NATURE|Vol 453|15 May 2008|doi:10.1038/nature07039

Wound repair and regeneration

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The repair of wounds is one of the most complex biological processes that occur during human life. After an injury, multiple biological pathways immediately become activated and are synchronized to respond. In human adults, the wound repair process commonly leads to a non-functioning mass of fibrotic tissue known as a scar. By contrast, early in gestation, injured fetal tissues can be completely recreated, without fibrosis, in a process resembling regeneration. Some organisms, however, retain the ability to regenerate tissue throughout adult life. Knowledge gained from studying such organisms might help to unlock latent regenerative pathways in humans, which would change medical practice as much as the introduction of antibiotics did in the twentieth century.

In the moments after an injury occurs, various intracellular and intercellular pathways must be activated and coordinated if tissue integrity and homeostasis are to be restored. Cellular components of the immune system, the blood coagulation cascade and the inflammatory pathways are activated in addition. Many types of cell — including immune cells (neutrophils, monocytes, lymphocytes and dendritic cells), endothelial cells, keratinocytes and fibroblasts — undergo marked changes in gene expression and phenotype, leading to cell proliferation, differentiation and migration[1,2]. If this response is successful and the injury does not result in the demise of the organism, these processes must be shut down in a precise sequence in the ensuing days as recovery progresses.

Given the complexity of the wound repair process, it is remarkable that it rarely becomes uncontrolled and that malignant transformation is an uncommon event in the wound environment[3,4]. For most injuries, repair results in once functional tissue becoming a patch of cells (mainly fibroblasts) and disorganized extracellular matrix (mainly collagen) that is commonly referred to as a scar. Surprisingly, in some eukaryotic organisms, the response to injury can completely recapitulate the original tissue architecture, through the poorly understood process of regeneration. Humans have this ability during prenatal development, but it is lost during adult life[5]. How regeneration occurs and why humans lose this ability remain a mystery[6]. In this review, we provide an overview of how multicellular organisms respond to tissue loss, highlighting areas in which developmental pathways have been used to foster tissue regeneration. Recent advances in epithelial stem-cell biology and developmental biology have begun to elucidate the pathways that need to be reactivated to effect regeneration in humans.

Clinical burden of fibrosis

In general, the wound repair process occurs in almost all tissues after exposure to almost any destructive stimulus. Thus, the sequence of events that follows a myocardial infarction (heart attack), for example, is remarkably similar to that following a spinal-cord injury, a burn or a gunshot wound, despite the different types of insult and the different organs affected. Likewise, scar formation that occurs during wound repair leads to similar tissue dysfunction wherever it takes place.

In the case of myocardial infarction, the formation of myocardial scar tissue is thought to result in congestive heart failure (a condition in which the heart cannot supply the body's tissues with enough blood) and/or abnormal heart rhythms (arrhythmias) (see page 322), which together account for nearly 100,000 deaths each year in the United States alone[7]. In addition, cirrhosis of the liver and some forms of fibrosis of the lungs are thought to result from fibrotic responses to toxin-mediated injury[8,9]. Interestingly, in other circumstances, the liver is one of the few organs in the human body that can regenerate up to 70% of itself without scar formation. Why the liver's regenerative capacity manifests only in some cases remains incompletely understood. A leading hypothesis is that the immune system is involved in the switch between regeneration and fibrotic healing, because human fetuses, which heal without scarring, have immature immune systems[10].

Healing by fibrosis, instead of regeneration, places a huge burden on public health. The total economic cost of diseases that result from fibrosis is difficult to calculate precisely but is of the order of tens of billions of dollars[11]. Importantly, dysfunctional healing often causes lifelong disability, which has a significant economic impact[2]. Thus, if fibrotic healing processes can be transformed into regenerative ones, in which the original tissues are restored, this would considerably improve human health.

Wound repair across phylogeny

The ability to respond to injury and to repair tissue is a fundamental property of all multicellular organisms. But there is tremendous diversity in how this process occurs. Studying wound repair in various phyla could improve our understanding of wound repair in humans and might help to identify molecules or pathways that can be targeted to restore the lost regenerative capacity.

The most primitive multicellular organism and the common ancestor of modern multicellular organisms is the sponge. Although wound repair has not been studied extensively in sponges, recent work on cellular patterning in these organisms has provided insight into the origin of embryonic patterning in metazoans. Patterning refers to the three-dimensional orientation of cells in an organism and is probably required for tissue regeneration. It is generally not thought to be involved in tissue repair with scarring. Sponges are simple organisms without a body axis, multiple layers of cells, or tissues[12–14]. Adult sponges have highly adaptable body shapes and lack an anteroposterior axis of symmetry,

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but embryonic and larval sponges have both an anteroposterior axis and radial symmetry. Certain soluble factors — WNT proteins and transforming growth factor-β (TGF-β) — together are required to pattern the embryonic sponge cells, and a hedgehog-like cell-surface signal, hedgling[15], is also produced in overlapping patterns with the WNT proteins and TGF-β.

Similarly, in *Drosophila melanogaster*, morphological events that occur during development, such as dorsal closure[16] and tracheal fusion[17], have a remarkable visual similarity to wound repair in humans. Unlike sponges, *D. melanogaster* has a complex, craniocaudal asymmetry in the adult stage. In addition, in *D. melanogaster* embryos, the same molecular machinery is used in these morphological developmental events and to close an epithelial gap in a wound[18]. That these intracellular signalling pathways and cell-adhesion pathways are highly conserved across species and components are present after injury in other multicellular organisms, including humans, indicates that the patterning cues for multicellular organization are present in the wound environment in adults but are not fully functional[19].

To examine the involvement of developmental pathways in wound repair more directly, morphogenetic events involving tissue movement have been used as models of wound repair. The two most commonly used models are dorsal closure in *D. melanogaster* embryos and ventral enclosure in *Caenorhabditis elegans*, both of which involve the elimination of epithelial gaps. In *D. melanogaster*, it seems that JUN amino-terminal kinase (JNK) signalling and the subsequent activation of the transcription factor activator protein 1 (AP1) in leading-edge epithelial cells, together with tight regulation of the associated cell division and cell–cell adhesion, are crucial for successful closure[20]. As discussed later, this process is analogous to the re-epithelialization (the recreation of an intact keratinocyte layer) of mammalian wounds, again emphasizing the common foundation of tissue repair across phylogeny.

Wound repair in amphibians has been the focus of recent excitement because of the relatively close relationship between these organisms and mammals and because of their remarkable ability to regenerate amputated appendages through the formation of a blastema, which is a mass of undifferentiated cells capable of regeneration. After wounding, differentiated cells in the mature tissues that surround the amputated region dedifferentiate into mononuclear blastemal cells that can proliferate and have the potential to differentiate into multiple cell types[21,22]. These cells then regenerate the limb through a process that mirrors embryonic development. Nerve stimulation is important for maintaining this regenerative state[23]. Recently, it has been shown in salamanders that nAG, a member of the anterior-gradient protein family, is produced by the Schwann cells of transected nerves and interacts with Prod 1 on the surface of blastemal cells, promoting their proliferation[24]. However, if the nerves proximal to the blastema (that is, near the spinal cord) are transected, then the blastema fails to undergo limb regeneration, and the cells fail to redifferentiate; therefore, wound repair occurs instead of limb regeneration. Similarly, planarians are simple animals that lack complex organ systems but can fully regenerate an intact organism from a minor remnant (as little as 1/289) of the original organism. Once again, undifferentiated cells (neoblasts) collect at the injury site and form a blastema, which can regenerate organ systems and tissues[25]. In planarians, it is thought that up to 20% of all cells are committed to the regeneration of adult tissues.

Mammals have retained much of the molecular machinery used by organisms such as salamanders, but their regenerative potential is only limited. In part, this seems to result from the rapid interposition of fibrotic tissue, which prevents subsequent tissue regeneration. For example, when the spinal cord of mice is injured, neurons begin to grow, but glial cells at the site of injury stimulate scar formation, preventing recovery. However, if the mouse spinal cord is cut so that scar formation is hindered, the neurons reconnect[26,27]. This rapid interposition of scar tissue probably confers a survival advantage by preventing infectious microorganisms from invading the wound and by inhibiting the continued mechanical deformation of larger tissues (a process that could compound the initial insult). To manipulate the wound repair process

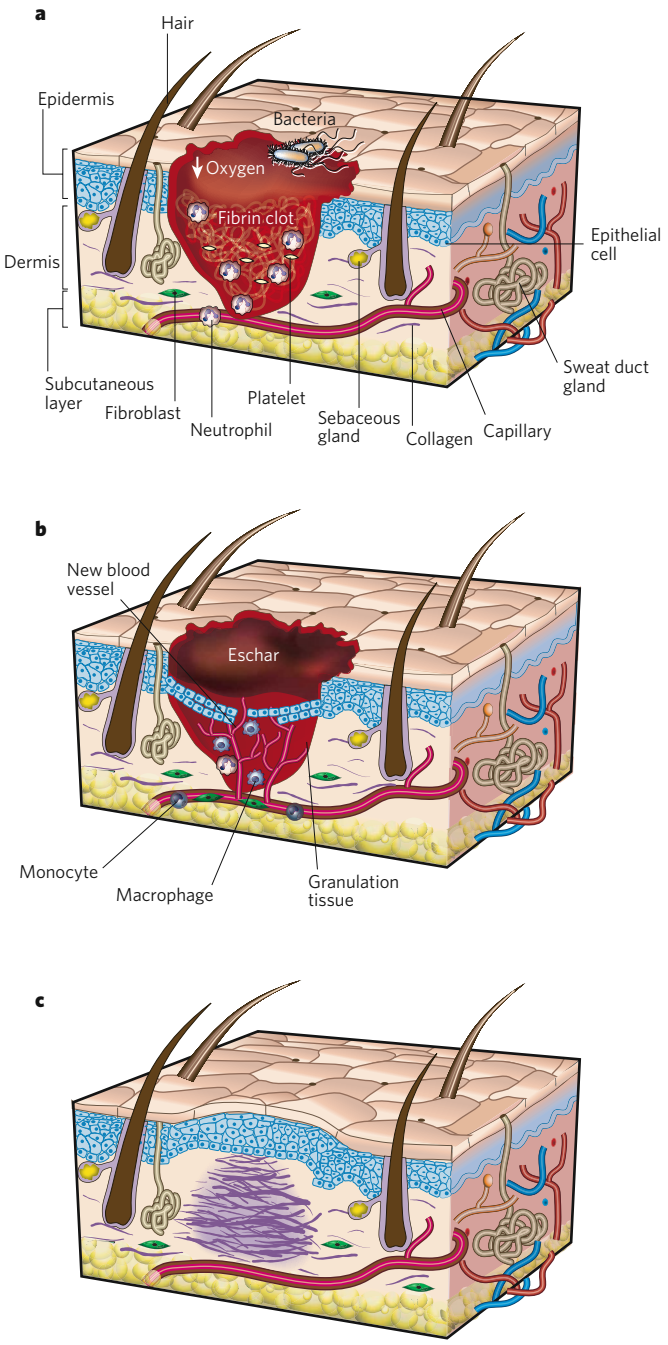

Figure 1 | Classic stages of wound repair. There are three classic stages of wound repair: inflammation (**a**), new tissue formation (**b**) and remodelling (**c**). **a**, Inflammation. This stage lasts until about 48 h after injury. Depicted is a skin wound at about 24–48 h after injury. The wound is characterized by a hypoxic (ischaemic) environment in which a fibrin clot has formed. Bacteria, neutrophils and platelets are abundant in the wound. Normal skin appendages (such as hair follicles and sweat duct glands) are still present in the skin outside the wound. **b**, New tissue formation. This stage occurs about 2–10 days after injury. Depicted is a skin wound at about 5–10 days after injury. An eschar (scab) has formed on the surface of the wound. Most cells from the previous stage of repair have migrated from the wound, and new blood vessels now populate the area. The migration of epithelial cells can be observed under the eschar. **c**, Remodelling. This stage lasts for a year or longer. Depicted is a skin wound about 1–12 months after repair. Disorganized collagen has been laid down by fibroblasts that have migrated into the wound. The wound has contracted near its surface, and the widest portion is now the deepest. The re-epithelialized wound is slightly higher than the surrounding surface, and the healed region does not contain normal skin appendages.

in mammals so that it shifts towards regeneration will probably require the ability to slow the rapid fibrotic response so that multipotent cells such as stem or progenitor cells can allow tissue regeneration rather than scar formation.

Classic stages of wound repair

In all organ systems, the normal mammalian response to injury occurs in three overlapping but distinct stages: inflammation, new tissue formation, and remodelling (Fig. 1). The first stage of wound repair — inflammation — occurs immediately after tissue damage, and components of the coagulation cascade, inflammatory pathways and immune system are needed to prevent ongoing blood and fluid losses, to remove dead and devitalized (dying) tissues and to prevent infection. Haemostasis is achieved initially by the formation of a platelet plug, followed by a fibrin matrix, which becomes the scaffold for infiltrating cells. Neutrophils are then recruited to the wound in response to the activation of complement, the degranulation of platelets and the products of bacterial degradation[28] (Fig. 1a). After 2–3 days, monocytes appear in the wound and differentiate into macrophages. Macrophages are thought to be crucial for coordinating later events in the response to injury, but the importance of neutrophils and macrophages in wound repair is incompletely understood. Recent data suggest, however, that a deficiency in either cell type can be compensated for by the redundancy in the inflammatory response[29]. In the absence of both cell types, the repair of small wounds can still occur, and the scarring response is even less[30].

The second stage of wound repair — new tissue formation — occurs 2–10 days after injury and is characterized by cellular proliferation and migration of different cell types. The first event is the migration of keratinocytes over the injured dermis (the inner layer of the skin) (Fig. 1b). New blood vessels then form (a process known as angiogenesis), and the sprouts of capillaries associated with fibroblasts and macrophages replace the fibrin matrix with granulation tissue, which forms a new substrate for keratinocyte migration at later stages of the repair process. The keratinocytes that are behind the leading edge proliferate and mature and, finally, restore the barrier function of the epithelium. The most important positive regulators of angiogenesis[31] are vascular endothelial growth factor A (VEGFA) and fibroblast growth factor 2 (FGF2; also known as bFGF). For example, the application of VEGFA alone to wounds in an animal model of diabetes (in which wound repair is dysregulated) can normalize healing[32]. Angiogenesis can also result from the recruitment of bone-marrow-derived endothelial progenitor cells, although the magnitude of this contribution is small — at least in non-ischaemic wounds (in which the concentration of oxygen is normal)[33] (Fig. 1b). In the later part of this stage, fibroblasts, which are attracted from the edge of the wound or from the bone marrow are stimulated by macrophages, and some differentiate into myofibroblasts[34]. Myofibroblasts are contractile cells that, over time, bring the edges of a wound together. Fibroblasts and myofibroblasts interact and produce extracellular matrix, mainly in the form of collagen, which ultimately forms the bulk of the mature scar[35].

The third stage of wound repair — remodelling — begins 2–3 weeks after injury and lasts for a year or more. During this stage, all of the processes activated after injury wind down and cease. Most of the endothelial cells, macrophages and myofibroblasts undergo apoptosis (that is, programmed cell death) or exit from the wound, leaving a mass that contains few cells and consists mostly of collagen and other extracellular-matrix proteins (Fig. 1c). Epithelial–mesenchymal interactions probably continuously regulate skin integrity and homeostasis[36]. And there must be additional feedback loops to maintain the other cell types in the skin. In addition, over 6–12 months, the acellular matrix is actively remodelled from a mainly type III collagen backbone to one predominantly composed of type I collagen[37]. This process is carried out by matrix metalloproteinases that are secreted by fibroblasts, macrophages and endothelial cells, and it strengthens the repaired tissue. However, the tissue never regains the properties of uninjured skin[38]. Interestingly, vertebrates such as zebrafish (*Danio rerio*) and *C. elegans* do not produce either of these collagen types; their extracellular matrix consists entirely of type VI and type XVIII collagens[39]. This finding suggests a degree of evolutionary plasticity that is not observed in the earlier stages of wound repair.

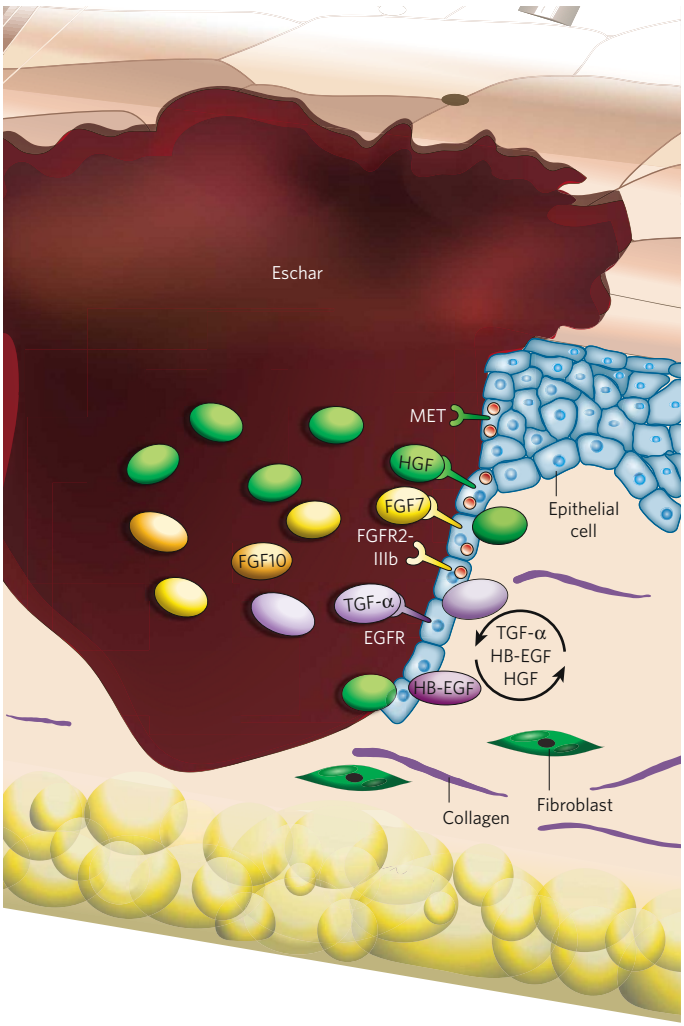

Figure 2 | Wound re-epithelialization. Half of an excisional wound in skin is shown at about 2 days after injury. Growth factors that are known to stimulate wound re-epithelialization are depicted: hepatocyte growth factor (HGF), which binds to MET; fibroblast growth factor 7 (FGF7) and FGF10, which bind to the IIIb isoform of FGF receptor 2 (FGFR2-IIIb), and ligands of the epidermal growth factor receptor (EGFR), such as transforming growth factor-α (TGF-α) and heparin-binding epidermal growth factor (HB-EGF). The signalling pathways initiated by these interactions activate the transcription factors signal transducer and activator of transcription 3 (STAT3) and AP1 proteins (pink circles), which help to regulate wound re-epithelialization. This process is further promoted by peroxisome-proliferator-activated receptors (PPARs), which are most probably activated by non-saturated fatty acids. The loop indicates autocrine stimulation.

Molecular mechanisms of wound repair

Following the observation that genes that are regulated in response to skin injury are functionally important for the wound repair process, DNA-microarray studies were carried out to identify such genes across the genome[40,41]. These studies showed that the gene-expression pattern of healing skin wounds strongly resembles that of highly malignant tumours[42], highlighting the importance of functional genomics studies for research into wound repair and cancer. The generation and analysis of genetically modified mice provided insight into the roles of some of the genes regulated during wound repair[28]. In addition, the observed similarities between the cell movements that occur during dorsal closure in *D. melanogaster* embryos and the healing of mammalian skin

Table 1 | Soluble mediators of re-epithelialization

Ligand	Receptor	Type of receptor	Signalling proteins	Role in re-epithelialization	References
HGF	MET	Receptor tyrosine kinase	Unknown, possibly ERK1 and ERK2, AKT, GAB1, PAK1 and/or PAK2	Stimulation of keratinocyte migration and probably proliferation	43
FGF7, FGF10 and FGF22	FGFR2-IIIb, possibly FGFR1-IIIb	Receptor tyrosine kinase	Unknown, possibly ERK1, ERK2, AKT and/or STAT3	Stimulation of keratinocyte proliferation and migration	44–46
Heparin-binding EGF and other EGF-family members	EGFR (also known as ERBB1), possibly ERBB2, ERBB3 and/or ERBB4	Receptor tyrosine kinase	Unknown, possibly ERK1 and ERK2, AKT and/or STAT3	Stimulation of keratinocyte proliferation and migration	30, 47
TGF-β	TGF-β receptor I and TGF-β receptor II	Receptor serine/threonine kinase	SMAD3 and others, including SMAD2 and MAPK	Inhibition of keratinocyte proliferation and survival	30, 51, 52
Acetylcholine	M3 receptor	G-protein-coupled receptor	Ca^{2+}-dependent guanylyl cyclase, cyclic GMP and PKG, leading to inhibition of RHO	Inhibition of keratinocyte migration	54
	M4 receptor	G-protein-coupled receptor	Adenylyl cyclase, cyclic AMP and PKA, leading to activation of RHO	Stimulation of keratinocyte migration	54
Catecholamines, including adrenaline	β_2-Adrenoceptor	G-protein-coupled receptor	Activation of phosphatase PP2A, resulting in dephosphorylation and inhibition of ERK1 and ERK2	Inhibition of keratinocyte migration	55
Polyunsaturated fatty acids	PPAR-α and PPAR-β*	Nuclear receptor	Direct activation of target genes by binding to the promoter/enhancer of these genes	Stimulation of keratinocyte migration and survival	56–58

EGF, epidermal growth factor; EGFR, EGF receptor; ERK, extracellular-signal-regulated kinase; FGF, fibroblast growth factor; FGFR1-IIIb, IIIb isoform of FGF receptor 1; GAB1, growth-factor-receptor-bound protein 2 (GRB2)-associated binding protein 1; HGF, hepatocyte growth factor; M3, muscarinic receptor subtype 3; PAK, p21-activated kinase; PKA, cyclic-AMP-dependent protein kinase; PKG, cyclic-GMP-dependent protein kinase; PPAR, peroxisome-proliferator-activated receptor; SMAD3, SMAD-family member 3; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-β. *PPAR-β ligands might be fatty acids.

wounds, together with the establishment of wound repair models in *D. melanogaster*[20], have allowed the genetically tractable *D. melanogaster* system to be used for identifying and functionally characterizing the molecules involved in wound repair, particularly in re-epithelialization. Many of the steps in wound repair are poorly characterized at the molecular level at present, so in this section we discuss recent data that reveal the underlying molecular mechanisms of re-epithelialization, a major event in the phase of new tissue formation (second stage).

The proteins involved in re-epithelialization include various extracellular-matrix proteins and their receptors, proteases (including matrix metalloproteinases), cytoskeletal proteins, and enzymes that regulate the cellular redox balance. The functions of these proteins in wound repair have been described in a recent review[43]. Other proteins that are known to be involved include growth factors and hormones, and we focus on the role of these proteins and their downstream targets in the repair of the injured epidermis (the outer layer of the skin) and the dermis (Fig. 2 and Table 1).

Peptide growth factors

One molecule that is particularly important in re-epithelialization is hepatocyte growth factor (HGF), which exerts its function by binding to and activating MET, a receptor tyrosine kinase[31,44]. Interestingly, mice in which the gene encoding MET had been deleted from keratinocytes showed strongly delayed re-epithelialization in response to skin wounds, and the cells that eventually covered the wounds in these mice were found to have escaped the recombination event in which *Met* was deleted and therefore still expressed MET[44]. This result was unexpected, because it was known that other growth factors are involved in re-epithelialization, but it is clear that these factors cannot compensate for a lack of HGF-mediated signalling. Other growth factors that positively regulate re-epithelialization include members of the FGF family and the epidermal growth factor (EGF) family. In addition, certain peptide growth factors, most notably TGF-β, negatively regulate re-epithelialization.

In terms of the FGF family, ligands for the IIIb variant of FGF receptor 2 (FGFR2-IIIb), in particular, contribute to wound repair. This is shown by the strong delay in re-epithelialization that occurs in transgenic mice expressing a dominant-negative mutant of FGFR2-IIIb in keratinocytes. The mutant receptor binds to FGF but cannot transduce the signal[45]. The most important FGFR2-IIIb ligands, the functions of which were abrogated in these mice, are likely to be FGF7 and FGF10 (ref. 46). In support of this idea, mice that lack dendritic epidermal T cells, which are a potent source of FGF7 and FGF10 (ref. 47), show decreased keratinocyte proliferation and wound closure after injury.

Many of the ligands for the EGF receptor (EGFR) are expressed at higher levels after skin injury, and one of these, heparin-binding EGF, has been shown to have a functional role in re-epithelialization[48] (Fig. 2). However, the large number of EGFR ligands at the wound site probably allows functional redundancy; therefore, ascertaining how the EGF family contributes to the wound repair process will require studies in mice in which the *Egfr* gene is knocked out just in keratinocytes.

These positive regulators of re-epithelialization (that is, HGF, FGFs and EGFs) are ligands of receptor tyrosine kinases, the activation of which most often stimulates keratinocytes to migrate, proliferate and survive. Some of these processes are mediated by AP1-family members and by another transcription factor, STAT3. These factors are activated in response to signalling through various receptor tyrosine kinases, as well as in response to cytokine-receptor activation. Loss of AP1 proteins or AP1 components impaired dorsal closure and wound repair in *D. melanogaster*[20,49]. Recent studies suggest that AP1 proteins have a similar role in mammalian wound repair[50], although some of their functions are probably masked by redundancy between the various members of the mammalian AP1 family[49]. STAT3, however, is clearly involved in re-epithelialization, because epidermis-specific deletion of *Stat3* in mice was found to retard epithelial repair after wounding[51].

In contrast to these mitogenic growth factors, TGF-β is a negative regulator of wound re-epithelialization. Re-epithelialization was shown to be strongly accelerated in mice expressing a gene encoding a dominant-negative TGF-β receptor in the epidermis[52] and in mice lacking the transcriptional regulator SMAD3, one of the main targets of TGF-β-mediated signalling[53]. Surprisingly, the TGF-β-family member activin, which also signals through SMAD3, does not inhibit keratinocyte proliferation at the wound site but, instead, promotes proliferation. This effect, however, is probably mediated through the mesenchyme[54], further highlighting the importance of epithelial–mesenchymal interactions in the wound repair process.

Hormones and other factors

In addition to peptide growth factors, several low-molecular-mass mediators are regulators of wound re-epithelialization. For example, the hormone acetylcholine and its receptors are produced by keratinocytes, resulting in an autocrine loop that both positively regulates migration (through muscarinic acetylcholine receptors of the M4 subtype) and negatively regulates migration (through M3 receptors)[55]. Keratinocytes also produce hormones known as catecholamines (including adrenaline) and their receptors, resulting in the inhibition of re-epithelialization in an autocrine manner[56].

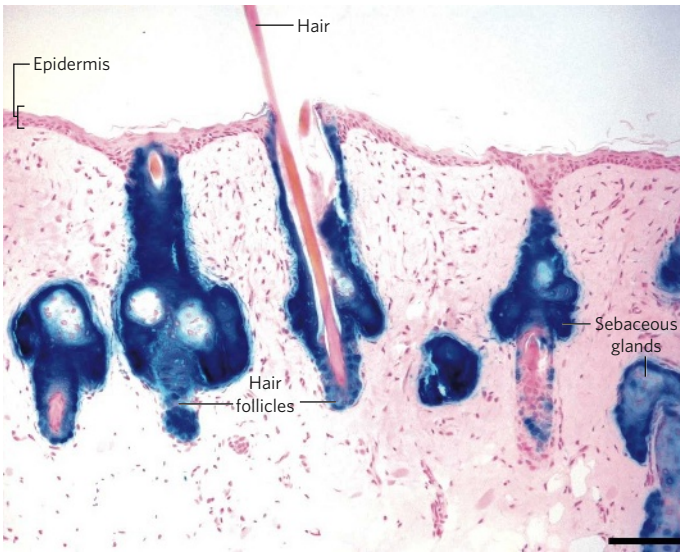

Figure 3 | Epithelial stem-cell-mediated skin regeneration. To examine which cell types contribute to epidermal renewal, a single multipotent stem cell was isolated from a whisker follicle of a rat, labelled with a retrovirus carrying *lacZ* (which encodes β-galactosidase), expanded in culture and transplanted into newborn mouse skin[61]. The skin was biopsied 7 months later, and the tissue section shown indicates β-galactosidase activity (blue) and cell nuclei (pink). The progeny of the labelled stem cell contributed to several hair follicles and sebaceous glands (blue) but not to the epidermis; it should be noted that the follicles are in telogen (resting phase). Hence, hair-follicle stem cells do not contribute to long-term epidermal renewal, and the epidermis contains its own stem cells. Scale bar, 100 μm. (Image courtesy of S. Claudinot, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.)

Unexpectedly, polyunsaturated fatty acids and fatty-acid derivatives that activate peroxisome-proliferator-activated receptors (PPARs) have also been found to be important regulators of re-epithelialization. Expression of PPAR-α and PPAR-β (also known as PPAR-δ) is increased in keratinocytes after skin injury[57] (Fig. 2). Upregulation of expression of the gene encoding PPAR-β and of as-yet unidentified ligands is achieved through the actions of pro-inflammatory cytokines, which in turn activate AP1 proteins through the stress-activated protein-kinase signalling cascade. This is functionally important, because mice in which the gene encoding PPAR-β was knocked out showed a significantly reduced rate of re-epithelialization, and this defect resulted from reduced migration and increased apoptosis of keratinocytes[58]. PPAR-β increases cell survival by upregulating expression of the genes encoding integrin-linked kinase and 3-phosphoinositide-dependent protein kinase 1, which in turn phosphorylate and activate the anti-apoptotic protein AKT[59].

Finally, an unexpected recent finding is that electrical signals also regulate wound re-epithelialization[60]. It is well known that disruption of an epithelial layer generates an endogenous electric field in a lateral plane. This field was found to be an important directional cue for the migration of keratinocytes in response to wounding of a monolayer *in vitro*. The receptors and signalling molecules shown to be involved include EGFR, $\alpha_6\beta_4$-integrin, phosphatidylinositol-3-OH kinase-γ and the phosphatase PTEN[43,60]. This mechanism regulates repair of the injured cornea and might have a similar role in wounded skin[43,60].

It is clear, therefore, that a variety of factors, including physical factors, can activate the intracellular signalling pathways that, ultimately, regulate the various steps of wound re-epithelialization.

Epithelial stem-cell biology

During re-epithelialization, renewal of the epidermis is required to provide keratinocytes, which then migrate and cover the healing wound. Similarly to other lining epithelia, such as those at the surface of the eye and the gut, the epidermis constantly and rapidly renews itself, a process that allows homeostasis and the maintenance of proper tissue function. This involves the proliferation of epidermal stem cells. Stem cells are defined by their capacity to self-renew for an extended period of time (like cancer stem cells) and their ability to differentiate into mature, adult cells. Stem cells can be defined as pluripotent (capable of forming all the cell types of the body, including germ cells) or multipotent (capable of forming many cell types). As is the case in haematopoiesis, multipotent epithelial stem cells can be distinguished from multipotent progenitor cells only by a combination of clonal analysis and serial long-term transplantation experiments[61]. By using this approach, it has been confirmed unambiguously that the upper permanent region of the hair follicle below the sebaceous glands (commonly referred to as the bulge or the niche) contains multipotent progenitor cells[62,63] (Fig. 3). The same experimental approach has also indisputably shown the presence of multipotent stem cells outside the bulge, confirming previous results indicating that stem cells are not exclusively located in the bulge[64].

Tissue stem cells can also broaden their capacity to form various lineages in response to physiological stimuli or injuries, a property that has great potential for regenerative medicine approaches. It has been argued that the epidermis is renewed by multipotent progenitor cells or stem cells generated in, and migrating from, the hair-follicle bulge[62]. Undoubtedly, multipotent stem cells from the bulge can contribute to epidermal repair[61,65], but this occurs only when a wound cannot spontaneously repair itself through the migration of epidermal cells from the neighbouring unwounded epidermis or from the infundibulum, the portion of the hair follicle between the epidermis and the sebaceous gland (Fig. 3). Indeed, the infundibulum — which can extend deep into the dermis, up to several hundred micrometres in human hair follicles — contains distinct epidermal and hair-follicle territories (Fig. 3). Furthermore, genetic analyses have confirmed that the epidermis is self-renewing and that it does not depend on cells generated from multipotent stem cells of the hair follicle[65,66]. Finally, lineage-tracing studies in mice have revisited the classic concept that epidermal renewal is based on a hierarchy of stem cells and transient amplifying cells[67], in support of the hypothesis that epidermal renewal relies on a single independent population of proliferative cells during normal homeostasis.

Another consideration is that the features of stem cells are still under debate, and this therefore influences experimental design and interpretation. Quiescence has been thought to be an important property of all stem cells, so retention of a label (indicating that cells are not actively dividing) has long been an indispensable criterion for the identification of epithelial stem cells[68]. However, there is now compelling evidence that stem cells can divide rapidly in some tissues but might not in others. Recently, it was shown that intestinal stem cells — as identified by expression of *Lgr5* (which encodes leucine-rich-repeat-containing G-protein-coupled receptor 5, a transmembrane protein downstream of the WNT-mediated signalling pathway) —are rapidly cycling[69]. Together with the observation that haematopoietic stem cells do not necessarily retain label[70], these findings have led some researchers to reconsider quiescence as a key feature of 'stemness'; hence, the absence of label-retaining cells, as is the case for the expression of differentiation markers, does not preclude the presence of stem cells in a tissue. Ultimately, function is the only property that can properly identify stemness. This could explain why mouse corneas can self-renew in the complete absence of limbus, the transitional zone at the junction of the conjunctiva and the cornea, which is thought to contain the corneal stem cells[71]. The identification of all cells that can function as stem cells is certain to influence future strategies for wound repair and tissue regeneration.

Adult corneal cells exposed to embryonic dermis can be induced to form hair follicles[72]. This finding, together with the observation that NOTCH1-deficient corneal epithelium forms an epidermis when wounded[71], indicates that epithelial cells have some plasticity, a property that has important clinical implications. Indeed, cultured grafts of epithelium obtained from the oral cavity have been used to reconstruct human corneas[73]. Advances in stem-cell biology have implications for improving skin therapies outside the traditional domain of wound repair. For example, the recent finding that stem cells from an individual

can be genetically engineered and then permanently engrafted in the individual for the treatment of incapacitating hereditary skin diseases is a considerable step forward[74]. It not only shows that *ex vivo* gene therapy is feasible but also brings new hope to patients with chronic wounds for which conventional wound repair therapy has failed. Despite such successes, there is much to accomplish before stem cells can be safely manipulated to reconstruct the function of the skin and other stratified epithelia fully.

In addition, the capacity to massively expand multipotent epithelial stem cells in culture, together with a better understanding of the epithelial–mesenchymal interactions that control hair-follicle morphogenesis[75,76], opens the door to the *ex vivo* reconstruction of skin appendages. In this regard, a recent report of spontaneous generation of hair follicles in the healing skin of adult mice emphasizes that WNT-mediated signalling is important for skin morphogenesis and repair[77] and provides hope that hair follicles can be newly generated. But *de novo* hair-follicle morphogenesis, which is known to occur in some species (for example, in deer during antler growth), has never been observed in humans. Of equal importance is the inductive role of dermal papilla cells in hair-follicle genesis and their relationship to the neural crest[78–80]. Dermal papilla cells can express markers of adipogenic, chondrogenic and osteogenic differentiation and can even form neurosphere-like spheres (which express markers of neuronal differentiation when cultured in the appropriate conditions). Therefore, dermal papilla cells seem to be functionally related to mesenchymal cells isolated from other tissues (for example, the adipose tissue or the bone marrow). Understanding the molecular control of epithelial and mesenchymal stem-cell fate will allow the design of new strategies for wound repair : for example, strategies to enhance the migration of stem cells, to induce the formation of epidermal appendages (such as hair follicles and sweat duct glands) and to decrease scarring[75,76,81].

Towards tissue regeneration in humans

In humans, problems with wound healing can manifest as either delayed wound healing (which occurs with diabetes or radiation exposure) or excessive healing (as occurs with hypertrophic and keloid scars). Excessive healing is characterized by the deposition of large amounts of extracellular matrix and by alterations in local vascularization and cell proliferation. These excessive fibrotic reactions manifest in humans as a 'bad scar'. Many such instances of 'overhealing' produce large disfiguring masses that can physically distort surface structures (such as the nose or eyelids). These commonly occur after major injuries such as burns, in which case they are referred to as hypertrophic scars. They can also appear for unknown reasons after a relatively minor trauma, as is the case for keloid scars, which might have a genetic basis[2]. Much interest has been generated by the observation that increased amounts of TGF-β are found in wounds that heal by scar formation as opposed to tissue regeneration as seen in bad scars[82,83]. This finding has led to clinical efforts to block scar formation with antibodies and small molecules directed against TGF-β and other pro-inflammatory mediators[84]. Recent evidence also suggests that changes in the physical environment might result in overhealing, by affecting the mechanical environment of the cells in the wound[85].

Although tremendous strides have been made in delineating the myriad factors involved in normal and pathological tissue repair, these findings have not led to substantial advances in patient care. It has become clear that single-agent therapies, such as administration of a growth factor, have only a moderate impact on wound repair in a clinical setting, most probably because of the considerable plasticity and redundancy of the components of the wound repair process or because of their rapid degradation at the wound site.

The ultimate solution to both underhealing and overhealing is likely to be administration of cells that retain the ability to elaborate the full complexity of biological signalling, together with the environmental cues that are needed to regulate the differentiation and proliferation of these cells. Previous attempts have used part of this approach (for example, the administration of cultured keratinocytes or fibroblasts), but until now it has not been possible to provide both 'seed and soil' in the same therapeutic agent. Recent advances in stem-cell and progenitor-cell biology have resulted in the isolation and characterization of skin progenitor cells in mammals. In parallel, advances in material science have made it possible to deliver extracellular or intracellular signals precisely in the appropriate temporal and spatial sequence[86]. One such approach is illustrated in Fig. 4. Adult epidermal progenitor cells (obtained from skin samples or standardized cell lines) are placed into a biomimetic matrix that reproduces the environment present during prenatal growth and development, when tissue regeneration occurs. Components of the environment include optimized mechanical stress and oxygen tension, and extracellular-matrix proteins. After grafting to the injured site, these progenitor cells can themselves provide the proper sequence of extracellular factors to accelerate tissue repair and regeneration. Using cells to integrate environmental signals and transduce them into biological effectors is likely to be crucial for new approaches to the ubiquitous problem of fibrotic wound repair. Thus, researchers and doctors stand at the dawn of a new era in which cell function can be controlled and regulated in clinical situations. ■

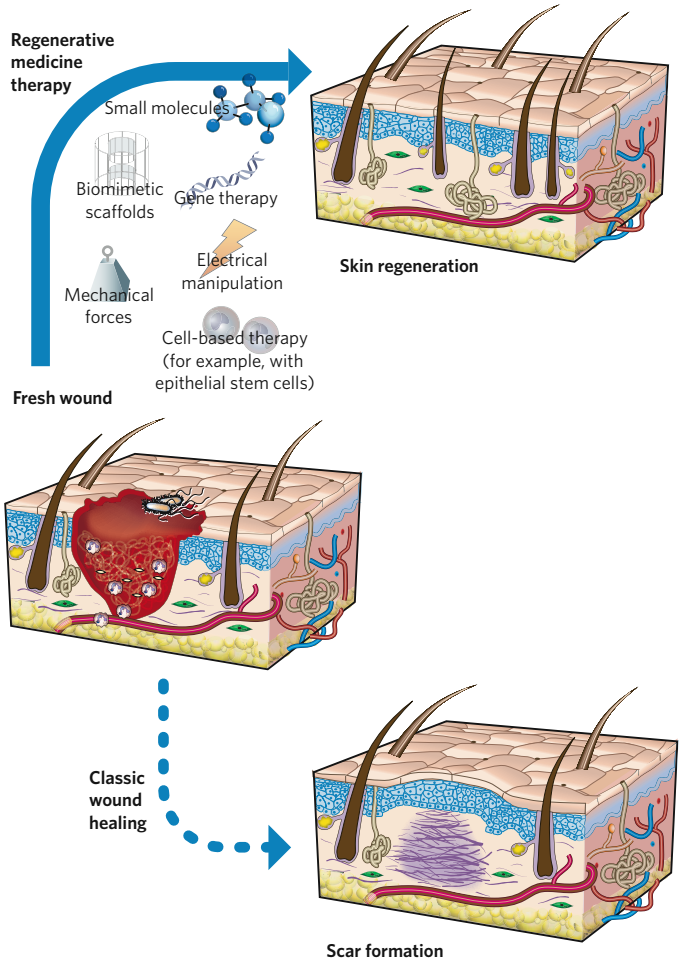

Figure 4 | Potential therapies for reducing scar formation during wound repair. To manipulate wound repair to become more regenerative than scar forming, strategies include the use of biomimetic scaffolds, the manipulation of the mechanical environment (for example, negative-pressure wound therapy to increase healing) or the electrical environment, the administration of small molecules, the use of gene-therapy approaches, and the use of cell-based strategies (including administration of epithelial stem cells). All of these elements have been demonstrated to have an effect on *in vitro* and *in vivo* models of wound healing as single-agent therapies. In theory, many of these elements could be combined to recreate a receptive environment (or 'soil') to promote regeneration. Combining these with the appropriate stem cells (or 'seed') will undoubtedly alter the result of wound healing in humans.

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Acknowledgements G.C.G. and M.T.L. are supported by grants from the National Institutes of Health and the Oak Foundation. S.W. is supported by the Swiss Federal Institute of Technology Zürich (ETHZ), the Swiss National Science Foundation (FNSNF), the European Union and Oncosuisse. Y.B. is supported by the École Polytechnique Fédérale de Lausanne (EPFL), the Centre Hospitalier Universitaire Vaudois Lausanne (CHUV) and the European Consortium for Stem Cell Research (EuroStemCell).

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