COMPREHENSIVE INVITED REVIEW

Epidermal Stem Cells in Skin Wound Healing

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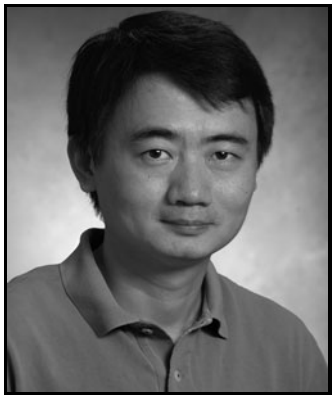
Significance: Skin serves as a protective barrier for mammals. Epidermal stem cells are responsible for maintaining skin homeostasis. When cutaneous injuries occur, skin homeostasis and integrity are damaged, leading to dire consequences such as acute, chronic, or infected wounds. Skin wound healing is an intrinsic self-saving chain reaction, which is crucial to facilitating the replacement of damaged or lost tissue.

Recent Advances: An immense amount of research has uncovered the underlying mechanisms behind the complex and highly regulated wound healing process. In this review, we will dissect the biological process of adult skin wound healing and emphasize the importance of epidermal stem cells during the wound healing.

Critical Issues: We will comprehensively discuss the current clinical practices used on patients with cutaneous wounds, including both traditional skin grafting procedures and advanced grafting techniques with cultured skin stem cells. The majority of these leading techniques still retain some deficiencies during clinical use. Moreover, the regeneration of skin appendages after severe injuries remains a challenge in treatment.

Future Directions: Understanding epidermal stem cells and their essential functions during skin wound healing are fundamental components behind the development of clinical treatment on patients with cutaneous wounds. It is important to improve the current standard of care and to develop novel techniques improving patient outcomes and long-term rehabilitation, which should be the goals of future endeavors in the field of skin wound healing.

Keywords: epidermal stem cells, skin, wound repair, tissue regeneration



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SCOPE AND SIGNIFICANCE

As a part of the largest organ in the adult mammalian body, the skin epidermis has significant functions in protecting the host from the external environment (*e.g.,* irradiation, pathogen, dehydration, and physical stress) and maintaining skin appendages. When cutaneous wounds occur, damaged epidermis requires an appropriate healing process to repair or regenerate skin. We will discuss the biological process of adult skin wound healing and the contributions of epidermal stem cells during these steps. We will also discuss the implementation of current clinical approaches in wound treatment that are closely based upon epidermal stem cells.

TRANSLATIONAL RELEVANCE

In adult skin wound healing, fibrosis and scar formation are the main forms of skin repair, during which many types of cells, growth factors, and cytokines orchestrate and fulfill the closure of the skin wound. Epidermal stem cells play essential roles during wound healing

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process, and the mechanisms controlling keratinocyte functions are fundamental questions that need to be answered. The standard of care for patients with cutaneous wounds is dependent on the crucial function of epidermal stem cells.

CLINICAL RELEVANCE

Although skin grafting is still commonly used to treat patients with cutaneous wounds, there are some limitations and deficiencies associated with these standard procedures. For instance, in patients with severe burns, there are insufficient skin areas to be harvested for skin grafting and the patient can be in critical conditions secondary to sepsis or fluid loss. In contrast, skin grafts generated from cultured epidermal stem cells have advantages compared to the traditional skin grafting. In this section, we will focus on these leading-edge techniques for the treatment of cutaneous wounds.

BACKGROUND

Mammalian skin epidermis is composed of interfollicular epidermis (IFE), hair follicles (HFs), sebaceous glands (SGs), and eccrine sweat glands, which have their own stem cell populations in each component.[1,2] For IFE, skin homeostasis and wound repair are sustained by stem cells and the progenitors, which are localized within the basal layer (Fig. 1) of the skin epithelium.[3–13] In addition to IFE stem cells, some epidermal stem cell populations are located at skin appendages, such as HFs and sweat glands.[9,10] Although IFE stem cells are unipotent in contrast to the multipotent HF stem cells, stem cells and progenitors in IFE are distinct from HF populations, which are essential for maintaining IFE homeostasis.[3,6] Based on previous studies, human IFE stem cells possess high level of β_1 and α_6 integrins, Leu-rich repeats and immunoglobulin-like domains 1 (LRIG1), and melanoma-associated chondroitin sulfate proteoglycan (MCSP).[8,14–18] In contrast, human and murine HF bulge stem cells possess markers of clusters of differentiation 34 (CD34) and keratin 15 (K15).[14,19–21] All the pools of skin stem cells contribute to epidermal homeostasis and wound healing.

A noteworthy aspect of wound healing process is that there are certain skin components that may not fully recover after wound closure upon adult skin wound healing. Although in adult mouse skin, HFs process the ability of neogenesis after a 1–2.25 cm^2 full-thickness skin excision,[22] the regeneration of HFs in patients with critical injuries, such as severe burns, has been a challenge in clinic. If the wound only affects epidermis, basal progenitors within sweat ducts have the ability of self-repair.[10] However, full-thickness skin wounds, such as severe burns, could cause complete loss of sweat gland regeneration.[23] Moreover, the scar tissue of the healed epidermis is different from intact skin, since the normal connections between the epidermis and dermis are lost after wound healing.[24–27] In addition, pigmentation commonly vanishes during wound healing, especially after severe wounds.

DISCUSSION

Skin wound healing is a highly regulated process that involves essential functions of various cell types

Adult skin wound healing is commonly divided into four phases (Fig. 2) that spatiotemporally overlaps:

Hemostasis. If the injury reaches beyond the epidermal layer, blood vessels are damaged and

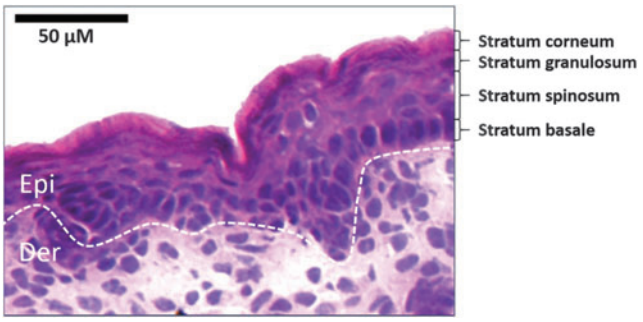

Figure 1. Epidermal stratification in mouse model. HE staining is performed in WT mouse embryonic 18.5-day skin section. Epidermal stratification layers are labeled as indicated. *Dotted lines* denote dermal–epidermal boundaries. Der, dermis; Epi, epidermis.

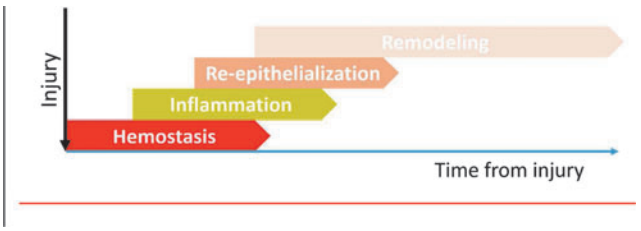

Figure 2. Diagram of wound healing stages. As the injury happens, wound healing process initiates immediately and is classically divided into four stages: hemostasis, inflammation, reepithelialization, and remodeling. These four stages are spatial–temporal overlapping and can continue up to 1 year after wounding.

cause hemorrhage. Instantly, blood clotting forms a provisional wound matrix. Thrombocytes not only assist the formation of a blood clot but also facilitate the activation of the second phase of wound healing by secreting mediators such as growth factors (discussed in the next section). Moreover, fibronectin, thrombospondin, and vitronectin, as well as the cleavage of fibrinogen, fill the blood clot with cross-linked fibrin molecules, which serve as a bridge for the migration of keratinocytes, blood cells, and endothelial cells.[28,29]

Inflammation. Growth factors and cytokines such as Interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, which are secreted by platelets and leukocytes, initiate the second stage of wound healing. Meanwhile, many other chemotactic molecules secreted during the inflammatory cascade are essential for the later phases of wound healing: Fibroblast growth factor (FGF)-2, insulin-like growth factor (IGF)-1, and transforming growth factor (TGF)-β activate the collagen synthesis; TGF-β stimulates fibroblast differentiation into myofibroblasts; FGF-2, vascular endothelial growth factor (VEGF)-A, and TGF-β initiate angiogenesis; epidermal growth factor (EGF), FGF-2, IGF-1, and TGF-α support reepithelialization.[30–45]

As soon as a couple of minutes after injury, neutrophils are recruited to the wound as essential warriors against potential pathogenic infections, since they secrete effective antimicrobial molecules and proteinases.[46] Neutrophils are also a source of cytokines (*e.g.,* IL-1α, IL-1β, and TNF-α) for the attraction and activation of other cells, amplifying the inflammatory cascade.[47,48]

Macrophages migrate to the wound shortly after neutrophils arrive, cleaning up pathogens and cell debris through phagocytosis.[49,50] In addition, macrophages will promote keratinocyte migration and fibroblast matrix synthesis, by secreting cytokines and growth factors like TGF-β, TGF-α, heparin-binding-EGF, FGFs, and platelet-derived growth factor.[51,52]

Reepithelialization: the contribution of epidermal stem cells during skin wound healing. The third phase of wound healing is dedicated to reepithelialization, or in other words, "the recreation of an intact keratinocyte layer".[53] The contribution of IFE stem cells and their progenitors is well illustrated in previous studies.[6,54] The long-term stem cells even contribute more extensively to skin regeneration compared to the progenitor cells during wound healing.[6] Since basal keratinocytes include IFE stem cells and all the proliferative progenitors at IFE, we simply refer the whole population as basal keratinocytes. First of all, basal keratinocytes at the wound's edges are activated and migrate along the upper layer of blood clot (Fig. 3). Detailed mechanisms regarding keratinocyte activation, migration, and fusion are reviewed by Jacinto *et al.*[55] In general, the hemidesmosome attachments through which basal keratinocytes anchor themselves to the basal lamina need to be dissolved before the migration can occur. The physical and physiological damages at the wound will directly cause calcium influx to the damaged front row cells, which activates AP-1 signaling.[55–57] Calcium influx also leads to the reorganization of the intracellular tonofilaments in keratinocytes, which prepares cell migration. Moreover, the expression of new integrins on leading edge keratinocytes,[58–60] the assembly of actin filament networks, as well as the formation and turnover of focal adhesions between keratinocytes and various extracellular matrix components, are required for keratinocyte migration.[61–63]

There is strong evidence showing that microtubules and filamentous actin (F-actin) are coordinately regulating cell migration, potentially through controlling focal adhesion dynamics.[64] Studies on the mechanisms coordinating cytoskeletal-focal adhesion dynamics during keratinocyte migration are crucial to understanding cell behaviors during wound healing. Our group is dedicated to this field, and we have uncovered the essential role of mammalian spectraplakin actin cross-linking factor 7 (ACF7) in regulating cytoskeletal-focal adhesion dynamics and cell migration.[64–67] We have found that ACF7 has ATPase activity, which is crucial in targeting microtubules to focal adhesions by tracking along F-actin.[64] In addition, the phosphorylation of ACF7 plays an essential role in promoting focal adhesion dynamics and *in vivo* epidermal migration.[66,67] We also found that keratinocyte migration and focal adhesion dynamics require interactions between HCLS1-associated protein X-1 (Hax1) and microtubule end-binding protein 2 (EB2).[65]

Keratinocytes at the leading edge need to dissolve the blood clot to pass through and migrate forward. It has been reported that migrating keratinocytes upregulate the expression of the activators and the receptor of urokinase-type plasminogen activator, which can activate plasmin, a fibrinolytic enzyme.[68–70] In addition, leading edge keratinocytes upregulate the expression of matrix metalloproteinase (MMP) family proteins, such as MMP-1, MMP-9, and MMP-10.[71–74] Furthermore,

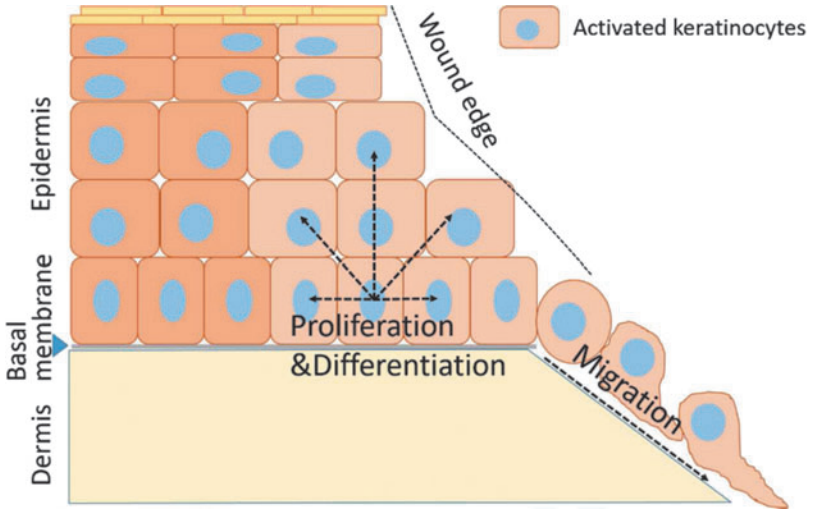

Figure 3. Epidermal stem cells contribute to cutaneous wound healing. Basal keratinocytes at the wound edges are activated and initiate changes in cell morphology, as well as migration ability. The keratinocytes at the leading edge migrate along the upper layer of blood clot, while the basal keratinocytes behind the front line start to proliferate and differentiate, restoring the barrier function of the epithelium.

the migration behavior of keratinocytes is directed by lamellipodia crawling, regulated by small GTPases such as Rho GTPases. Ras is an indispensable regulator of Rho GTPases, cycling and inhibiting Ras will impede cell movement.[55,75]

As the keratinocytes at the leading edge migrate, IFE stem cells and the progenitors behind the front line begin to proliferate and restore the barrier function of the epithelium.[53,61] The EGF families of growth factors are key regulators of basal keratinocyte proliferation, and they are abundantly released at the wound.[41] Gradually, the migrating keratinocytes generate a barrier between the eschar and the underlying tissue, while epithelia from opposing directions fuse and close the wound. Then keratinocytes undergo a standard differentiation process and restore the epithelium.

If the stumps of HFs and SGs at the wound site are intact, skin stem cells at the HF and sweat glands also play a role in facilitating wound healing.[10,22,76] After injury, stem cells at the HF bulge and sweat duct spread out. The release of contact inhibition and the migration process are similar to basal keratinocytes: in parallel to the basal keratinocytes behind the leading edge, which are undergoing fast proliferation, these dispersed HF and sweat gland stem cells provide a source of extra proliferating cells to facilitate the covering of wound surface. However, the contribution of HF stem cells is transient during acute wound repair since these cells disappeared from the newly formed epidermis after several weeks.[77] Moreover, as mentioned earlier, the regeneration of HFs and sweat glands in patients with severe wounds is challenging.

Besides basal keratinocytes, local fibroblasts immigrate through the provisional wound matrix, proliferate and synthesize collagen III, fibronectin, and other components to form a new mechanical structure, closing the tissue gap at the wound.[78] There is a paracrine signaling loop between keratinocytes and fibroblasts, which promotes keratinocyte proliferation and the fibroblast secretion of cytokines and growth factors important in wound healing.[79–84] Furthermore, neovascularization/angiogenesis also occurs during the reepithelialization process, restoring the vascular system and resuming nutrient supply.[29,85–89] By the end of the third phase, a granulation tissue comprising fibroblasts, immune cells, capillaries, and collagens forms at the wound site.

Remodeling. The last phase of wound healing approximately occurs between 1 week and up to 1 year after wounding. At this stage, collagen III is replaced by collagen I while fibroblasts differentiate into myofibroblasts stimulated by TGF-β1 and other growth factors.[35,90] Myofibroblasts express α-smooth muscle actin and generate wound contractions like smooth muscle cells by combining collagen I, which decreases the scar surface.[91,92] In addition, the granulation tissue stops growing due to cell apoptosis within it, which as a result makes the mature wound tissue avascular and acellular, also known as scar formation.[93]

Epidermal stem cells are essential components when skin grafting over an open cutaneous wound

Skin grafting is commonly used when the wound site is largely open or the wound has been unable to heal by secondary intentional alone. There are two types of skin grafting, full-thickness skin grafts (FTSGs) and split-thickness skin grafts (STSGs). While both FTSGs and STSGs contain the entire epidermis, STSGs only comprise part of the dermis and FTSGs retain the full dermis. Skin grafts can be autografts, homografts, or xenografts, although autografts are preferred when feasible due to the critical immune compatibility between the grafting and recipient tissues. Although both FTSGs and STSGs are standard of care, they can cause significant morbidity of the donor site after harvesting. Therefore, epidermal skin grafts (ESGs) act as an alternative since only the epidermal layer is harvested without anesthesia at donor site.[94] The donor area heals quickly with decreased morbidity and additionally provides rapid coverage, as well as restores skin functions at the wound site. However, ESGs are only applicable when the wound size is not too large and when only the epidermis is needed. Moreover, it requires a more optimized wound environment to support the grafted epidermis.

In these different types of skin grafts, the process through which donor skin tissue is taken in the wound is identical to that of wound healing.[95] Therefore, IFE stem cells and the progenitors within the grafts are important in reassuring the rapid healing of graft skin when the stem cell populations on the original wound edges are too far apart or lose the ability to heal the wound. Moreover, epidermis in ESGs might simulate wound healing through growth factors and cytokines secreted by the keratinocytes in the graft.[96]

Skin grafts generated from cultured keratinocytes (IFE stem cells)

Cultured epithelial autograft. Since the 1940's, many studies focusing on cultured human epidermal cells have been carried out.[97–99] It was reported in 1975 that human keratinocytes can be cultured in a serial culture, and keratinocytes in the culture will form stratified squamous epithelium.[100] Later, another study reported that multiple stratified colonies would ultimately fuse and form an epithelium suitable for grafting.[101] Cultured human keratinocytes display three distinct morphologies as follows: holoclone, paraclone, and meraclone.[102] The holoclone cells have long-term proliferative potential and have been considered as epidermal stem/progenitor cells.[102] The ability of cultured keratinocytes to generate skin grafts relies on epidermal stem cells.[5,8,15,102–106]

Since then, scientists have developed a procedure called cultured epithelial autograft (CEA) where the patient's own healthy skin is harvested, cultured, and grown *in vitro* on human, mouse, or artificial matrices. Then, cultured epidermis (or with culture matrices) is transferred back to the patient's wound location. CEA is based on the rapid multiplying ability of epithelial cells, more specifically IFE stem cells and the progenitors.

Through years of development,[103,107–112] CEA has become an alternative method to obtaining epidermal graft and treating major burns (burns ≥20% of total body surface area [TBSA] in adult). This is because in patients with severe burns, there are insufficient skin areas to be harvested for STSGs. Moreover, there is urgent need to protect the patient from infection and fluid loss. In 2010, it was reported that the final graft take of CEA in 88 patients is 72.7%, with a 91% overall survival (OS) rate.[113] In 2007, Epicel® (Genzyme Biosurgery) was approved by the U.S. Food and Drug Administration (FDA) to be used in patients with deep dermal or full thickness burns (TBSA ≥30%).

Recently, CEA has been suggested to be used in the treatment of chronic wounds.[114,115] Zöller *et al.* showed that functional CEA has equivalent features of a differentiated epidermis, and together with wound dressings comprising of fibroblasts and other important cells, CEA can be suitable in treating patients with severe chronic wounds.[115]

However, there are debates concerning the disadvantages of CEA.[116–120] First of all, CEA requires a long expansion time (3 weeks) *in vitro* and requires labor-intensive preparation.[117,118] Moreover, patients with CEA are vulnerable to infections, which cause variabilities and early graft failures.[120] In addition, one case report argues that CEA lacks long-term durability due to the occurrence of blistering and sloughing after an early successful coverage of CEA.[116] Therefore, patients with CEA may stay in the hospital longer and require further reconstructive surgeries.[119] Nevertheless, CEA is still a valuable strategy for lifesaving and the early treatment of severe wounds.[119]

Isolated keratinocyte grafts. To resolve the deficiencies of CEA, instead of expanded differentiated keratinocytes, cultured preconfluent keratinocytes are used for transplantation, known as isolated keratinocyte grafts. Since the differentiation and merging process of the grafts occur *in vivo* instead of in culture, it reduces the amount of time required for *in vitro* culture. Moreover, the grafted keratinocytes

multiply more actively *in vivo* and produce a more robust epithelialization since the limited *in vitro* culture keeps the cells more intact.[121]

There are two types of isolated keratinocyte grafts: keratinocyte autografts and allografts. Keratinocyte autografts were proposed in the 1950's in animal models.[122] In 1994, cell spray of an aerosol cell suspension prepared from *in vitro* cultured autologous keratinocytes was introduced to treat patients with burns involving 50% TBSA.[123] The same group of clinicians, through 11 years of experience, developed a unique method for harvesting and applying cell suspension to help wound closure, called ReCell® (Avita Medical). First, a postage stamp size of a split-thickness skin biopsy is harvested from the patient. Then, the cell suspension is generated after dissolving the cell-to-cell connections using a proprietary solution. Finally, a spray applicator or a syringe is used to apply the suspended cell to the wound site.

The cell suspension derived from patient's healthy skin contains critical cell types required for wound healing, such as undifferentiated basal keratinocytes (IFE stem cells), fibroblasts, melanocytes, and immune cells.[124] Once applied, the responsive epithelial cells migrate across the wound surface area (similar to the canonical wound healing process), helping to generate healing tissues that have normal color and textures. In addition, the cytokine cross-talks between the damaged tissue and the newly introduced cells, as well as among cell suspension populations, might accelerate the wound healing process. Therefore, ReCell is proposed to have benefits such as minimizing the size of donor skin needed, expediting the healing process, minimizing scar formation, reintroducing pigmentation to the skin, and having the potential to be used by a single clinician in poorer area (Avita Medical).

There are convincing data stating that keratinocyte autografts have beneficial effects. In a randomized trial published in 2007, keratinocyte autografts produce similar results to standard skin grafting, but with the benefits of minor harvesting areas and significantly less postoperative pain.[125] Another pilot study using keratinocyte autografts to treat chronic nonhealing wounds of more than 6 weeks' duration showed a 73% healing rate.[126] More recently, Mcheik *et al.*[127] reported a preliminary study during which boys with partial deep severe burns (10% TBSA) were grafted with foreskin-derived autologous keratinocyte suspensions. They discovered accelerated wound healing with an improved quality of scarring (lack of hypertrophy and pruritus) and pigmentation at the wound site, compared to traditional skin grafts.[127] They are planning to conduct a further multicenter trial study for severe deep burns.[119]

Although ReCell has been approved in Europe for various dermatologic purposes and has been used in more than 10,000 patients, it has not been approved by FDA. However, there are multiple clinical trials that are either still in progress or have been recently finished (https://clinicaltrials.gov). For instance, there is a prospective randomized pilot study evaluating the safety and effectiveness of ReCell device for venous insufficient leg ulcers. Another clinical trial focuses on the safety and efficacy of the ReCell device in vitiligo and piebaldism patients after conventional CO_2 laser ablation or fractional laser treatment. Although the reports are not yet available, various active studies highlight the broad-spectrum usage of keratinocyte autografts in the treatment of diverse dermatologic diseases.

In 2008, a medical device similar to ReCell called SkinGun™ was developed by Gerlach and colleagues. It is slightly different from ReCell since after undamaged skin is taken through biopsy, healthy stem cells are isolated and cultured in a laboratory, whereas ReCell is a one step process. Moreover, SkinGun utilizes an electronically controlled spraying device that helps to keep the stem cells intact during procedures. Since 2008, SkinGun have been used to treat over a dozen patients in Germany and America who have suffered from second degree burns. However, SkinGun still remains an experimental method that requires further testing and research.

Another type of isolated keratinocyte grafts is keratinocyte allografts. When patients with severe epidermal or dermal injuries lack available donor sites, allogeneic keratinocytes become an alternative in such situations. Since allogeneic cells express foreign class II histocompatibility molecules, immune response can cause complications. However, studies have shown that cultivated allogeneic keratinocytes can largely facilitate wound healing, likely through their interactions with other cell populations and the production of critical cytokines and growth factors by keratinocytes,[96] although the allocation of allogeneic keratinocytes to the wound is temporary.[128–130] In a phase 2, multicenter, and randomized clinical trial, HP802-247 ("a novel spray-applied cell therapy containing growth-arrested allogeneic neonatal keratinocytes and fibroblasts") was used to treat chronic venous leg ulcers (VLU).[129] The primary outcome showed that compared to the placebo group, the reduction in wound area is significantly greater in the patients treated with HP802-247, and the most optimal dosage is 0.5×10^6 cells/mL every 14 days.[129]

More importantly, new skin ulcers and cellulitis only occurred in 5–6% patients.[129] However, HP802-247 failed to show efficacy in two phase 3 clinical trials of treating chronic VLU.[131] After investigation, the most possible cause of the failure was the age of the cell banks used in phase 3 trials compared to that used in phase 2 trials; the cell banks used in phase 3 trials were 9–12 years old, while the cell banks used in phase 2 trials were 6–8 years old.[131] Cell behavior test indicated phenotypic changes primarily in the keratinocytes (longer doubling time with decreased cell viability), as well as the changes in culturing conditions to restore keratinocyte doubling time and VEGF production.[131] It was highly recommended that new cell banks should be established before the new trial and robust characterization of the keratinocyte phenotypes should be ensured.[131]

In terms of treating chronic wounds, Apligraf® (Organogenesis, MA) has been approved by FDA to treat VLU and diabetic foot ulcers (DFU).[132–134] Apligraf is a bi-layered bioengineered skin substitute; cultured human foreskin-derived neonatal fibroblasts form the "neodermis" layer, while human foreskin-derived neonatal epidermal keratinocytes are cultured and induced to stratify *in vitro* to form the "neoepidermis" layer. Although Apligraf does not persist permanently after grafting (<4 weeks) in most patients,[135,136] it significantly promotes wound healings, likely through mechanisms such as cytokine secretion and optimizing wound environment. Other than being used to treat VLU and DFU, Apligraf has been reported to facilitate other type of wound healing. For instance, Apligraf was reported to be effective in treating a patient with necrobiosis lipoidica who had multiple chronic wounds.[137] In addition, Apligraf was applied with hyperbaric oxygen therapy in a patient with a degloving injury, who was also under chronic steroid treatment.[138] Recently, Apligraf has been reported to facilitate wound healing in premature neonates with full thickness skin and soft tissue necrosis at the dorsum of the right hand caused by total parenteral nutrition induced extravasation.[139]

TAKE-HOME MESSAGE

Epidermal stem cells are crucial in maintaining skin homeostasis and facilitating wound healing process. The implementation of current clinical approaches in wound treatment is closely based upon epidermal stem cells and their functions.

SUMMARY

The biological process of cutaneous wound healing is regulated by many types of cellular functions. Epidermal stem cells are crucial for wound coverage and restoring epidermal function. Therefore, the mechanisms controlling keratinocyte activation, migration, and fusion give rise to fundamental questions that need to be answered. Our group is dedicated to understanding the mechanisms coordinating cytoskeletal dynamics during keratinocyte migration, which is crucial to comprehending the cell behaviors during wound healing. Our previous study has provided critical insights into the mechanics of cell-adhesion dynamics.[64–67] We will look further into the complex signaling networks controlling the cross talk between different cytoskeletal networks.

The crucial function of epidermal stem cells during skin wound healing has intrigued the development of stem cell therapies, as well as tissue engineering for therapeutic purposes in clinic. As mentioned, both traditional skin grafting procedures and advanced keratinocyte grafting techniques are based upon the fundamental role of somatic skin stem cells during wound healing process. However, the majority of leading techniques still retain some deficiencies during clinical use, such as requiring improvement in decreasing vulnerability to infections and increasing long-term durability. Most importantly, the appendages under the epidermis, such as HFs and sweat glands, merely have the ability to regenerate after severe injuries. Although pigmentation is improved by keratinocyte grafting, the regaining of skin cosmesis, sensitivity, elasticity, and normal appendage is still difficult to pursue through available treatment approaches. Therefore, there are urgent inquires to improve the current standard of care and to develop novel techniques such as human skin substitutes to improve patient outcomes and long-term rehabilitation.

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Abbreviations and Acronyms

ACF7 = actin cross-linking factor 7
CD34 = clusters of differentiation 34
CEA = cultured epithelial autograft
DFU = diabetic foot ulcers
EB2 = microtubule end-binding protein 2
EGF = epidermal growth factor
ESGs = epidermal skin grafts
F-actin = filamentous actin
FDA = Food and Drug Administration
FGF = fibroblast growth factor
FTSGs = full-thickness skin grafts
Hax1 = HCLS1-associated protein X-1
HFs = hair follicles
IFE = interfollicular epidermis
IGF = insulin-like growth factor
IL = interleukin
K15 = keratin 15
LRIG1 = Leu-rich repeats and immunoglobulin-like domains 1
MCSP = melanoma-associated chondroitin sulfate proteoglycan
MMP = matrix metalloproteinase
OS = overall survival
SGs = sebaceous glands
STSGs = split-thickness skin grafts
TBSA = total body surface area
TGF = transforming growth factor
TNF = tumor necrosis factor
VEGF = vascular endothelial growth factor
VLU = venous leg ulcers