Published in final edited form as:
Nat Cell Biol. 2019 January 01; 21(1): 18–24. doi:10.1038/s41556-018-0237-6.

Stem cell dynamics, migration and plasticity during wound healing

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Abstract

Tissue repair is critical for animal survival. The skin epidermis is particularly exposed to injuries which necessitates rapid repair. The coordinated action of distinct epidermal stem cells recruited from various skin regions together with other cell types including fibroblasts and immune cells is required to ensure efficient and harmonious wound healing. A complex crosstalk ensures the activation, migration and plasticity of these cells during tissue repair.

Introduction

The skin is the first barrier protecting animals against UV radiation and pathogens from the external environment. It is composed of an epithelial layer, the epidermis, and the underlying dermis, which are separated by a basement membrane[1]. The epidermis contains pilo-sebaceous units that include a hair follicle and sebaceous glands, and are connected with the interfollicular epidermis (IFE) through the infundibulum[1]. The skin epidermis also contains other appendages, such as sweat glands, which regulate the body temperature through perspiration[1]. The dermis is composed of an upper (papillary) and a lower layer (reticular) of fibroblasts, blood vessels, immune cells and extracellular matrix (ECM)[2, 3]. Specialized fibroblasts form the dermal papilla, which regulates hair follicle growth and the erector pili muscle, responsible for pilo-erection. Partially integrated into the reticular dermis is a layer of dermal adipocytes that form the dermal white adipose tissue (DWAT)[4]. Underneath the dermis, the hypodermis (or subcutaneous adipose tissue) is composed of adipocytes, blood vessels and inflammatory cells[4]. This layer is important for thermoregulation and mechanical protection[2, 4] (Fig. 1a).

Upon tissue damage, the skin has to be repaired as quickly as possible to prevent excessive blood loss and infection. Wound healing occurs through distinct overlapping phases: haemostasis, inflammation, proliferation and remodelling[5]. Haemostasis occurs immediately after tissue damage and results in the formation of a blood clot, which stops the haemorrhage and triggers the recruitment of different immune cells, including neutrophils, macrophages and lymphocytes, to prevent infection and further activate the inflammatory

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Competing interests
The authors declare no competing interests.

response[5, 6] (Fig. 1b). The proliferation phase coordinates epidermal re-epithelialization and dermal repair[5] (Fig. 1c). The remodelling phase removes cells that are no longer necessary and induces ECM remodelling[6]. In small excisional wounds (<1cm diameter in mice), hair follicles are not re-formed and dermal scar tissue compensates for skin loss[7] (Fig. 1d). However, in large wounds (>1cm diameter), regeneration of hair follicles (wound-induced hair follicle neogenesis, WIHN) can be observed after re-epithelialization during the remodelling phase, resembling hair follicle embryonic development and a regeneration phase, typically 13-14 days after injury in mice [7, 8] (Fig. 1d).

Although the key steps of wound healing are well-described at the tissue level, a more in-depth characterization of the behaviour of individual cells at the clonal level and their fate transitions has only yet begun. The emergence of lineage tracing and intravital microscopy, coupled with transcriptional and epigenetic profiling, provide important insights about cellular and molecular mechanisms responsible for wound healing[9–12]. In this Perspective article, we describe recent advances with an emphasis on skin epithelial stem cell populations, their heterogeneity, clonal dynamics and remarkable plasticity during wound healing. Finally, we discuss the role of fibroblast populations and immune cells during repair and regeneration.

Skin epithelial stem cells during homeostasis

The skin epithelium renews throughout life in a continuous turnover ensured by stem and progenitor cells that balance proliferation and differentiation to replace dead and terminally differentiated cells[1, 13, 14]. Epithelial stem cells reside in a specific microenvironment called niche that is composed of a variety of cell types. Niche cells influence stem cell behaviour directly by cell contact or indirectly via ECM components and growth factors[15]. Although skin stem cells are able to regenerate the entire repertoire of skin epithelial lineages upon transplantation, lineage tracing has demonstrated that during physiological conditions, epidermal compartments are sustained by their own pool of resident stem cells[14–16] (Fig. 2a).

During adult homeostasis, hair follicles undergo cycles of growth (anagen) and degeneration (catagen), followed by a resting stage (telogen)[1]. The hair follicle stem cells (HFSC) responsible for cyclic regeneration are located in the permanent non-cyclic follicle portion called bulge[17–20]. HFSCs were first identified based on their slow-cycling properties[19, 21, 22]. They have higher clonogenicity *in vitro* and give rise to IFE, hair follicle and sebaceous gland lineages upon transplantation[17, 18, 20, 23, 24]. Slow-cycling HFSCs were first isolated and characterised using *K5-rtTA/TRE-H2BGFP and Krt15-EGFP* transgenic mice[25, 26], revealing expression of specific markers, such as *Cd34*[18, 23], *Krt15*[27, 28], *Krt19*[29], *Lgr5*[30], *Sox9*[31, 32] and *Tcf3*[33]. In sharp contrast with transplantation experiments, lineage tracing using *Krt15-CrePR*[34], *Shh-Cre*[35] Lgr5-CreER[30], *K19-CreER*[29], *Sox9-Cre*[32] and *Tcf3-CreER*[33] mouse strains established that HFSCs only contribute to hair follicle regeneration during physiological conditions, and do not maintain the sebaceous gland, infundibulum or IFE (Fig. 2a).

The IFE is composed of a single layer of proliferative basal cells and several layers of differentiated non-proliferative cells[1]. Basal cells replenish the suprabasal cells that are lost as terminally differentiated squames. In mice, it takes about a week for a basal cell to transit to the surface of the skin and about a month to replenish the whole IFE[36]. Early proliferation kinetic experiments reported maintenance of the IFE by small proliferative units that contain stem cells and progenitors[37]. However, lineage-tracing at clonal density later demonstrated that these units do not have a fixed size or predictable proliferation kinetics[38, 39]. Instead, in these studies IFE homeostasis was sustained by a single population of committed progenitors that balance renewal and differentiation in a stochastic manner[38, 40–43]. However, further studies provided evidence that basal epidermal cells are heterogeneous and some cells, depending on the skin regions, exhibit stem cell characteristics[44, 45]. These cells were more quiescent, persisted longer, and could give rise to more rapidly cycling committed progenitors with a shorter life-span[44, 45] (Fig. 2a). Profiling of murine stem and progenitor cells showed that the two populations are molecularly different and that stem cells express higher level of basal integrins, as do human epidermal stem cells, whereas committed progenitors are primed toward differentiation[44, 46].

The isthmus, a region located between bulge and sebaceous gland, contains its own pool of resident stem cells that express *Blimp1*[47], *Lgr6*[48], *Lrig1*[49], *Gata6*[50] or *Plet1*[51]. These multipotent cells give rise to all epidermal lineages upon transplantation[48–50, 52]. Lineage tracing using *Blimp1-cre*[47], *Lgr6-CreER*[48, 53] and *Lrig1-CreER*[54] has confirmed that these cells maintain the isthmus and sebaceous gland during homeostasis (Fig. 2a). In addition, *Lrig1*-expressing cells also give rise to cells of the infundibulum[54] (Fig. 2a), whereas *Gata6*-expressing stem cells only contribute to the maintenance of the sebaceous gland ducts but not the gland itself during homeostasis[50] (Fig. 2a). Altogether, these data show that during physiological conditions skin stem cells are confined to restricted compartments. Presently, the molecular mechanisms that restrain the movement of these cells across different territories remain unclear. As in the intestine[55], cell-specific expression of different guidance molecules might confine cell types in specific territories.

Skin epithelial stem cells during wound healing

During wound healing stem cells are activated and recruited from different skin regions. Interestingly, lineage restriction and spatial confinement of resident skin stem cells are transiently lost during repair, allowing contribution of multiple epidermal stem cells[15, 34, 54, 56, 57] (Fig. 2b).

The involvement of HFSCs in wound healing was already proposed 40 years ago after dermabrasion experiments in mice[58] and further confirmed by more recent analyses of proliferation kinetics[22]. Lineage tracing targeting label retaining cells[26] or using *Krt15-CrePR*[34], *Shh-Cre*[57], *K19-CreER*[54] and *Lgr5- CreER*[54] reporter strains showed that HFSCs rapidly migrate from the bulge to the wound and contribute to epidermal repair (Fig. 2b). These data demonstrate that HFSCs are highly plastic during wound healing, similarly to their expanded fate potential upon transplantation[17, 20, 30, 49, 52].

Clonal analysis of IFE stem cells (IFESCs) and committed progenitor cells following injury of mouse tail skin showed that IFESCs are recruited to the wound, contribute to epidermal repair and persist up to 35 days[44] (Fig. 2b). By contrast, committed progenitors are initially recruited but their progeny do not remain in the wound long-term[44]. Additional lineage tracing with *Dlx1-CreER* and *Slc1a3-CreER* reporters, which mark slow and rapidly cycling stem cells from different micro-domains of tail and back-skin IFE, demonstrated that upon wounding, both stem cell populations repopulate the two IFE regions[59]. However, in the long-term both cell populations only persist in their region of origin and not the region they migrate to during wound healing[59]. These observations suggest that during repair all basal cells present some degree of plasticity, a change in behaviour and functional contribution, but the wound does not reset the clock completely and cells keep a memory of their original location and hierarchy.

Similarly to HFSCs, *Lrig1* and *Lgr6*-expressing stem cells from the upper isthmus are mobilized following wounding and possibly activated even more rapidly than HFSCs[54]. HFSCs have been assumed to be quickly lost during regeneration and to only serve as a transient bandage that allows other stem cells from the IFE and upper isthmus/infundibulum to sustain long-term repair[34]. However, a more recent study showed that the proportion of hair follicle and Lrig1-derived cells located in the epidermis drops dramatically 3 weeks after an injury, whereas remaining cells can persist up to one year thereafter[54] (Fig. 2b). The persistence of these cells in the re-epithelialized IFE is proportional to the amount of stem cells labelled in the beginning and suggests a stochastic competition between equipotent stem cells rather than a hard-wired process[54]. Importantly, glabrous skin, such as the ventral (or palmar) part of the paw, heals correctly with slower kinetics compared to human skin, showing that HFSCs are dispensable for wound healing[60]. Moreover, similar to the contributions of hair follicles and infundibulum in skin with hair, sweat gland duct progenitors help to regenerate the injured epidermis in mouse paws[61]. Altogether, these studies suggest that the vacant niche created by an injury activates a broad range of stem cells to assume characteristics that differ from their homeostatic roles. Additional studies will be required to better understand the signals that activate distant stem cells, respective timelines and the mechanisms that disrupt and re-establish the boundaries between skin compartments. It further remains unclear how differentiation programmes get rewired during wound healing and how the balance between proliferation, migration and differentiation is achieved.

Migration, proliferation and compartmentalization

Epidermal injury is typically followed by increased keratinocyte proliferation[5]. Interestingly, proliferation is not observed at the wound edge but rather at a distance of 0.5 to 1.5 mm away from the edge[9, 10, 62], in a proliferative zone that surrounds the wound. At the leading edge, keratinocytes do not proliferate but migrate as cellular sheet[9, 10] (Fig. 3).

Intravital microscopy during wound healing demonstrated that both basal and suprabasal layers migrate during wound healing[10]. The speed of migration is greatest closer to the leading edge and decreases thereafter[10]. At a distance of 0.5 mm from the edge both migration and proliferation co-occur[10]. In this mixed region, basal cells are elongated toward

the wound and orient their division in this direction[10]. In tail epidermis, cells present at the leading edge are initially elongated parallel to the direction of the wound, suggesting active migration, but assume perpendicular orientation 2 to 4 days after wounding, possibly because they are pushed and compressed by cells behind[9]. Whether proliferation is necessary for cell migration remains unclear. Pharmacological inhibition of cell proliferation prevents wound closure and cell compression at the leading edge in tail skin[9]. By contrast, proliferation is dispensable for wound closure in mouse ear epidermis[10]. However, cells also display a more elongated shape in wounds with inhibited proliferation, suggesting a compensatory effect [10]. Differences in wound size and region-specific dermal populations could explain the discrepancies observed between ear and tail IFE. However, in Rac1 knockout mice with perturbed cell migration and elongation, a defect in the orientation of cell division is evident, suggesting its control by migration of the leading edge[10]. Altogether, these observations imply that cell migration at the leading edge comes first after wounding and that the displacement of cells in this region triggers orientated cell division of the cells following behind. Increased proliferation can itself generate a surplus of migrating cells that later push the leading edge toward the wound centre.

Transcriptional profiling of cells from migration and proliferation zones indicated two molecularly distinct and transient regions[9]. Cells at the leading edge expressed transiently higher levels of matrix metalloproteinases, pro-inflammatory molecules, genes controlling the cytoskeleton, microtubule and actin remodelling, ECM ligands and cell adhesion molecules such as integrin α5[9] (Fig 3). The constant size of the leading edge and its independence of wound size or skin area suggest that the signals controlling marker expression are local and potentially propagated from cell to cell within the epidermis. The leading edge might act as a transient scaffold enabling harmonious wound healing. By secreting higher lever of proteins that control ECM remodelling and blood clot dissolution, the leading edge might promote the progression of tissue regeneration toward the wound centre and protect stem cells and their progeny from tissue remodelling.

In addition, the acquired migratory phenotype of keratinocytes displays some features of epithelial-to-mesenchymal transition (EMT) including downregulation of cell adhesion molecules, increased motility and upregulation of EMT markers, such as Slug[63, 64]. Whether EMT is required for efficient wound healing or leading edge migration will require further analysis.

Stem cell population dynamics

During homeostasis, IFESCs and committed progenitors divide asymmetrically at the population level to maintain a constant number of epidermal cells[65]. However, during wound healing, cell numbers need to increase to compensate for lost cells until re-epithelialization is completed. Excess of renewal over differentiation can be achieved by increasing symmetric renewal or decreasing the proportion of cells that undergo differentiation, as during oesophageal wound repair[66] or *in vitro* culture of keratinocytes[67]. Upon tail injury, clonal analysis of *K14-CreER* (IFESCs and progenitors) and *Lrig1-CreER* (infundibulum stem cells) mouse strains demonstrated streaks of labelled cells arising from single IFE or infundibulum cells, both basal and suprabasal, that project toward the wound centre[9].

IFE-derived clones decreased by more than 90% during the first week, due to rapid terminal differentiation of committed progenitors, but overall cell ratios demonstrated that committed progenitors continued to divide mostly asymmetrically at the population level[9]. The proportion of basal and suprabasal cells from *Lrig1-CreER*-derived clones was similar to IFE-derived clones, although the size of the infundibulum-derived clones was slightly bigger[9]. Clonal persistence, clone size and basal/suprabasal cell ratio were consistent with a hierarchical model in which rare stem cells reside at the top of the hierarchy, dividing asymmetrically at a much more rapid pace compared to homeostasis and give rise to progenitors, so that the equilibrium between proliferation and differentiation remains balanced. Irrespective of their initial locations, wounding seems to induce the activation of a minor stem cell population, whereas lineage hierarchy and balance between self-renewal and differentiation of committed progenitors remain unchanged from homeostasis[9].

Recently, clonal analysis of human skin epidermis was performed after grafting in vitro reconstructed, genetically engineered skin into a patient with a severe form of the genetic skin disorder Epidermolysis Bullosa[68]. Sequencing of the integration site of the retrovirus used to replace the mutated gene revealed that viral integration in keratinocyte colonies originating from progenitors rapidly decreased over time[68]. By contrast, the integration sites of the most clonogenic colonies derived from stem cells increased, supporting the notion that only human skin stem-cell-derived colonies are able to renew and expand long-term *in vivo*, whereas progenitor cells possess limited potential to self-renew and revert back into a stem cell like state[68].

Stem cell plasticity

When stem cells from the hair follicle and infundibulum are recruited to the IFE upon injury, they progressively lose their initial identity and are reprogrammed to an IFE fate[34]. The molecular mechanisms responsible for this plasticity are still incompletely understood. In a comparison of chromatin landscapes of injured IFE and homeostatic HFSCs and IFESCs, the wounded IFE exhibited a hybrid signature between HFSCs and IFESCs, in which the open chromatin regions were enriched for both IFESC (Klf5) and HFSC (Sox9) transcription factors[12]. This hybrid stage called ‘lineage infidelity’ seems to ensure proper re-establishment of the epidermal barrier[12]. Although this hybrid state is transient during repair, it persists in skin cancer[12, 69].

Differentiated suprabasal epidermal cells are able to revert back to a stem cell state upon wounding[70, 71], a phenomenon also observed in airway epithelium after lineage ablation of basal stem cells[72]. However, lineage tracing and photolabelling of suprabasal IFE cells demonstrated that these cells cannot adopt a basal state again under wound healing conditions[9,10]. Contrastingly, a population of *Gata6*-expressing cells residing in the isthmus, which during homeostatic conditions give rise to the sebaceous duct, can be mobilized during wound healing to migrate toward the injured IFE and revert from a differentiated to a basal stem cell fate[50]. This reversion does not occur immediately after injury, as the suprabasal cells require a few days to access the basal layer and undergo stem cell reprograming[50]. This intriguing observation raises the question of whether other differentiated epidermal cells are also able to revert back to a stem cell state or

whether this is a unique property of the *Gata6*-expressing population. It is possible that the timing of reversion is important and that experiments performed on the tail and ear epidermis induced the labelling of the suprabasal cells too early to observe the reversion[9, 10]. Further experiments will be necessary to identify the mechanisms underlying this cellular plasticity and reprogramming of differentiated cells during wound healing. Other cases of dedifferentiation have been previously described in the hair follicle[73, 74]. After depilation or laser ablation to induce the loss of bulge HFSCs, hair germ cells[73] as well as infundibulum or sebaceous gland cells[74] are able to repopulate the stem cell niche and establish functional HFSCs. Similarly to skin, cells in the gut epithelium that are committed to terminal differentiation can revert back to a progenitor-like state and contribute to tissue repair following injury[75–77]. However, intestinal stem cells are required to ensure tissue repair following ionizing radiation[78], demonstrating that despite the ability of committed cells to re-assume stemness, regular tissue resident stem cells are essential for repair.

The degree of damage can also influence cellular plasticity. In relatively small wounds, re-epithelialization occurs without reforming hair follicles, whereas *de novo* hair follicle formation is apparent in large wounds[8]. Lineage tracing confirmed that these *de novo* hair follicles do not originate from HFSCs but IFE cells[8]. Analogous to hair follicle morphogenesis during embryonic development[79–81], WIHN depends on Wnt signalling as overexpression of Dickkopf Wnt signalling pathway inhibitor 1 (Dkk1), or β-catenin deletion in IFE basal cells prevents *de novo* hair follicle regeneration[8]. Epidermal deletion of *Wntless,* a gene required for the secretion of Wnt ligands, suppresses WIHN, suggesting that keratinocyte-derived Wnt is essential[82]. Different mouse strains have different susceptibilities to WIHN[83, 84]. Toll like receptor 3 (Tlr3) is increased in mouse strains with enhanced WIHN and dsRNA is a key signal that triggers skin regeneration through Tlr3[84]. Msh homeobox 2 (Msx2) is also crucial for WIHN[85]. Fibroblast growth factor 9 (Fgf9), secreted by γδ T cells, triggers Wnt expression by wound-induced fibroblasts, further amplifying the Wnt signal required for WIHN. These data illustrate the importance of a crosstalk between immune cells, fibroblasts and keratinocytes to enable successful WIHN[86].

Plasticity upon wound healing is also observed in other skin lineages. In the dermis, myofibroblasts, located close to *de novo* formed hair follicles, are converted into adipocytes in large wounds[87]. This cell fate conversion depends on BMP signalling originating from newly formed hair follicles that activate the expression of Zfp423, a transcription factor regulating adipocyte development[87]. Cell plasticity has also been described in other epithelia, such as the mammary gland, lung and intestinal epithelium[88]. It will be important to define whether generic mechanisms conserved across different tissues and species control cellular plasticity after lineage ablation and during tissue repair, inflammation or tumorigenesis.

Crosstalk between stem cells and the niche—During wound repair, fibroblasts are responsible for ECM synthesis in the dermis and for the fibrotic response leading to scar formation[5]. However, during embryonic development and in certain body locations, such as the oral cavity, wounds heal without forming scars[89]. The dermis of mouse back skin is composed of fibroblasts of different developmental origin[90, 91]. At least two

fibroblastic lineages give rise to the upper (papillary fibroblasts, dermal papillae, and erector pili muscle) and lower dermis (reticular fibroblasts, preadipocytes, hypodermal adipocytes), respectively[90]. Upon wounding, fibroblasts of the lower dermis are recruited first, followed by fibroblasts of the upper dermis[90] (Fig. 1). Reticular fibroblasts secrete the collagens responsible for scar tissue formation and are unable to regenerate the hair follicle after transplantation[90]. This observation may potentially explain why *de novo* hair follicle formation rarely occurs during wound healing. The upper papillary dermis is the only fibroblast lineage competent to regenerate hair follicles after transplantation, but it is recruited late during repair[90]. Interestingly, activation of Wnt/β-catenin signalling in epidermal cells increases the recruitment of papillary fibroblasts and also the number of hair follicles regenerated in the wound bed[90]. In sharp contrast, inhibition of β-catenin in fibroblasts promotes hair follicle regeneration during wound healing and correlates with a decrease of reticular fibroblasts and an increase of papillary fibroblasts[92]. In addition to different locations, two distinct fibroblastic lineages can also be identified on the basis of embryonic expression of Engrailed-1 (*En-1*)[91]. The En-1 positive lineage of fibroblasts (EPFs) produces ECM composed of collagen type I and III, becomes more abundant postnatally and is responsible for fibrotic scarring after injury[91]. By contrast, the En-1 negative lineage becomes less abundant after birth but has a higher regenerative capacity and their dominance during embryonic development might explain why embryos are able to regenerate scarless skin[91, 93]. Of note, the preferential location of EPFs in the reticular dermis after birth suggests they encompass the reticular populations previously described[90, 91]. Altogether, these data support the importance of surrounding dermal fibroblasts in skin regeneration.

A crosstalk between epidermal and immune cells also plays a major role during wound healing. Interestingly, after epidermal stem cells have been challenged with an inflammatory stimulus, the skin retains a memory of past inflammation and heals faster upon wounding. This memory is associated with chromatin remodelling that primes stem cells to respond more rapidly upon injury, in particular by promoting cell migration, which is partly mediated by the Aim2 inflammasome[11]. This remarkable observation is reminiscent of an ‘alert state’, as described in muscle stem cells, in which stem cells contralateral to the injured muscle undergo accelerated cell cycle entry upon tissue damage[94]. By contrast, ageing is associated with a defect in wound healing, linked to a decrease in epidermal migration and impaired signalling between epidermal and dendritic epidermal T cells[95]. Aged epidermal cells demonstrate a defect in Stat3 activation following wounding, which in turn prevents the activation of SKINTS genes that code for immunoglobulins and activate dendritic epidermal T cells in the wound bed[95].

Upon wounding, skin stem cell populations are also activated by Activin A, which is overexpressed in the dermis and the epidermis[9, 96]. Overexpression of Activin A enhances wound healing[96, 97], promotes keratinocyte migration and the formation of granulation tissue by dermal cells[98, 99]. Moreover, Activin A recruits Foxp3-expressing regulatory T cells, which further support accelerated healing[97]. These data illustrate the importance of the crosstalk between immune cells, dermal cells and keratinocytes during wound healing[97].

Conclusions and outlook

Taken together, this body of work provides previously unappreciated insights into the cellular and molecular mechanisms regulating wound healing and the importance of a crosstalk between different skin cell populations. However, many open questions remain regarding the signals that stimulate stem cells to migrate to the wound centre. We also know little about the control of skin compartmentalization during homeostasis and repair. How rapidly after wounding are these boundaries re-established? Mechanistically, it will be interesting to further elucidate how cells move between and within basal and suprabasal layers and learn more about the role of the leading edge. Factors controlling chromatin remodelling and lineage infidelity in chronic wounds and cancer also remain to be discovered.

Lineage ablation experiments at the leading edge should aid in defining the role and function of involved compartments during wound repair. Moreover, single-cell RNA-sequencing and ATAC-sequencing of stem cell populations isolated during different regeneration stages should provide insights into the chromatin and transcriptional landscape associated with cellular heterogeneity. These studies might also elucidate further mechanisms that mediate the reprogramming of cells from the hair follicle and infundibulum as they are recruited to the IFE. Finally, understanding the role of the niche in the reprogramming of differentiated cells toward a stem cell fate will be an important challenge for the future.

Acknowledgements

We apologize to all authors whose work could not be cited due to space constraints.

C.B. is an investigator of WELBIO. S.D. is supported by a fellowship of the FNRS/ TELEVIE.

This work was supported by a consolidator grant of the European Research Council.

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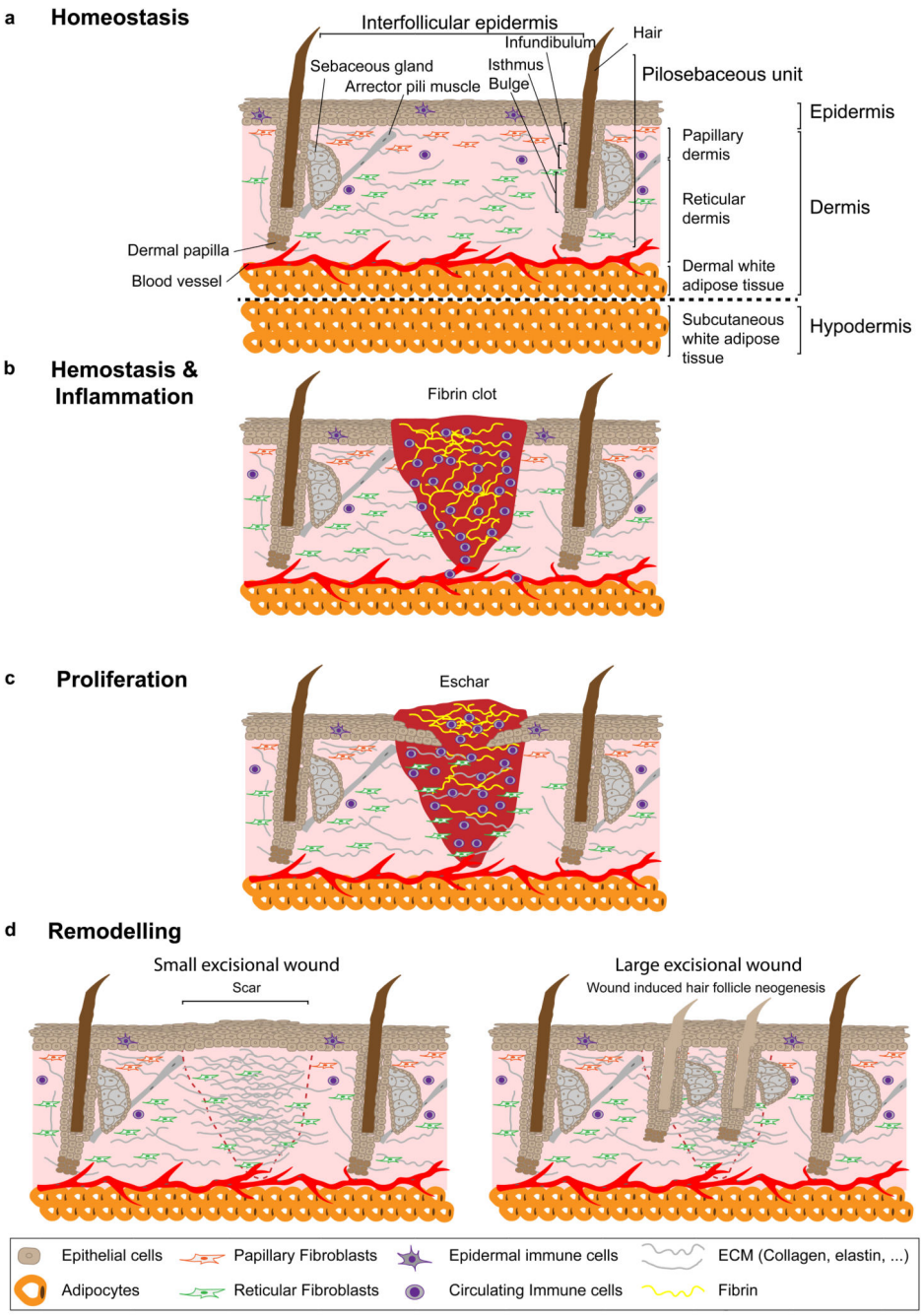

Figure 1. Overview of skin homeostasis and wound healing phases.
a, The skin is composed of dermis and epidermis. In the epidermis, epithelial cells are organized into a pilo-sebaceous unit, the hair follicle and its associated sebaceous glands, and the surrounding tissue, the IFE. The dermis consists of a papillary and a reticular layer located in the upper and lower part, respectively. Dermal papilla controls the hair follicle cycle and arrector pili muscle ensures its movement. The dermis includes fibroblasts, blood vessels, immune cells, sensory nerves and in its lower portion, the DWAT, which contains adipocytes. Below the skin lies the hypodermis or SWAT. **b**, Haemostasis and

inflammation start immediately after wounding. The fibrin clot prevents further blood loss and provides a scaffold for the migration of immune, dermal and epidermal cells. **c**, During the proliferation phase, keratinocytes, fibroblasts and endothelial cells proliferate and migrate to the wound site and reform the ECM. **d**, During the remodelling phase the collagen in the dermis is remodelled and cells from earlier stages are removed. In small excisional wounds in mice, hair follicles are not regenerated and dermal scar tissue compensates for skin loss (left panel). In large excisional injuries, WIHN can be observed after complete re-epithelialization (right panel). DWAT, dermal white adipose tissue; ECM, extracellular matrix; SWAT, subcutaneous white adipose tissue; WIHN, wound induced hair follicle neogenesis.

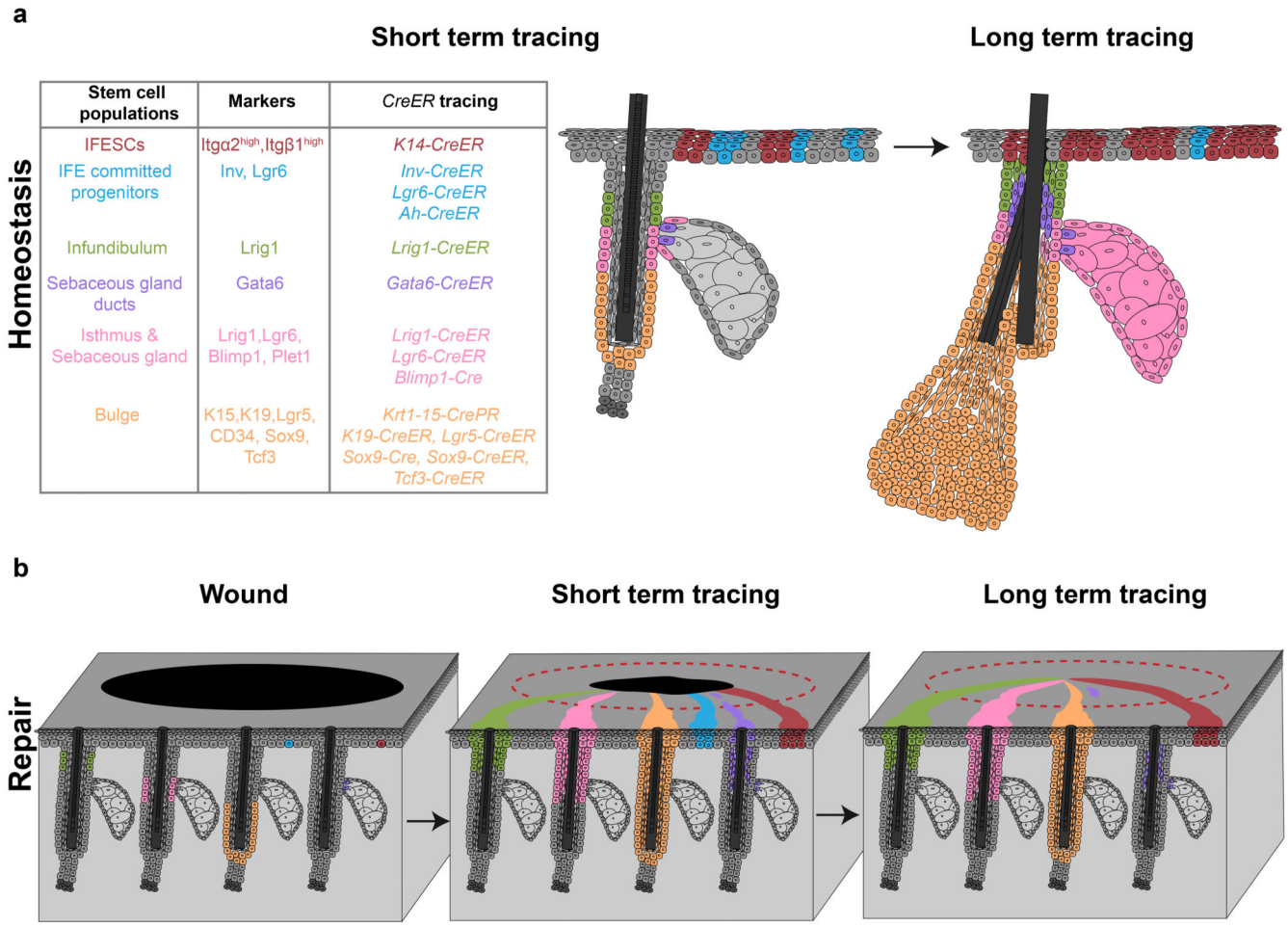

Figure 2. Skin epithelial stem cell populations during homeostasis and repair.
a, Skin epithelial stem cells express specific markers and can be lineage-traced with *CreER* mouse strains (left table). IFE stem cells and committed progenitors are located in the basal layer of the IFE and give rise to suprabasal, differentiated cells. Stem cells and committed progenitors can be traced using a *K14-CreER* or *Inv-CreER* mouse strains induced at low dose respectively. IFE committed progenitors also express *Lgr6.* Infundibulum stem cells are located in the upper part of the isthmus and express *Lrig1.* A population of sebaceous gland duct stem cells expressing *Gata6* are located at the entrance of the gland but only maintains the junctional zone. Isthmus and sebaceous gland stem cells are basal cells located at the junction between the hair follicle and the gland, express *Lrig1, Lgr6* and *Blimp1* and give rise to the entire sebaceous gland and the isthmus. Bulge stem cells are located in the permanent lowest portion of the hair follicle, express *K15, K19, Lgr5, CD34, Sox9 and Tcf3* and give rise to the entire hair follicle. **b**, Upon wounding, both IFESCs and committed progenitors are recruited and contribute to tissue repair. Only IFESCs will reside in the newly formed IFE long-term. Isthmus, sebaceous gland and infundibulum stem cells are recruited, contribute to IFE repair and remain long-term. Bulge stem cells are recruited to the IFE and a small proportion can remain long-term as IFESCs. *Gata6+* sebaceous gland duct stem cells are recruited to the IFE, migrate suprabasally, de-differentiate and

are re-established as IFESCs in the long-term. IFE, interfollicular epidermis; IFESCs, interfollicular epidermis stem cells.

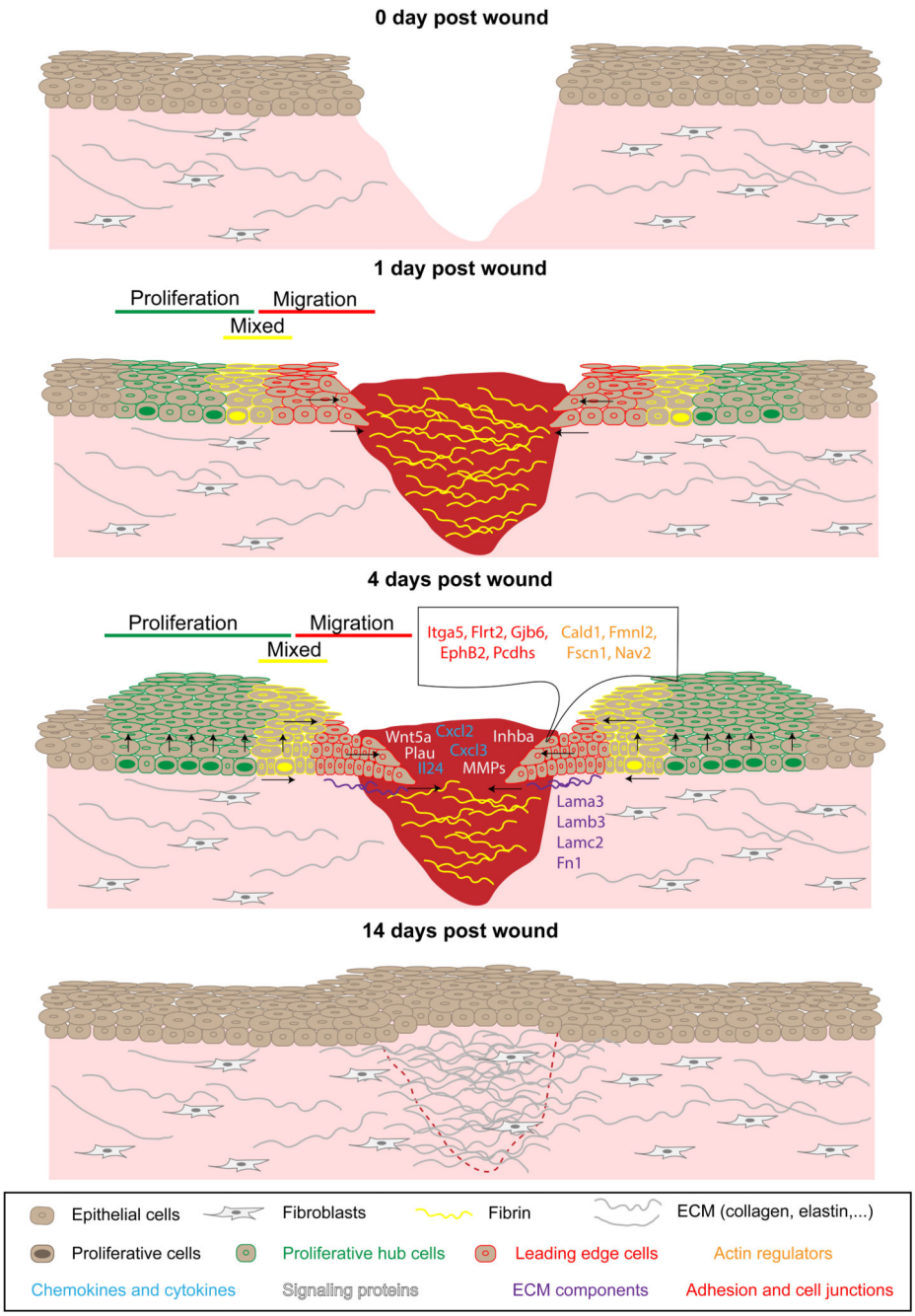

Figure 3. Epidermal migration, proliferation and compartmentalization during wound healing. Epithelial cells start to migrate into the wound bed within 12 hours after injury. The day after wounding, IFE cells located close to the wound show an elongated shape toward the direction of the wound and are quiescent, whereas cells located at a distance start to proliferate, which leads to the establishment of a proliferative and a migrating leading edge compartment. Between these two zones, a mixed region is observed containing both migrating and proliferative cells. Four days after wounding, leading edge cells are compressed and upregulate the expression of specific genes that promote inflammation and

regeneration. This gene signature is transient and disappears when the IFE is healed. IFE, interfollicular epidermis; ECM, extracellular matrix.