Wound Healing—Aiming for Perfect Skin Regeneration

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The healing of an adult skin wound is a complex process requiring the collaborative efforts of many different tissues and cell lineages. The behavior of each of the contributing cell types during the phases of proliferation, migration, matrix synthesis, and contraction, as well as the growth factor and matrix signals present at a wound site, are now roughly understood. Details of how these signals control wound cell activities are beginning to emerge, and studies of healing in embryos have begun to show how the normal adult repair process might be readjusted to make it less like patching up and more like regeneration.

Adult skin consists of two tissue layers: a keratinized stratified epidermis and an underlying thick layer of collagen-rich dermal connective tissue providing support and nourishment. Appendages such as hairs and glands are derived from, and linked to, the epidermis but project deep into the dermal layer. Because the skin serves as a protective barrier against the outside world, any break in it must be rapidly and efficiently mended. A temporary repair is achieved in the form of a clot that plugs the defect, and over subsequent days steps to regenerate the missing parts are initiated. Inflammatory cells and then fibroblasts and capillaries invade the clot to form a contractile granulation tissue that draws the wound margins together; meanwhile, the cut epidermal edges migrate forward to cover the denuded wound surface (*1*) (Fig. 1). Fundamental to our understanding of wound-healing biology is a knowledge of the signals that trigger relatively sedentary cell lineages at the wound margin to proliferate, to become invasive, and then to lay down new matrix in the wound gap. Studies in the last decade have provided a list of the growth factors and matrix components that are available to provide these "start" signals, and one of the tasks now begun is to relate these factors specifically to the starting and stopping of each of the many cell activities by which the wound is healed.

Most skin lesions are healed rapidly and efficiently within a week or two. However, the end product is neither aesthetically nor functionally perfect. Epidermal appendages that have been lost at the site of damage do not regenerate, and when the wound has healed there remains a connective tissue scar where the collagen matrix has been poorly reconstituted, in dense parallel bundles, unlike the mechanically efficient basket-weave meshwork of collagen in unwounded dermis. A major goal of wound-healing biology is to figure out how skin can be induced to reconstruct the damaged parts more perfectly. Clues as to how this might be achieved come from studies of wound healing in embryos, where repair is fast and efficient and results in essentially perfect regeneration of any lost tissue.

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The Fibrin Clot

Most wounds to the skin will cause leakage of blood from damaged blood vessels. The formation of a clot then serves as a temporary shield protecting the denuded wound tissues and provides a provisional matrix over and through which cells can migrate during the repair process. The clot consists of platelets embedded in a mesh of cross-linked fibrin fibers derived by thrombin cleavage of fibrinogen, together with smaller amounts of plasma fibronectin, vitronectin, and thrombospondin (*2*). Importantly, the clot also serves as a reservoir of cytokines and growth factors that are released as activated platelets degranulate. This early cocktail of growth factors (Table 1) "kick starts" the wound closure process: It provides chemotactic cues to recruit circulating inflammatory cells to the wound site, initiates the tissue movements of reepithelialization and connective tissue contraction, and stimulates the characteristic wound angiogenic response.

Recruitment of Inflammatory Cells to the Wound Site

Neutrophils and monocytes are attracted to wound sites by a huge variety of chemotactic signals. These include not only growth factors released by degranulating platelets, but also cues as diverse as formyl methionyl peptides cleaved from bacterial proteins and the by-products of proteolysis of fibrin and other matrix components (*3*). Both neutrophils and monocytes are recruited from the circulating blood in response to molecular changes in the surface of endothelial cells lining capillaries at the wound site. Initially, members of the selectin family of adhesion molecules are expressed to allow rapid but light adhesion so that leukocytes are slowed and pulled from rapid circulation in the blood; then tighter adhesions and arrest, mediated by the β2 class of integrins, lead to diapedesis, whereby the activated leukocytes crawl out between endothelial cells into the extravascular space (*4*). Transgenic mouse studies are beginning to pinpoint the crucial adhesion interactions in this process; for example, in the p-selectin knockout mouse, leukocyte rolling and extravasation are severely impaired (*5*). Neutrophils normally begin arriving at the wound site within minutes of injury; their role has long been considered to be confined to clearing the initial rush of contaminating bacteria, but recent studies have shown that neutrophils are also a source of pro-inflammatory cytokines that probably serve as some of the earliest signals to activate local fibroblasts and keratinocytes (*6*). Unless a wound is grossly infected, the neutrophil infiltration ceases after a few days, and expended neutrophils are themselves phagocytosed by tissue macrophages. Macrophages continue to accumulate at the wound site by recruitment of blood-borne monocytes and are essential for effective wound healing; if macrophage infiltration is prevented, then healing is severely impaired (*7*). Macrophage tasks include phagocytosis of any remaining pathogenic organisms and other cell and matrix debris. Once activated, macrophages also release a battery of growth factors and cytokines at the wound site (Table 1), thus amplifying the earlier wound signals released by degranulating platelets and neutrophils.

Reepithelialization

In unwounded skin, the basal keratinocyte layer attaches to a carpet of specialized matrix, the basal lamina. The keratinocyte's primary anchoring contacts are hemidesmosomes, which bind to laminin in the basal lamina by way of α6β4 integrins and have intracellular links with the keratin cytoskeletal network. The hemidesmosome attachments have to be dissolved and leading edge keratinocytes have to express new integrins, primarily the α5β1 and αvβ6 fibronectin/tenascin receptors and the αvβ5 vitronectin receptor, and relocalize α2β1 collagen receptors, in order to grasp hold of, and crawl

over, the provisional wound matrix and underlying wound dermis (8) (Fig. 2). Forward locomotion involves contraction of intracellular actinomyosin filaments that insert into the new adhesion complexes (9). This rearrangement of integrin receptors and assembly of associated actin filament networks may account for the lag of several hours before epidermal migration begins (10). It remains unclear which cells lead the keratinocytes' forward march; recent evidence from an organotypic model of wound healing in which keratinocytes were genetically labeled with retroviruses suggests that migration is not solely of basal cells, but that suprabasal cells may also "leapfrog" over basal cells (11). Other evidence that suprabasal keratinocytes at the leading edge might be capable of more than just terminal differentiation comes from their atypical expression of integrins that are restricted to the proliferating basal layer in unwounded skin (12).

If a skin wound leaves the stumps of hair follicles intact, then a large contribution to the healed epidermis derives from these hair follicle remnants. They act as normal cut epidermal wound edges and spread out like growing islands from the follicle stump. Some hours after the onset of migration, epidermal cells just back from the wound margin undergo a proliferative burst (11, 13) which, although not strictly required for the reepithelialization movement, provides a pool of extra cells to replace those lost during the injury. Indeed, the proliferative capacity of just a small patch of adult skin is immense, as exemplified by the ability of autologous grafts of cultured keratinocytes to rescue patients who have received full-thickness burn wounds covering up to 98% of their body surface (14). Recent research has clarified the location and proliferative capacity of the epidermal stem cells both in hair follicles (15) and in the basal keratinocyte layer proper (16). These data, together with a knowledge of differences in stem cell potential dependent on anatomical location, will be important in the management of wound healing and skin replacement therapies in the clinic.

Protease Expression at the Wound Margin

In order to cut a path through the fibrin clot or along the interface between clot and healthy dermis, the leading-edge keratinocytes have to dissolve the fibrin barrier ahead of them. The chief fibrinolytic enzyme is plasmin, which is derived from plasminogen within the clot itself and can be activated either by tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA). Both of these activators and the receptor for uPA are up-regulated in the migrating keratinocytes (17). In transgenic mice where the gene encoding plasminogen has been knocked out, wound reepithelialization is almost completely blocked (18). Various members of the matrix metalloproteinase (MMP) family, each of which cleaves a specific subset of matrix proteins, are also up-regulated by wound-edge keratinocytes. MMP-9 (gelatinase B) can cut basal lamina collagen (type IV) and anchoring fibril collagen (type VII), and is thought to be responsible for releasing keratinocytes from their tethers to the basal lamina (19). MMP-1 (interstitial collagenase) is up-regulated only in those basal keratinocytes that have migrated beyond the free edge of the basal lamina (20), suggesting that cell-matrix interactions may control expression of this MMP, which specifically degrades native collagens and presumably aids keratinocyte crawling by cutting collagens I and III at sites of focal adhesion attachment to the dermal substratum. MMP-10 (stromelysin-2) has a wider substrate specificity and is also up-regulated by keratinocytes at the wound margin, but its expression is increased in situations of impaired healing (21); this finding, together with the observation of high levels of proteolytic activity in chronic wound fluid, has led to the speculation that misregulated proteases may contribute to the inability of some chronic wounds to heal even when treated by application of exogenous matrix or growth factors (22).

The End Point of Reepithelialization

Once the denuded wound surface has been covered by a monolayer of keratinocytes, epidermal migration ceases and a new stratified epidermis with underlying basal lamina is reestablished from the margins of the wound inward (23). Suprabasal cells cease to express integrins and basal keratins and instead un-

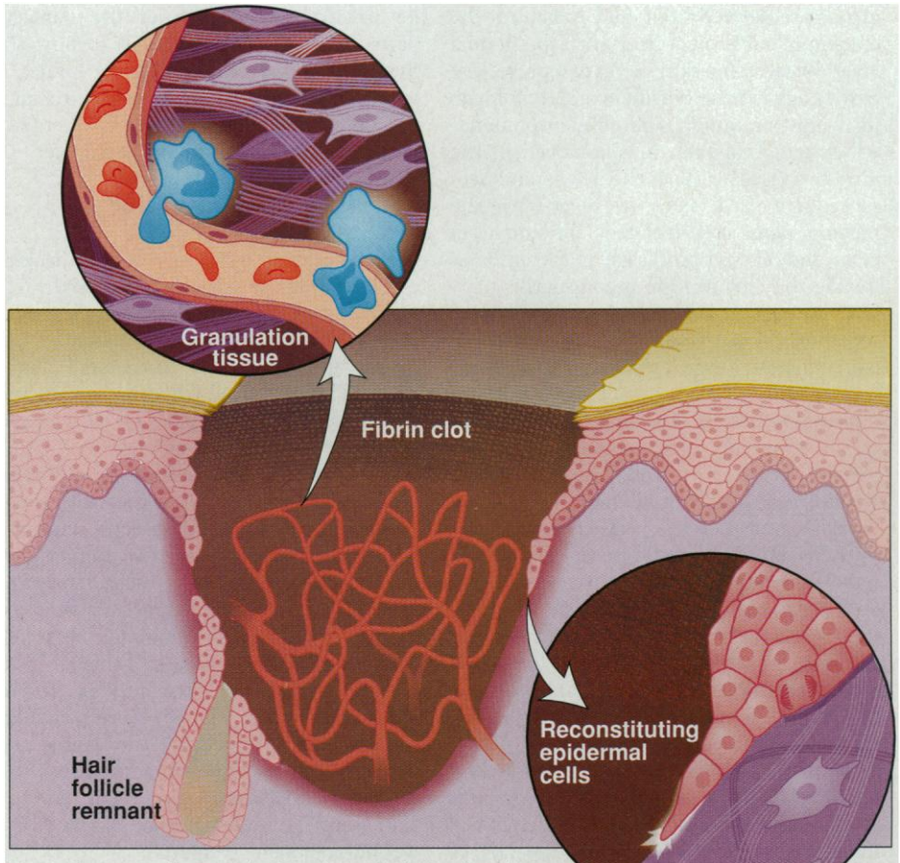

Fig. 1. Cartoon to illustrate the key players in the healing of a skin wound. The defect is temporarily plugged with a fibrin clot, which is infiltrated by inflammatory cells, fibroblasts, and a dense capillary plexus of new granulation tissue. An epidermal covering is reconstituted from the edges of the wound and from the cut remnants of hair follicles. At the migrating keratinocyte leading edge, cells bore a passageway enabling them to crawl beyond the cut basal lamina and over provisional matrix and healthy dermis. Cell division occurs back from the leading edge. Monocytes emigrate from wound capillaries into the granulation tissue, which contracts by means of smooth muscle–like myofibroblasts that tug on one another and the surrounding collagen matrix.

dergo the standard differentiation program of cells in the outer layers of unwounded epidermis. We know little about keratinocyte "stop" signals except that they probably include contact inhibition arising from mechanical cues. Coincident with the onset of basal lamina synthesis, MMP expression is shut off, and new hemidesmosomal adhesions to the basal lamina reassemble. Biopsies from healed skin established by grafting cultured keratinocytes onto naked wound beds suggest that the last components of the epidermal attachment machinery to reach maturity are the anchoring fibrils that link basal lamina to underlying connective tissue (*14*).

Growth Factors Regulating Reepithelialization

For many years the EGF family of growth factors, comprising epidermal growth factor (EGF) itself, transforming growth factor–α (TGF-α), and more recently heparin binding epidermal growth factor (HB-EGF), all acting as ligands for the EGF receptor, were considered the key regulators of keratinocyte proliferation at a wound edge. Indeed all three of these factors are released in abundance at a site of injury (Table 1). Moreover, exogenous application of EGF or TGF-α to burn wounds on the backs of pigs enhances reepithelialization (*24*). Study of keratinocyte responsiveness to EGFs in culture suggests that these growth factors act on the epidermis as motogens as well as mitogens to drive wound closure (*25*). Until recently it was not clear how extracellular signals might affect cell motility, but it is now known that some growth factors, including EGF, are able to activate the small guanosine triphosphatase (GTPase) Rac, which mediates lamellipodial extension and the assembly of focal adhesion complexes as part of the crawling response of tissue culture fibroblasts and epithelial cells (*26*).

Recently the EGFs have had to share their status as chief epidermal wound regulators with keratinocyte growth factor (KGF), or FGF7, which acts specifically on keratinocytes through a constitutively expressed splice-variant of FGFR2. KGF is upregulated more than 100-fold within 24 hours by dermal fibroblasts at the wound margin, possibly in response to pro-inflammatory cytokines (*27*). In glucocorticoid-treated mice and genetically diabetic mice with impaired healing, KGF (but not KGFR) expression is reduced, suggesting that a defect in KGF regulation might underly various wound-healing disorders (*28*). Transgenic knockout mice lacking KGF seem not to suffer impaired healing (*29*), but this may reflect genetic redundancy, because a dominant-negative mutant form of the FGFR2 expressed in the basal keratinocyte layer (making these cells unresponsive to KGF) blocks cell proliferation at the wound margin and delays reepithelialization (*30*). Exogenous KGF applied to skin wounds has mitogenic and motogenic effects on the healing epidermis (*31*) and stimulates high plasminogen activator and MMP-10 expression in the motile keratinocytes, which might speed up the rate of healing in vivo by enhancing the capacity of the epithelial edge to cut through the clot (*32*).

Other growth factors may also regulate epidermal repair. For example, TGF-β1 and some pro-inflammatory cytokines appear to stimulate expression of some of the integrin subunits that facilitate keratinocyte migration (*33*).

Reepithelialization of Embryonic Wounds

Early embryos show a remarkable capacity to rapidly reepithelialize wounds, but the basal epidermal cells do not move forward by lamellipodial crawling. Rather, they are drawn forward by contraction of an actin cable that acts like a purse-string to pull the wound edges together (*34*) (Fig. 3). Thus, embryonic epidermal cells have no need to alter their integrins and may begin moving promptly, without a lag phase. The actin cable assembles within minutes of wounding. It is not yet clear what signals regulate embryonic reepithelialization, but they are mediated by another of the small GTPases, Rho. Inactivation of Rho prevents cable formation and results in a failure of reepithelialization (*35*). It will be fascinating to discover whether adult wound keratinocytes can be induced to move by a purse-string mechanism rather than by crawling. In particular, one might expect a purse string to be an effective means of repairing small wounds, where the high curvature of the wound margin will allow a purse string to generate a strong centripetal force. Hints that this might be the case come from studies of gut epithelium in which closure of small wounds is necessarily rapid and efficient and may also use purse-string reepithelialization (*36*).

A Role for Keratins

Although the actin cytoskeleton is critical for crawling motility of adult keratinocytes and purse-string closure of embryonic epidermal wounds, it might be presumed that the keratin cytoskeletal network would supply essential cell and tissue strength during such strenuous epithelial movements. Indeed, in mice with a deletion of the gene encoding the bullous pemphigoid antigen (BPAG1), which mediates linkage between keratin filaments and the hemidesmosomal α6β4 integrins, incisional wounds are unable to reepithelialize (*37*). Some keratins may play rather more subtle roles than simply providing cell

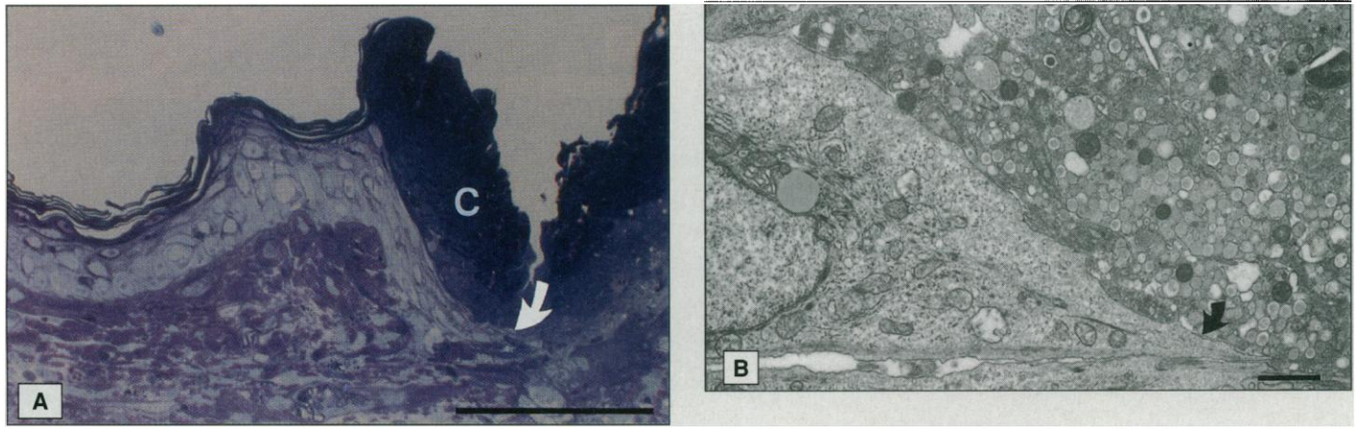

Fig. 2. Histology of adult skin repair. (**A**) Resin section through the leading front of keratinocytes (arrow) as they cut their way through a clot (C). (**B**) Transmission electron micrograph of a front row cell showing classic lamellipodial crawling morphology (arrow). Bars: 100 μm (A) and 1 μm (B). [(A) and (B) courtesy of M. Turmaine]

strength. As with integrins (*12*), keratins that are normally basally restricted appear suprabasally in keratinocytes at the wound margin. New, short-filament keratins 6, 16, and 17 are also induced and appear to help retract other cellular keratins into juxtanuclear aggregates within actively crawling cells (*38*). Keratins may be less important in the embryo. In mouse embryos lacking keratin 8 and supposedly missing all keratin filaments, reepithelialization of a wound appears to proceed exactly as in wild-type embryos, suggesting that the embryonic epidermis does not need intermediate filament support during the repair process (*39*).

Regeneration of Hair and Sweat Glands

If an adult skin wound is deeper than the level of hair bulbs in the dermis so that no remnants of hair follicles remain, the repairing epithelium does not regenerate hairs; the same is also true for sweat glands lost at the site of injury. During embryogenesis, the dermal connective-tissue fibroblasts supply permissive and instructive signals that govern the positions and types of hairs and other cutaneous appendages that will differentiate from the overlying epidermis (*40*). The timing and nature of these signals remain unclear, but some clues come from accounts of the expression of patterning genes—notably lymphoid enhancer factor–1 (LEF-1), sonic hedgehog (Shh), bone morphogenetic protein–2 (BMP-2) and FGF-4—in the developing hair and feather buds of mouse and chick embryos (*41*), and from reports that transgenic knockout mice null for various FGF and EGF family members exhibit a range of defects in hair development (*29*, *42*). Adult wound epidermis fails to regenerate hairs, not because it is unable to respond to hair-inducing signals, but because it does not receive such signals from the underlying wound dermis. Competence to make hairs has been demonstrated by seeding the wound site with inductive dermal papilla cells (*43*).

Contraction of the Wound

The job of reepithelializing a wound is made easier by the underlying contractile connective tissue, which shrinks in size to bring the wound margins toward one another. As an early response to injury, resident dermal fibroblasts in the neighborhood of the wound begin to proliferate, and then 3 or 4 days after the wound insult they begin migration into the provisional matrix of the wound clot where they lay down their own collagen-rich matrix (*44*). The premigratory lag phase appears to be largely due to the time required for fibroblasts to emerge from quiescence, because it does not occur a second time if the wound is re-wounded and a new provisional matrix laid down (*45*). Many of the growth factors present at a wound site can act either as mitogens or as chemotactic factors for wound fibroblasts, and some, notably isoforms of platelet-derived growth factor (PDGF) (*46*) and TGF-β (*47*), may do both (Table 1). The β_A and β_B isoforms of the TGF-β–related growth factor activin are induced in the proliferative fibroblasts of a wound margin and in the adjacent wound-edge keratinocytes, respectively; it is not yet clear which cells respond to these activin signals, but almost certainly there will be significant functional overlap with the TGF-β signals (*48*). Connective-tissue growth factor (CTGF), which is homologous to the product of the *Drosophila* morphogenesis gene *twisted gastrulation*, is expressed at high levels by wound fibroblasts as an immediate-early gene response to TGF-β1. In *Drosophila*, *twisted gastrulation* may lie genetically downstream of *decapentaplegic*, a TGF-β family member, suggesting that some of the signaling cascades of *Drosophila* embryogenesis have been conserved and are reused during vertebrate

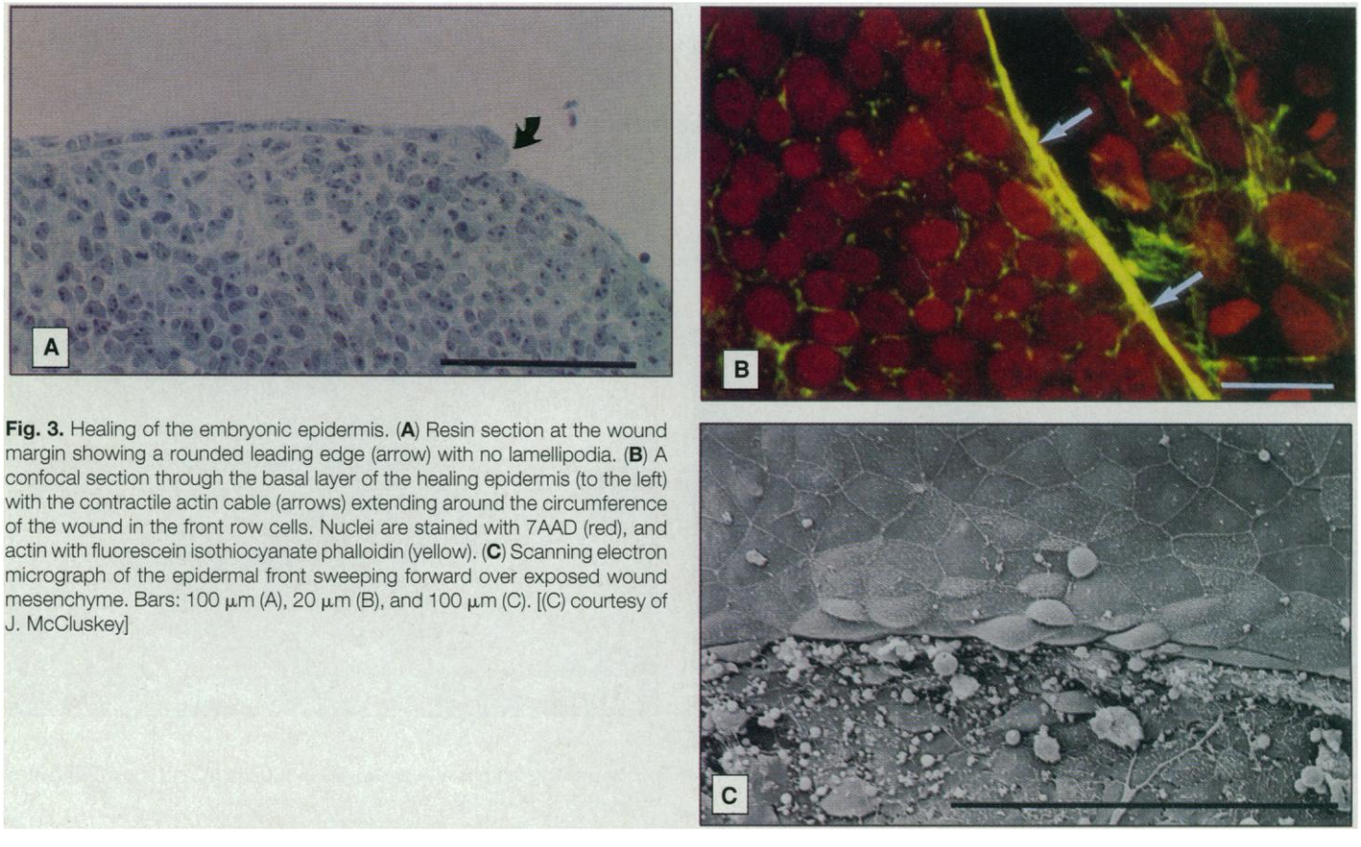

Fig. 3. Healing of the embryonic epidermis. (**A**) Resin section at the wound margin showing a rounded leading edge (arrow) with no lamellipodia. (**B**) A confocal section through the basal layer of the healing epidermis (to the left) with the contractile actin cable (arrows) extending around the circumference of the wound in the front row cells. Nuclei are stained with 7AAD (red), and actin with fluorescein isothiocyanate phalloidin (yellow). (**C**) Scanning electron micrograph of the epidermal front sweeping forward over exposed wound mesenchyme. Bars: 100 μm (A), 20 μm (B), and 100 μm (C). [(C) courtesy of J. McCluskey]

tissue repair programs (*49*).

Just as wound-edge keratinocytes have to adjust their integrin profile before migration, dermal fibroblasts, which normally lie in a collagen-I–rich matrix, must down-regulate their collagen receptors and up-regulate integrins that bind fibrin, fibronectin, and vitronectin in order to crawl into the clot. Fibroblasts read and act according to dual signals from their matrix surroundings and from the growth factor milieu in which they are bathed. If fibroblasts are cultured in a fibrin-fibronectin gel, then exposure to PDGF will trigger up-regulation of the provisional-matrix integrin subunits, α3 and α5, whereas in a collagen gel the same growth factor signal instead supports expression of collagen-specific α2 subunits and not provisional-matrix receptors (*50*). Fibroblasts may use a fibronectin conduit to lead them into the fibrin clot (*51*), and in this regard it is interesting to note that the predominant splice-variant of fibronectin expressed by fibroblasts and macrophages at the wound interface is a form otherwise unique to sites of embryonic-cell migrations (*52*), suggesting that this fibronectin is an exceptionally good substratum for cell migration. Little is known about wound-triggered regulation of the actinomyosin cytoskeleton, which must be crucial in fibroblast migration, but almost certainly of relevance is the observation that fibroblasts from transgenic knockout mice lacking the actin severing and capping protein gelsolin have impaired migratory response in culture (*53*). Because PDGF activates the small GTPase Rac in fibroblasts (*26*), and gelsolin is a downstream effector of Rac (*54*), it seems likely that Rac may be one of the key molecular switches responsible for the onset of fibroblast migration into a wound.

By about a week after wounding, the wound clot will have been fully invaded and all but replaced by activated fibroblasts that are stimulated by TGF-β1 and other growth factors to synthesize and remodel a new collagen-rich matrix (*44*); at this stage, a proportion of the wound fibroblasts transform into myofibroblasts, which express α-smooth muscle actin and resemble smooth muscle cells in their capacity for generating strong contractile forces (*55*). This conversion is triggered by growth factors such as TGF-β1 (*56*) and mechanical cues related to the forces resisting contraction (*57*).

The various tensile forces acting on and exerted by wound fibroblasts before, during, and after contraction have been studied in collagen-gel model systems. For example, a number of growth factors at the wound site are potent stimulators of fibroblast-driven gel contraction and presumably signal granulation tissue contraction in vivo (*58*). Potential "stop" signals for wound contraction are being analyzed by releasing mechanically stressed anchored gels from their substrate attatchments to simulate the loss of resistance after a wound has closed. Within minutes of release from resisting forces, fibroblasts activate an adenosine 3′,5′-monophosphate (cAMP) signal transduction pathway, which involves influx of extracellular Ca^{2+} ions and production of phosphatidic acid by phospholipase D (*59*). Subsequently, PDGF and EGF receptors on the cell surface become desensitized (*60*) and the relaxed cells return to a quiescent state similar to that existing before the injury. Programmed cell death occurs in some of the wound fibroblasts, probably the myofibroblasts, after wound contraction has ceased (*61*).

Wound Angiogenesis and the Neural Response

The wound connective tissue is known as granulation tissue because of the pink granular appearance of numerous capillaries that invade the wound neodermis. FGF2 and vascular endothelial growth factor (VEGF) released at the wound site promote angiogenesis. FGF2, or basic FGF, is released at the wound site by damaged endothelial cells and by macrophages (*62*); when this growth factor is experimentally depleted with monospecific antibodies raised against FGF2, wound angiogenesis is almost completely blocked (*63*). VEGF, also called vascular permeability factor, is induced in wound-edge keratinocytes and macrophages, possibly in response to KGF and TGF-α, and synchronously at least one of its receptors, flt-1, is up-regulated by endothelial cells at the site of injury (*64*). Evidence that VEGF may promote healing comes from a study of genetically diabetic mice, in which VEGF expression fails at the wound site and healing is impaired (*65*).

Endothelial cells must up-regulate $\alpha_v\beta_3$ integrins if they are to respond to any wound angiogenic signal. $\alpha_v\beta_3$ is expressed transiently at the tips of sprouting capillaries in the granulation tissue, and the presence of blocking peptides or antibodies against this integrin causes angiogenesis to fail and results in severely impaired wound healing (*66*). Just as with all other cell migrations at the wound site, capillary morphogenesis is also dependent on tightly regulated proteolysis of the matrix surrounds during the invasion phase (*67*).

As the embryo develops, its skin becomes densely innervated by a plexus of sensory and sympathetic nerves serving the blood vessels and cutaneous appendages as well as supplying sensation. The sensory nerve termini are exquisitely sensitive to signals released after injury, resulting in transient nerve sprouting at the site of an adult skin lesion and more dramatic, permanent hyperinnervation after wounding of neonatal skin (*68*). The wound-induced signal controlling this nerve overgrowth may be nerve growth factor (NGF) (*69*), and because NGF is up-regulated after exposure to any of the TGF-β isoforms (*70*), it is tempting to consider nerves as another in-

Table 1. Growth factor signals at the wound site.

Growth factor	Source	Primary target cells and effect	Refs.
EGF	Platelets	Keratinocyte motogen and mitogen	(*88*)
TGF-α	Macrophages; keratinocytes	Keratinocyte motogen and mitogen	(*88, 89*)
HB-EGF	Macrophages	Keratinocyte and fibroblast mitogen	(*90*)
FGFs 1, 2, and 4	Macrophages and damaged endothelial cells	Angiogenic and fibroblast mitogen	(*27, 62*)
FGF7 (KGF)	Dermal fibroblasts	Keratinocyte motogen and mitogen	(*27, 62*)
PDGF	Platelets; macrophages; keratinocytes	Chemotactic for macrophages, fibroblasts; macrophage activation, fibroblast mitogen, and matrix production	(*46*)
IGF-1	Plasma; platelets	Endothelial cell and fibroblast mitogen	(*89, 91*)
VEGF	Keratinocytes; macrophages	Angiogenesis	(*64*)
TGF-β1 and -β2	Platelets; macrophages	Keratinocyte migration; chemotactic for macrophages and fibroblasts; fibroblast matrix synthesis and remodeling	(*47*)
TGF-β3	Macrophages	Antiscarring	(*47, 82*)
CTGF	Fibroblasts; endothelia	Fibroblasts; downstream of TGF-β1	(*49*)
Activin	Fibroblasts; keratinocytes	Currently unknown	(*48*)
IL-1α and -β	Neutrophils	Early activators of growth factor expression in macrophages, keratinocytes, and fibroblasts	(*6*)
TNF-α	Neutrophils	Similar to the IL-1s	(*6*)

direct target for TGF-β at the wound site. Given the importance of nerves in regeneration of limbs in urodele amphibians (*71*), it is interesting to wonder whether sprouting nerves may play some stimulatory role in the healing process by delivering neuropeptides and other factors to the wound site (*72*). Indeed, sparsely innervated regions of the body tend to heal poorly, and transgenic mice lacking the low-affinity NGF receptor p75 suffer from impaired wound healing (*73*).

TGF-β and Scarring

Connective-tissue contraction closes embryonic as well as adult wounds; but in embryos there is no apparent conversion from fibroblast to myofibroblast (*34*, *74*), and neither is there a significant angiogenic response. Amazingly, until late fetal stages there is generally no sign of a connective-tissue scar where the wound has healed: The repair is perfect. Numerous studies have compared embryonic and adult healing in a search for molecular differences that could explain why this is so (*75*). Trivial explanations such as differences in exposure to bacterial infection or to the dryness of the atmosphere are ruled out by grafting adult skin to a fetal environment, where it still heals with a scar (*76*), and by observations of healing in marsupials, which are born at developmental stages equivalent to young amniote fetuses and heal wounds without a scar for the first few days of their postnatal period (*77*). There is a strong correlation between the the age of onset of scarring and the first stage in development when a noticeable inflammatory response is raised after wounding (*78*); TGF-β1 may again provide the link. In the embryo, TGF-β1 is expressed transiently and at low levels after injury (*79*), but at the adult wound site it is present at high levels for the duration of healing and beyond (*47*). TGF-β1 is implicated in pathogenic fibrotic conditions in kidney, liver, and lung disease (*80*), and now in scarring of skin wounds as well. Delivery of antibodies that neutralize TGF-β1 and -β2 at the time of wounding reduces scarring (*81*), as does exogenous application of TGF-β3, which down-regulates the other two TGF-β isoforms (*82*), suggesting that a balance among the TGF-β isoforms may be critical. A recent understanding of TGF-β activation, in particular the permissive involvement of the mannose-6-phosphate (M-6-P)–IGFII receptor, has suggested further ways to block this signal: M-6-P directly applied to wounds will also prevent scarring (*83*).

Future Prospects

Our understanding of wound-healing mechanisms has progressed considerably in recent years. What remaining questions are tractable in the foreseeable future, and what more do we need to know in order to help clinicians deal with problems of skin healing?

Part of the difficulty in unraveling tissue repair mechanisms is a consequence of redundancy and cross-talk in the system: Most wound signals probably control more than one cell activity, and most cell activities are responses to cocktails of signals. The redundancy of the multiple signals is becoming more apparent through study of transgenic mice. Although only a trickle of knockout mice have been wounded so far, there have been some surprisingly normal healing phenotypes reported (Table 2). Other candidate wound-healing genes turn out to be so important in normal development that a full gene knockout is lethal to the embryo. Nonetheless, interbreeding of knockout mice and the careful design of transgenic mice with gene knockouts or dominant-negative receptor constructs targeted to particular skin cell types will provide a wealth of further insight.

We know little about how the various wound signals are translated and transduced into changes in cell activity. Transcription factors such as c-*fos* and *Egr*-1 are induced after wounding in embryos and in tissue culture monolayers (*84*), but little is known about their roles in adult healing. The same applies to the small GTPase molecular switches Rho, Rac, and Cdc42, which regulate actin reorganization in tissue culture cells (*26*) and may also govern cell motility during wound closure.

It is almost certain that growth factor and matrix signals are not the only relevant influences. Changes of gap-junctional connections between keratinocytes at the healing margin (*85*) may help to coordinate cell proliferative and migratory activities at the wound edge. Mechanical signals in the form of cell stretching and even ripping of the plasma membrane at the time of wounding may prove to be important activators of the wound response. Mechanical stresses at the wound site may also play a role in guiding collagen fibrillogenesis because altered tensions during wound closure affect the extent of scarring (*86*).

A differential display study designed to find novel genes induced in keratinocytes after KGF exposure (*87*) identified a glutathione peroxidase that does indeed become up-regulated soon after skin injury. In hindsight, it makes sense that cells should synthesize such enzymes to protect themselves from oxidative damage at the wound site; nonetheless, the result was a surprise. Innovative studies of this sort will certainly come up with more surprises, offering new potential targets for therapeutic intervention.

The next few years in wound-healing research will be exciting as we test whether we can improve on nature and induce adult wounds to heal like embryonic wounds—without delay, without scarring, and with full regeneration of hairs and glands.

Table 2. Wounding of transgenic knockout mice. A full and regularly updated compendium of wounding studies in knockout mice will be available to *Science* Online subscribers at http://sciencemag.org. Please e-mail details of your study, published or not, to p.martin@ucl.ac.uk.

Gene knockout	Healing phenotype	Refs.
Plasminogen	Reepithelialization blocked	(*18*)
Tenascin	Skin healing normal	(*92*)
KGF	Skin healing normal	(*29*)
TGF-α	Skin healing normal	(*29*)
Gelsolin	Fibroblast migration hindered in vitro	(*53*)
BPAG1	Reepithelialization fails	(*37*)
Keratin 8	Embryonic healing normal	(*39*)

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Amphibian Limb Regeneration: Rebuilding a Complex Structure

Jeremy P. Brockes

The ability to regenerate complex structures is widespread in metazoan phylogeny, but among vertebrates the urodele amphibians are exceptional. Adult urodeles can regenerate their limbs by local formation of a mesenchymal growth zone or blastema. The generation of blastemal cells depends not only on the local extracellular environment after amputation or wounding but also on the ability to reenter the cell cycle from the differentiated state. The blastema replaces structures appropriate to its proximodistal position. Axial identity is probably encoded as a graded property that controls cellular growth and movement through local cell interactions. The molecular basis is not understood, but proximodistal identity in newt blastemal cells may be respecified by signaling through a retinoic acid receptor isoform. The possibility of inducing a blastema on a mammalian limb cannot be discounted, although the molecular constraints are becoming clearer as we understand more about the mechanisms of urodele regeneration.

Many larval and adult animals are able to regenerate large sections of their body plan after transection or amputation (*1*), and this usually restores the structures that were removed by the operation. In some invertebrates this occurs in a bidirectional fashion (Fig. 1). Thus, if a planarian worm is transected, the head fragment regenerates tail structures, whereas the tail fragment grows a new head. The importance of ani-

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