derived MSCs can express a fibroblast-like morphology and a "secretome," a collection of protein which includes several growth factors and cytokines. These signaling proteins modulate the inflammatory response to promote accelerated cell migration and neovascularization. They also function as survival factors on neighboring differentiated cells to promote epithelialization, increase granulation tissue formation, and inhibit scar formation [2, 13, 30, 31, 32•]. As MSCs are free of many of the ethical concerns surrounding use of embryonic stem cells, they have become attractive modalities for research (Fig. 2).

Categories of Stem Cells and Utility in Wound Healing

Endogenous stem cells, being multipotent and self-renewing, hold significant therapeutic potential in wound healing, particularly through secretion of pro-regenerative cytokines and ability to modulate cell-signaling pathways. Unlike tissue-engineered skin substitutes, stem cells allow for continually adaptive tissue properties to best support wound healing. Several studies have shown stem cell lineages that are active throughout all four phases of wound healing [4, 33]. However, due to the multipotent nature of stem cells, risks associated

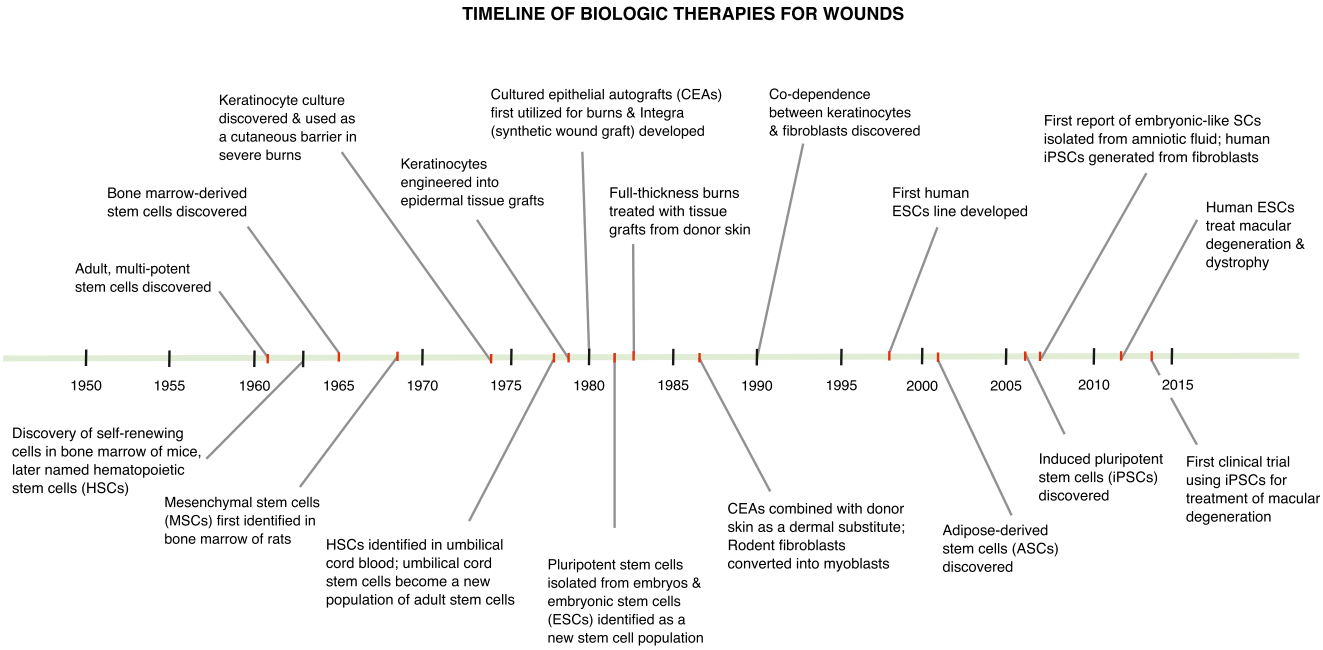

Fig. 2 Timeline of biologic therapies for wounds

with this therapeutic modality include concerns of potential immunogenicity and tumorigenicity. In this section, we provide an overview of seven stem cell populations and their applications in wound healing: mesenchymal stem cells derived from skin, MSC from bone marrow, MSC from adipose tissue, stem cells derived from cord blood, stem cells derived from extra fetal tissue, embryonic stem cells, and induced pluripotent stem cells (Fig. 3) [4, 24, 33]. It should be noted that at this time all of these therapies are in research stages and are not yet commercially available.

For cutaneous wound healing, the most well-studied stem cell populations are of mesenchymal origin. Mesenchyme, derived from embryonic mesoderm, is able to differentiate into either connective tissue or hematopoietic cells, whereas MSCs or multipotent stromal progenitor cells may only differentiate into connective tissue constituents. Per definitions by the International Society of Cellular Therapy, MSCs express CD73, CD90, and CD105, which allow them to adhere to a plastic surface, and lack hematopoietic markers CD14, CD34, CD45, CD11b/CD79, and CD19/HLA-DR [34]. MSCs preferentially reside in bone marrow and adipose tissue but may also be found in the skin. They also localize to synovial fluid, traumatized muscle, corneal stroma, and fetal tissue. MSCs aid epithelialization through paracrine effects rather than direct structural contributions to wound sites [35–38]. MSCs contribute to extracellular matrix formation, angiogenesis, and cell differentiation by secreting matrix

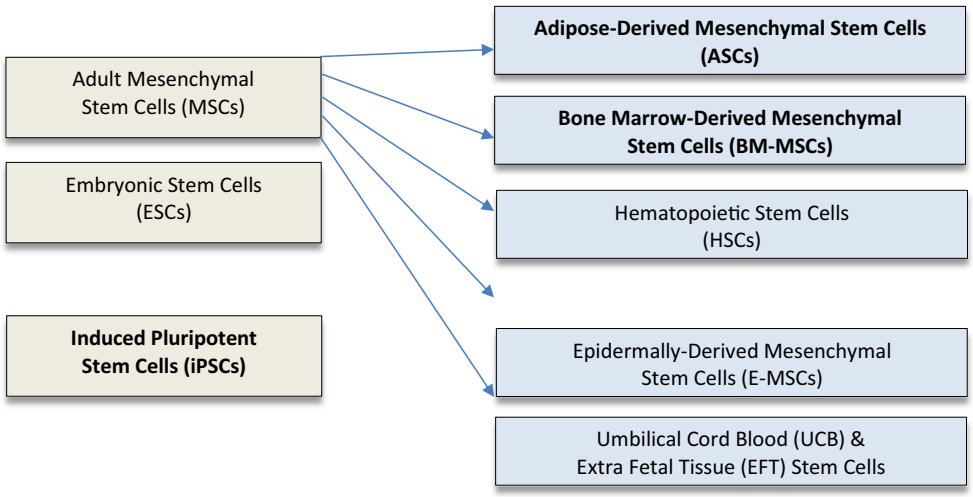

Fig. 3 Categories of stem cells. Those with most promising potential for rapid translation to clinical care in bold

metalloproteinase-9 (MMP), IL-6, IL-10, TNF-a, VEGF, EGF, IGF, and Ang-1. MSCs modulate immune cells via regulation of migration and proliferation and reduce scar formation through prostaglandin E2 secretion [2, 31, 32•, 39, 40]. Of MSCs affecting the inflammatory phase, bone marrow-derived stem cells (BM-MSCs) appear to be the major contributor. BM-MSCs produce higher amounts of procollagen, growth factors, and angiogenic factors compared to dermal fibroblasts [4].

In a randomized control trial, Chen et al. utilized both topical and injectable MSC-conditioned medium, with cells derived from bone marrow and adipose tissue, on full-thickness wounds in mice which demonstrated accelerated wound closure and improved tensile strength [41]. Wu et al. obtained similar results with diabetic wounds and excisional wounds, respectively [42]. Falanga et al. showed improved wound healing in both acute surgical and chronic lower extremity wounds with topical fibrin polymer spray mixed with BM-MSCs [43]. Findings were attributed to increased neovascularization, attenuation of the inflammatory response, and differentiation of wound cells (keratinocytes, endothelial cells, and pericytes) by MSCs. However, large wounds may be impractical to treat with this modality as they require substantial quantities of MSC culture.

Adipose-derived mesenchymal stem cells (ASCs) are another promising population of stem cells for wound healing, due to their multipotent nature, allowing them to differentiate into bone, fat, cartilage, and muscle tissue. These cells localize to the stromal-vascular portion of enzymatically digested fat [44•]. ASCs contribute to accelerated wound healing and scar reduction by stimulating angiogenesis and subcutaneous tissue production [45]. Adipose tissue may be extracted with minimal donor morbidity from solid tissue specimens, through excision, or in liposuction aspirates. Several studies have shown no malignant transformation from ASC transplantation within at least 120 days, and the procedure is generally well-tolerated. In comparison to bone marrow aspiration, it is relatively easy to obtain high yields of ASCs from subcutaneous tissue or lipoaspirate. Due to all these benefits, ASCs have become a useful modality for treatment of acute wounds, such as burns, as well as refractory wounds, such as diabetic foot ulcers [2, 46, 47].

Recent studies have also shown accelerated wound healing with MSCs from both umbilical cord blood (UCB) and extra-embryonic fetal tissue. As with ASCs, UCB stem cells are an attractive treatment modality due to easy accessibility of large numbers of stem cells [33]. UCB stem cells express CD24+ surface markers which accelerate wound healing through inhibition of several matrix metalloproteinases, causing decreased inflammatory response, as well as promoting neovascularization and collagen production. UCB stem cells have been utilized for refractory wounds with good success in large traumatic wounds and in diabetic ulcers. Multipotent MSCs may also be derived from extra-embryonic fetal tissue and have been cultured from amniotic fluid, Wharton's jelly, and placental tissue [48–50]. These sources, however, are considered less favorable due to risk of immune-mediated rejection, transmission of genetic diseases, and potential for malignant transformation.

Embryonic stem cells (ESCs) are also a less favorable source due to ethical considerations. These pluripotent cells originate in human blastocysts and possess the ability to differentiate into any germ layer: ectoderm, endoderm, or mesoderm. They have been used to successfully foster keratinocytes and subsequently rebuild sections of epidermis [51]. Because of their pluripotency, they hold significant potential in both wound healing and constructing bioengineered skin substitutes [11]. However, when compared with commercially available skin substitutes, such as xenografts or hydrocolloid dressings, ESCs have shown increased costs and few advantages. Their robust pluripotent capacity also results in increased risk of immunogenicity and tumorigenicity [4, 52]. Finally, debate persists regarding ethically acceptable use of cells harvested from human embryos, largely centered on the metaphysical and controversial questions of precisely when an embryo becomes a person and whether embryos that would otherwise be discarded are ethically appropriate to use for research purposes. Fortunately, advances in procurement of adult stem cells and the development of induced pluripotent stem cells have reduced the acuity of this debate, as acceptable alternatives not fraught with the ethical concerns surrounding embryonic stem cells are now available [53].

Induced pluripotent stem cells (iPSC) are a relatively recently engineered type of multipotent and hybrid stem cell population. They originate from adult somatic cells (such as keratinocytes, fibroblasts, lymphocytes, or hepatocytes) that are de-differentiated through application of a cocktail of transcription factors, such as Oct-3, Oct-4, Sox2, c-Myc, and KLF4 [48, 54, 55]. Accordingly, iPSCs may be obtained from adult tissue and cultured indefinitely and, thus, may be an invaluable source for regenerative medicine. When a patient's somatic cells are harvested, reprogrammed, and replaced as iPSCs, little to no immunogenicity has been found [55]. However, some potential for genetic instability and tumorigenicity has been observed, though ongoing work attempts to improve these risks. Use of iPSCs in wounds rats has demonstrated increased angiogenesis and collagen production [56]. iPSCs have been successfully utilized for treatment of wounds from recessive dystrophic epidermolysis bullosa [57, 58]. As iPSCs are derived from adult cells, they present less ethical controversy than stem cells derived from umbilical cord blood, extra-embryonic fetal tissue, or embryonic stem cells.

Special Cases: Chronic Wounds, Burns, and Corneal Ulcerations

Cutaneous wound healing involves a complex interplay of several cell populations and signaling molecules through sequential phases of repair. This well-orchestrated process may be impaired due to local or systemic factors, such as age, systemic diseases, continued trauma, ischemia, pressure, or exposure to substances such as tobacco [17]. Chronic wounds, though still incompletely understood, arise from an inability to meet the biologic demands of cutaneous tissue repair. Inadequate neovascularization is often a primary characteristic of chronic wounds, and a well-vascularized dermal wound bed appears essential for viability of treatment with stem cells or keratinocytes [59]. Human cells require adequate nutrients and oxygen to thrive, whereas microbes can proliferate in anaerobic conditions and immune response is compromised without sufficient oxygen. Cutaneous wounds typically require oxygen tension of at least 20 mmHg to heal, but chronic wounds often demonstrate oxygen tension of less than 5 mmHg [10]. Hypoxia immediately following traumatic injury stimulates angiogenesis via the release of cytokines and growth factors, but sustained healing thereafter requires restoration of adequate oxygenation [17]. Overwhelming or chronic inflammation also hinders wound healing by dysregulating cell signaling within the wound. For example, inflammation deregulates protease and fibroblast proliferation, decreasing collagen formation, increasing ECM deposition, and ultimately inducing hypertrophic scars.

Historically, treatments for chronic wounds have been limited to optimizing local wound health through debridement and dressing changes, surgical intervention, antibiotics, or use of compression and pressure-relieving devices. Improvement of systemic factors, such as obesity, hyperglycemia, venous stasis, decreased cardiac output, or smoking, has also been helpful in healing of chronic wounds [60–62]. More recently, stem cell therapies have shown promising results in the treatment of chronic wounds. As detailed previously, MSCs stimulate angiogenesis and modulate the immune response, anti-microbial properties, and structural integrity of wounds [63, 64]. In chronic wounds in rats, application of MSCs causes an increase in IL-10, an anti-inflammatory cytokine, and LL-37, an antimicrobial peptide [65–67].

MSCs have shown similar promise in the treatment of burn wounds, with decreased inflammation and subsequently faster healing, and decreased fibrosis, contraction, and scar formation [42, 67]. Commonly, full-thickness burns are repaired with full- or split-thickness skin grafts. However, these treatments are limited by availability of suitable skin grafts, and the risks of infection, fluid loss, and graft loss. MSCs have been used to rebuild both dermal and epidermal layers in full-thickness burns [68]. Stimulation of MSCs in hair bulbs of scalp burns can lead to re-epithelialization of skin layers and return of functioning of hair follicles and sebaceous glands [69, 70]. Stem cells within burns are induced by human alpha defensin 5, CXCL12, and CXCL4 pathways [71]. Substantial hair and epithelial growth in burn wounds has been obtained through use of progenitor cells and cytokines in amniotic fluid [72]. Intravenous injection of umbilical cord blood in rats has demonstrated increases in IL-10, VEGF, and healing of severe burns [65]. BM-MSCs were first used in human burns in 2004 and showed signs of faster healing and angiogenesis in extensive burns. In subsequent rat studies, BM-MSCs also caused decreased formation of granulation tissue in burn wounds [73]. Addition of BM-MSCs to skin treated with bleomycin demonstrated decreased fibrosis during healing [42]. Finally, autologous adipose-derived MSCs appear to accelerate healing and result in decreased pain and necrosis in burns [46, 74].

Corneal wounds have also been treated with stem cell therapy. Corneal opacity, often due to infection, burns, or trauma, is a common cause of blindness. Blindness ensues when the extent of injury outweighs the regenerative capacity of native corneal epithelial stem cells [75]. Autologous limbal stem cells (corneal MSCs) have been utilized in several trials of corneal injuries from burns and trauma, with substantial benefits in revascularization, re-epithelialization, decreased irritation, and improved vision in human and animal trials [72, 76].

Delivery Approaches of Stem Cells for Wound Healing

In attempting to deliver stem cells to wounds, several approaches have been used. Systemic administration is appealing for extensive wounds, but somewhat limited by specificity of tissue targeting and difficulties with proportionately high cell loss. Concomitant administration of other factors may help improve targeting: for instance, early clinical trials in bone healing have shown that addition of intermittent parathyroid hormone therapy to MSC therapy speeds repair of rib fractures [77].

In general, local delivery approaches for stem cells have been preferred due to ease of targeting. However, inflammation within wounds makes them difficult environments for single cells to engraft and survive. Trials involving injection of stem cells into wounds have shown durable engraftment rates as low as 0%, likely due to shear injury to fragile cells during application [78]. Topical sprays have been used to apply stem cells, but they do not provide any protection to cells either and do not allow for fine control of cell spacing [43].

Delivery of stem cells within bioscaffolds has become the most popular and marketable technique. Numerous products exist, consisting of both naturally derived and synthetic molecules, with stem cells seeded within these matrices. These provide a framework for stem cells and thus grant structural protection; they also appear to maintain stem cells in a pre-differentiated state for longer, extending expression of genes unique to stem cells [79]. Several decellularized bioscaffold allogenic skin substitutes are currently commercially available, though none of

these products contain living stem cells at this time [80–85]. Though all of these products appear to accelerate wound healing, problems with wound contracture or hypopigmentation due to loss of melanocytes are common following healing. In experimental settings, similar bioscaffolds have been used for delivery of living stem cells and appear to confer improved cellular survival and engraftment [68, 78, 79, 84].

More recently, devices such as the CelluTome™ have been used to update and standardize the traditional technique of "pinch grafting" [86]. Epidermal micrografts containing epidermal MSCs are obtained from normal skin with minimal donor site morbidity and are then minced and distributed over wounds, sometimes in combination with a hydrogel. The transplanted cells proliferate and expand to heal the wound [87•].

Conclusion

Wound healing occurs physiologically through a straightforward sequence, but this process may often become derailed due to age, disease, local factors, or other causes. Stem cell therapy in many forms has emerged as a promising approach for these difficult wounds (Fig. 2). Growth factors and cytokines released by stem cells introduced to wounds promote healing through improved angiogenesis and immune modulation. No consensus has yet been reached on the optimal types of stem cell or delivery methods for various types of wounds, but much translational work has already been done with direct benefit to patients. Research in this aspect of regenerative medicine continues to actively progress. Further studies are likely to build on the findings discussed in this review, refining techniques to harvest and deliver stem cells to optimize engraftment and wound healing. These advances may revolutionize the treatment of problematic wounds.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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