Mechanisms of Disease

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Cutaneous Wound Healing

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The primary function of the skin is to serve as a protective barrier against the environment. Loss of the integrity of large portions of the skin as a result of injury or illness may lead to major disability or even death. Every year in the United States more than 1.25 million people have burns and 6.5 million have chronic skin ulcers caused by pressure, venous stasis, or diabetes mellitus.

The primary goals of the treatment of wounds are rapid wound closure and a functional and aesthetically satisfactory scar. Recent advances in cellular and molecular biology have greatly expanded our understanding of the biologic processes involved in wound repair and tissue regeneration and have led to improvements in wound care. We review here the biology of the healing of traumatic and nontraumatic wounds and discuss the use of skin substitutes and growth factors to promote wound healing.

Biology of Wound Healing

Wound healing is a dynamic, interactive process involving soluble mediators, blood vessels, extracellular matrix, and parenchymal cells (Fig. 1 and 2). Wound healing has three phases — inflammation, tissue formation, and tissue remodeling — that overlap in time.

Inflammation

Tissue injury causes the disruption of blood vessels and extravasation of blood constituents. The blood clot reestablishes hemostasis and provides a provisional extracellular matrix for cell migration (Fig. 1). Platelets not only facilitate the formation of a hemostatic plug but also secrete several mediators of wound healing, such as platelet-derived growth factor, that attract and activate macrophages and fibroblasts (Table 1). However, in the absence of hemorrhage, platelets are not essential to wound healing. Numerous vasoactive mediators and chemotactic factors are generated by the coagulation and activated-complement pathways and by injured or activated parenchymal cells. These substances recruit inflammatory leukocytes to the site of injury.

Infiltrating neutrophils cleanse the wounded area of foreign particles and bacteria and are then extruded with the eschar or phagocytosed by macrophages. In response to specific chemoattractants, such as fragments of extracellular-matrix protein, transforming growth factor β, and monocyte chemoattractant protein 1, monocytes also infiltrate the wound site and become activated macrophages that release growth factors such as platelet-derived growth factor and vascular endothelial growth factor, which initiate the formation of granulation tissue. Macrophages bind to specific proteins of the extracellular matrix by their integrin receptors, an action that stimulates phagocytosis of microorganisms and fragments of extracellular matrix by the macrophages.

Adherence to the extracellular matrix also stimulates monocytes to undergo metamorphosis into inflammatory or reparative macrophages. Adherence induces monocytes and macrophages to express colony-stimulating factor 1, a cytokine necessary for the survival of monocytes and macrophages; tumor necrosis factor α, a potent inflammatory cytokine; and platelet-derived growth factor, a potent chemoattractant and mitogen for fibroblasts (Table 1). Other important cytokines expressed by monocytes and macrophages are transforming growth factor α, interleukin-1, transforming growth factor β, and insulin-like growth factor I. The monocyte- and macrophage-derived growth factors are almost certainly necessary for the initiation and propagation of new tissue formation in wounds, because macrophage-depleted animals have defective wound repair. Thus, macrophages appear to have a pivotal role in the transition between inflammation and repair.

Epithelialization

Reepithelialization of wounds begins within hours after injury (Fig. 3). Epidermal cells from skin appendages such as hair follicles quickly remove clotted blood and damaged stroma from the wound space (Fig. 2). At the same time, the cells undergo marked phenotypic alteration that includes retraction of intracellular tonofilaments, dissolution of most inter-
cellular desmosomes, which provide physical connections between the cells; and formation of peripheral cytoplasmic actin filaments, which allow cell movement. Furthermore, epidermal and dermal cells no longer adhere to one another, because of the dissolution of hemidesmosomal links between the epidermis and the basement membrane, which allows the lateral movement of epidermal cells. The expression of integrin receptors on epidermal cells allows them to interact with a variety of extracellular-matrix proteins (e.g., fibronectin and vitronectin) that are interspersed with stromal type I collagen at the margin of the wound and interwoven with the fibrin clot in the wound space. The migrating epidermal cells dissect the wound, separating desiccated eschar from viable tissue. The path of dissection appears to be determined by the array of integrins that the migrating epidermal cells express on their cell membranes.

The degradation of the extracellular matrix, which is required if the epidermal cells are to migrate between the collagenous dermis and the fibrin eschar, depends on the production of collagenase by epidermal cells, as well as the activation of plasminogen by plasminogen activator produced by the epidermal cells. Plasminogen activator also activates collagenase (matrix metalloproteinase 1) and therefore facilitates the degradation of collagen and extracellular-matrix proteins.

One to two days after injury, epidermal cells at the wound margin begin to proliferate behind the actively migrating cells (Fig. 3). The stimuli for the migration and proliferation of epidermal cells during reepithelialization have not been determined, but several possibilities exist. The absence of neighbor cells at the margin of the wound (the “free edge” effect) may signal both migration and proliferation of epidermal cells. Local release of growth factors and increased expression of growth-factor receptors may also stimulate these processes. Leading contenders include epidermal growth factor, transforming growth factor α, and keratinocyte growth factor. As reepi-
Reepithelialization ensues, basement-membrane proteins reappear in a very ordered sequence from the margin of the wound inward, in a zipperlike fashion. Epidermal cells revert to their normal phenotype, once again firmly attaching to the reestablished basement membrane and underlying dermis.

Formation of Granulation Tissue

New stroma, often called granulation tissue, begins to invade the wound space approximately four days after injury. Numerous new capillaries endow the new stroma with its granular appearance. Macrophages, fibroblasts, and blood vessels move into the wound space at the same time. The macrophages provide a continuing source of growth factors necessary to stimulate fibroplasia and angiogenesis; the fibroblasts produce the new extracellular matrix necessary to support cell ingrowth; and blood vessels carry oxygen and nutrients necessary to sustain cell metabolism.

Growth factors, especially platelet-derived growth factor and transforming growth factor β1, in concert with the extracellular-matrix molecules, presumably stimulate fibroblasts of the tissue around the wound to proliferate, express appropriate integrin receptors, and migrate into the wound space. Indeed, platelet-derived growth factor accelerates the healing of chronic pressure sores and diabetic ulcers, and basic fibroblast growth factor has been used with some success to treat chronic pressure sores.

The structural molecules of newly formed extracellular matrix, termed the provisional matrix, contribute to the formation of granulation tissue by providing a scaffold or conduit for cell migration. These molecules include fibrin, fibronectin, and hyaluronic acid. In fact, the appearance of fibronectin and the appropriate integrin receptors that bind fibronectin, fibrin, or both on fibroblasts appears to be the rate-limiting step in the formation of granulation tissue. The fibroblasts are responsible for the synthesis, deposition, and remodeling of the extracellular matrix. Conversely, the extracellular matrix can have a positive or negative effect on the ability of fibroblasts to synthesize, deposit, remodel, and generally interact with the extracellular matrix.
Cell movement into a blood clot of cross-linked fibrin or into tightly woven extracellular matrix may require an active proteolytic system that can cleave a path for cell migration. A variety of fibroblast-derived enzymes, in addition to serum-derived plasmin, are potential candidates for this task, including plasminogen activator, collagenases, gelatinase A, and stromelysin.\(^{17,33}\)

After migrating into wounds, fibroblasts commence the synthesis of extracellular matrix.\(^{32,34}\) The provisional extracellular matrix is gradually replaced with a collagenous matrix,\(^{32,34}\) perhaps as a result of the action of transforming growth factor \(\beta1.\)^{32}

Once an abundant collagen matrix has been deposited in the wound, the fibroblasts stop producing collagen, and the fibroblast-rich granulation tissue is replaced by a relatively acellular scar. Cells in the wound undergo apoptosis\(^{35}\) triggered by unknown signals. Dysregulation of these processes occurs in fibrotic disorders such as keloid formation, morphea, and scleroderma.

**Neovascularization**

The formation of new blood vessels is necessary to sustain the newly formed granulation tissue. Angiogenesis is a complex process that relies on extracellular matrix in the wound bed as well as migration and mitogenic stimulation of endothelial cells.\(^{36}\)

The induction of angiogenesis was initially attributed to acidic or basic fibroblast growth factor. Subsequently, many other molecules have also been found to have angiogenic activity, including vascular endothelial growth factor, transforming growth factor \(\beta\), angioatin, angiotropin, angiopoietin 1, and thrombospondin, to name but a few.\(^{37,38}\)

Low oxygen tension\(^{40}\) and elevated lactic acid may also stimulate angiogenesis. Many of the molecules mentioned above appear to induce angiogenesis by stimulating the production of basic fibroblast growth factor and vascular endothelial growth factor by macrophages and endothelial cells. Activated epidermal cells of the wound secrete large quantities of vascular endothelial-cell growth factor.\(^{41}\) Basic fibroblast growth factor may set the stage for angiogenesis during the first three days of wound repair, whereas vascular endothelial-cell growth factor is critical for angiogenesis during the formation of granulation tissue on days 4 through 7.\(^{42}\)

In addition to angiogenesis factors, appropriate extracellular matrix and endothelial receptors for the provisional matrix are necessary for angiogenesis. Proliferating microvascular endothelial cells adjacent to and within wounds transiently deposit increased amounts of fibronectin within the vessel wall.\(^{43}\) Since angiogenesis appears to require the expression of functional fibronectin receptors by endothelial cells,\(^{44}\) the perivascular fibronectin may act as a conduit for the movement of endothelial cells into the wound. Protease expression and activity are also necessary for angiogenesis.\(^{45}\)

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**Table 1. Cytokines That Affect Wound Healing.**

<table>
<thead>
<tr>
<th>CYTOKINE</th>
<th>MAJOR SOURCE</th>
<th>TARGET CELLS AND MAJOR EFFECTS</th>
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<tr>
<td>Epidermal growth factor family</td>
<td>Platelets</td>
<td>Epidermal and mesenchymal regeneration</td>
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<td>Epidermal growth factor</td>
<td></td>
<td>Pleiotropic-cell motility and proliferation</td>
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<tr>
<td>Transforming growth factor (\alpha)</td>
<td>Macrophages, epidermal cells</td>
<td>Pleiotropic-cell motility and proliferation</td>
</tr>
<tr>
<td>Heparin-binding epidermal</td>
<td>Macrophages</td>
<td>Pleiotropic-cell motility and proliferation</td>
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<td>growth factor</td>
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<tr>
<td>Fibroblast growth factor family</td>
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<td>Basic fibroblast growth factor</td>
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<td>Acidic fibroblast growth factor</td>
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<td>Keratinocyte growth factor</td>
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<td>Transforming growth factor (\beta) and (\beta2)</td>
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<tr>
<td>Transforming growth factor (\beta3)</td>
<td>Macrophages</td>
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</tr>
<tr>
<td>Other</td>
<td>Platelet-derived growth factor</td>
<td>Fibroblast proliferation and chemotaxis, macrophage chemotaxis and activation</td>
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<tr>
<td>Vascular endothelial growth factor</td>
<td>Epidermal cells, macrophages</td>
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<td>Tumor necrosis factor (\alpha)</td>
<td>Neutrophils</td>
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<tr>
<td>Interleukin-1</td>
<td>Neutrophils</td>
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<td>Insulin-like growth factor 1</td>
<td>Fibroblasts, epidermal cells</td>
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<td>Colony-stimulating factor 1</td>
<td>Multiple cells</td>
<td>Macrophage activation and granulation-tissue formation</td>
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The series of events leading to angiogenesis may be as follows (Fig. 2). Injury causes destruction of tissue and hypoxia. Angiogenesis factors such as acidic and basic fibroblast growth factor are immediately released from macrophages after cell disruption, and the production of vascular endothelial-cell growth factor by epidermal cells is stimulated by hypoxia. Proteolytic enzymes released into the connective tissue degrade extracellular-matrix proteins. Fragments of these proteins recruit peripheral-blood monocytes to the site of injury, where they become activated macrophages and release angiogenesis factors. Certain macrophage angiogenesis factors, such as basic fibroblast growth factor, stimulate endothelial cells to release plasminogen activator and procollagenase. Plasminogen activator converts plasminogen to plasmin and procollagenase to active collagenase, and in concert these two proteases digest basement membranes. The fragmentation of the basement membrane allows endothelial cells stimulated by angiogenesis factors to migrate and form new blood vessels at the injured site (Fig. 4). Once the wound is filled with new granulation tissue, angiogenesis ceases and many of the new blood vessels disintegrate as a result of apoptosis. This programmed cell death probably is regulated by a variety of matrix molecules, such as thrombospondins 1 and 2, and antiangiogenesis factors, such as angiostatin, endostatin, and angiopoietin 2.

**Wound Contraction and Extracellular-Matrix Reorganization**

Wound contraction involves a complex and superbly orchestrated interaction of cells, extracellular matrix, and cytokines. During the second week of healing, fibroblasts assume a myofibroblast phenotype characterized by large bundles of actin-containing microfilaments disposed along the cytoplasmic face of the plasma membrane of the cells and by cell–cell and cell–matrix linkages. The appearance of the myofibroblasts corresponds to the commencement of connective-tissue compaction and the contraction of the wound. The contraction probably requires stimulation by transforming growth factor β1 or β2 and platelet-derived growth factor, attachment of fibroblasts to the collagen matrix through integrin receptors, and cross-links between individual bundles of collagen.

Collagen remodeling during the transition from granulation tissue to scar is dependent on continued synthesis and catabolism of collagen at a low rate. The degradation of collagen in the wound is controlled by several proteolytic enzymes termed matrix metalloproteinases, which are secreted by mac-
rophones, epidermal cells, and endothelial cells, as well as fibroblasts.\textsuperscript{17} The various phases of wound repair rely on distinct combinations of matrix metalloproteinases and tissue inhibitors of metalloproteinases.\textsuperscript{54}

Wounds gain only about 20 percent of their final strength in the first three weeks, during which time fibrillar collagen has accumulated relatively rapidly and has been remodeled by contraction of the wound. Thereafter the rate at which wounds gain tensile strength is slow, reflecting a much slower rate of accumulation of collagen and, more important, collagen remodeling with the formation of larger collagen bundles and an increase in the number of intermolecular cross-links.\textsuperscript{55} Nevertheless, wounds never attain the same breaking strength (the tension at which skin breaks) as uninjured skin. At maximal strength, a scar is only 70 percent as strong as normal skin.\textsuperscript{56}

**ABNORMAL WOUND HEALING**

Although a detailed discussion of the many conditions associated with abnormal wound healing is beyond the scope of this review, several examples will illustrate the multifactorial nature of these conditions. Diabetic ulcers are an excellent example of how multiple physiologic and biochemical defects can lead to impaired healing. They usually occur in patients who are unable to sense and relieve cutaneous pressure because of neuropathy. Ischemia secondary to vascular disease impedes healing by reducing the supply of oxygen and other nutrients. Diabetic ulcers are also prone to infection because of impaired granulocytic function and chemotaxis.\textsuperscript{57} Other abnormalities associated with diabetic ulcers include prolonged inflammation, impaired neovascularization, decreased synthesis of collagen, increased levels of proteinases, and defective macrophage function.\textsuperscript{58,59}

Keloids and hypertrophic scars that are characterized by excess accumulation of collagen within the wound are examples of fibroproliferative disorders. In these conditions, abnormalities in cell migration and proliferation, inflammation, synthesis and secretion of extracellular-matrix proteins and cytokines, and remodeling of the wound matrix have all been described.\textsuperscript{60} Increased activity of fibrogenic cytokines (e.g., transforming growth factor \(\beta_1\), insulin-like growth factor 1, and interleukin-1) and exaggerated responses to these cytokines have also been noted.\textsuperscript{51,62} In addition, abnormal epidermal–mesenchymal interactions and mutations in regulatory genes (such as \(p53\)) have recently been proposed to help explain abnormal healing.\textsuperscript{63,64}

**CLINICAL EXPERIENCE WITH GROWTH FACTORS**

The overall clinical experience with growth factors and other mediators to accelerate wound healing has been discouraging. This is not surprising, considering that wound repair is the result of a complex set of interactions among soluble cytokines, formed blood elements, extracellular matrix, and cells (Table 1). It is possible that combinations of various growth factors given at precisely timed intervals would be more effective in promoting healing. Indeed, synergistic effects on wound repair have been demonstrated for several growth-factor combinations.\textsuperscript{65} Among these factors, only recombinant platelet-derived growth factor has been approved by the Food and Drug Administration (FDA) for the treatment of wounds.

**INSIGHTS FROM FETAL WOUND HEALING**

Fetal wounds recapitulate rapidly. Unlike adult epidermal cells, which resurface the wound by “crawling” across it, embryonic epidermal cells are pulled forward by the contraction of actin fibers that draw the wound edges together as the opening of a purse is closed by a purse string.\textsuperscript{66} Fetal wounds also heal without scarring. One reason for this may be the small amount of transforming growth factor \(\beta_1\), a scar-promoting cytokine, in fetal skin. The addition of transforming growth factor \(\beta_1\) to fetal wounds results in scarring.\textsuperscript{67} Furthermore, fetal skin is rich in metalloproteinases that may promote scarless healing.\textsuperscript{68} Scarring is reduced in adult rats given neutralizing antibodies to transforming growth factors \(\beta_1\) and \(\beta_2\) and those given transforming growth factor \(\beta_3\), which down-regulates the other transforming growth factor \(\beta\) isoforms.\textsuperscript{69} This result supports the central role of transforming growth factor \(\beta_1\) in scar formation.

**SKIN SUBSTITUTES**

Immediate wound coverage is one of the cornerstones of wound management. Acute or chronic wounds can usually be covered by any of a number of synthetic and natural dressings. For more extensive or recalcitrant wounds, a variety of skin substitutes are available (Table 2). These are of three types. The first type consists of grafts of cultured epidermal cells with no dermal components. The second type has only dermal components. The third type is a bilayer containing both dermal and epidermal elements.

Most skin substitutes do not survive indefinitely. Their chief effect is to promote wound healing by stimulating the host to produce a variety of cytokines. These cytokines promote healing by stimulating the production of components of the basement membrane, preventing dehydration, increasing inflammation, and increasing the formation of granulation tissue. Skin substitutes are attractive alternatives to autografts, especially since they do not require painful and invasive procedures and may be used in outpatients.

**Epidermal Skin Substitutes**

In 1975 wound management was revolutionized by the development of a technique that used cul-
tured human epidermal cells to form sheets suitable for grafting. This development was soon followed by the use of autologous cultured epidermal-cell grafts for the treatment of burns as well as other acute and chronic wounds, and junctional epidermolysis bullosa. Autologous grafts provide permanent coverage for large areas with reasonable cosmetic results. The disadvantages of these grafts include the two to three weeks needed to grow enough epidermal cells, the need for skin biopsies to obtain autologous donor cells, and the high cost. Furthermore, success is quite variable, being dependent on the status of the wound, the patient’s overall health, and the experience of the physician.

Allografts of cultured epidermal cells were developed to avoid the long time required to produce autologous grafts. In 1983 burns were successfully treated with grafts of cultured epidermal cells from cadavers. Since then there have been many reports of the use of cultured epidermal cells from cadavers and unrelated adult donors for the treatment of burns, skin-graft donor sites, and chronic leg ulcers. The use of cultured allogeneic epidermal cells has resulted in wound healing without any evidence of rejection, probably because cultured epidermal cells do not express major histocompatibility complex class II HLA-DR antigens and are not contaminated with Langerhans’ cells, the antigen-presenting cells of the epidermis. Since cultured epidermal-cell allografts are eventually replaced by host cells, their use is mainly limited to temporary coverage of burns, skin-graft donor sites, and chronic open wounds, such as pressure sores and venous stasis ulcers. Unlike autologous grafts, they are inappropriate for permanent coverage of full-thickness wounds.

Neonatal epidermal cells, unlike adult cells, release growth factors that stimulate other epidermal cells. Allografts of cultured neonatal foreskin cells accelerate healing and relieve pain in patients with acute and chronic skin ulcers. Patients with chronic skin ulcers can be treated with fresh or cryopreserved cultured epidermal-cell allografts with equal efficacy.

### Dermal Skin Substitutes

Inclusion of a dermal component in skin substitutes helps prevent wound contraction and provides greater mechanical stability. Allografts of cadaver skin containing dermis that has been chemically treated to remove the antigenic epidermal cellular elements have been used alone or in combination with cultured autologous epidermal cells for closure of various wounds.

A composite skin graft made of a collagen-based dermal lattice (containing bovine collagen and chondroitin 6-sulfate) with an outer silicon covering has been used successfully to treat burns. The dermal component is slowly degraded, and several weeks later the Silastic sheet is removed and covered with an autograft. This composite graft recently received FDA approval for the treatment of burns. A similar
product, in which the Silastic outer covering is replaced with human epidermal cells and the dermal component includes viable fibroblasts, has also been used successfully for burns and chronic wounds. A nylon mesh in which viable human fibroblasts are embedded, covered with an outer Silastic layer to limit evaporation, has been used successfully for temporary wound coverage after excision of burn wounds. It has recently been approved by the FDA for this indication.

**Combined Dermal and Epidermal Skin Substitutes**

A composite graft consisting of type I bovine collagen and live allogeneic human skin fibroblasts and epidermal cells has been developed and used successfully in patients with surgical wounds and venous ulcers.

**CLINICAL IMPLICATIONS**

In general, conservative and time-honored methods of wound care should be attempted first. These methods are successful in the majority of patients with skin wounds and include the use of standard wound dressings, remediation of underlying problems such as hyperglycemia, debridement of nonviable tissue, restoration of adequate tissue perfusion, limitation of pressure at the wound site, and control of infection. Particularly large and life-threatening skin wounds (such as extensive burns) may require the use of cultured autologous epidermal-cell grafts or biologic skin substitutes. There are not yet enough clinical data to support the routine use of growth factors or other wound mediators. However, further refinement and development of substances that stimulate wound healing are likely.

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