Pathophysiology of acute wound healing

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Abstract Wound healing is a complex process that can be divided into at least 3 continuous and overlapping processes: an inflammatory reaction, a proliferative process leading to tissue restoration, and, eventually, tissue remodeling. Wound healing processes are strictly regulated by multiple growth factors and cytokines released at the wound site. Although the desirable final result of coordinated healing would be the formation of tissue with a similar structure and comparable functions as with intact skin, regeneration is uncommon (with notable exceptions such as early fetal healing); healing however results in a structurally and functionally satisfactory but not identical outcome. Alterations that disrupt controlled healing processes would extend tissue damage and repair. The pathobiologic states may lead to chronic or nonhealing wounds or excessive fibrosis.

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Introduction

Wound healing is a complex process that can be roughly divided into 3 overlapping phases of inflammatory reaction, proliferation, and remodeling. The inflammatory phase involves vascular responses characterized by blood coagulation and hemostasis as well as cellular events, including infiltration of leukocytes with varied functions in antimicrobial and cytokine release, which initiates the proliferative response for wound repair. Some authors have divided wound healing into 4 stages, with the first stage being hemostasis, highlighting the importance of vascular responses. During the proliferative phase, there is formation of the epithelium to cover the wound surface with concomitant growth of granulation tissue to fill the wound space. Granulation tissue formation involves proliferation of fibroblasts, deposition of collagens and other extracellular matrices, and development of new blood vessels. Once the new tissue within the wound is formed, the remodeling phase begins to restore tissue structural integrity and functional competence. The 3 phases of wound repair are however not simple linear events but rather overlapping in time (Fig. 1).

Acute wounds refer to those wounds, such as burns, other traumatic injuries, and surgically created wounds, that heal in a timely fashion. An example of a common acute wound is a clean and uninfected surgical incisional wound approximated by surgical sutures. Although the desirable final result of coordinated healing would be the formation of tissue with a similar structure and comparable functions as with intact skin, regeneration is uncommon (with notable exceptions such as early fetal healing); healing however results in a structurally and functionally satisfactory but not identical outcome. Wound healing processes seem to be strictly regulated by multiple growth factors and cytokines released at the wound site. Alterations that disrupt controlled timely healing processes would extend tissue damage and prolong repair.

Inflammatory phase

Inflammation is a highly effective component of the innate initial reaction of the body to injury. It is an important
consequence of injury and one that normally leads to tissue repair and restoration of function. The inflammatory response can be subdivided into vascular and cellular responses. Early in the wounding process, local vasodilatation, blood and fluid extravasation into the extravascular space, and blocking of lymphatic drainage can produce cardinal signs of inflammation, including redness, swelling, and heat. This acute inflammatory response usually lasts between 24 and 48 hours and may persist for up to 2 weeks in some cases. Tissue injury causes blood vessel disruption and bleeding. Platelets adhere, aggregate, and release many mediators to facilitate coagulation. Although hemostasis is the major function of blood coagulation, a secondary but equally important function of platelets is to initiate the healing cascade via release of chemoattractants and growth factors. At the same time, the clot provides a matrix scaffold for the recruitment of cells to an injured area. In responding to these important mediators, leukocytes, including neutrophils and macrophages, infiltrate the wounded area and assist in cleaning and removing damaged tissue debris and foreign particles. Once in the wound site, activated macrophages release several important growth factors and cytokines, initiating granulation tissue formation.

The vascular response and hemostasis

Bleeding occurs immediately after tissue injuries as a result of the disruption of blood vessels. The first step in wound healing is thus hemostasis. Hemostasis consists of 2 major processes: development of a fibrin clot and coagulation. Platelets are the first cells to appear after an injury and play a central role in normal hemostasis. With vascular injury, platelets are exposed to and activated by the extracellular matrix in the vascular wall, such as fibrillar collagen, fibronectin, and other adhesive matrix proteins. Upon activation, platelets undergo adhesion as well as aggregation and at the same time release many mediators (eg, serotonin, adenosine diphosphate, and thromboxane A₂) and adhesive proteins (eg, fibrinogen, fibronectin, thrombospondin, and von Willebrand factor VIII). These mediators and locally generated thrombin induce further platelet aggregation and secretion and form the platelet plug. With the conversion by thrombin of fibrinogen to fibrins during platelet aggregation, a fibrin clot is formed to stop the bleeding.

The second component of hemostasis is coagulation achieved via intrinsic and extrinsic coagulation pathways. Platelet aggregation triggers a specific enzyme in blood known as Hageman factor XII to initiate the intrinsic coagulation cascade through a series of conversions of proenzymes to activated enzymes that culminates in the transformation of prothrombin into thrombin. This in turn converts soluble fibrinogen to insoluble fibrin. Damaged tissue releases a lipoprotein known as tissue factor, which activates the extrinsic coagulation pathway. Activated monocytes and endothelial cells also express this tissue factor in their surface and participate in the coagulation.

While performing critical functions of hemostasis, platelets also significantly contribute to other processes of wound healing, including inflammation, reepithelialization, fibroplasia, and angiogenesis. For example, the fibrin clot acts as a scaffold matrix for the migration of leukocytes, keratinocytes, fibroblasts, and endothelial cells and serves as a reservoir of growth factors. Platelets influence wounds
through an infiltration of leukocytes by releasing platelet-
derived chemotactic factors. Platelets also promote new
regenerative tissue by releasing several growth factors
strongly implicated in wound repair, including transforming
growth factor (TGF-α), TGF-β, and platelet-derived growth
factor (PDGF; Table 1).

The cellular response and inflammation

The cellular response of the inflammatory phase is
caracterized by the influx of leukocytes into the area of
injury. In the early inflammatory state, neutrophils and
monocytes are the predominant cells at the site of injury.
Shortly after injury, neutrophils and monocytes begin to
emigrate from capillaries into the wounded tissue, with
neutrophils being the first to arrive in great numbers. Later
in inflammation, the number of neutrophils declines and
macrophages (tissue-derived monocytes) predominate.

Neutrophils and monocytes are recruited to the wound by
chemotactic factors released during hemostasis and by mast
cells. Chemotactic factors generated during the coagulation
process, such as kallikrein, fibrinopeptides released from
fibrinogen, and fibrin degradation products, also serve to
up-regulate the expression of important intercellular adhe-
sion molecules. Substances released by mast cells, such as
tumor necrosis factor, histamine, proteases, leukotrienes
(LTs), and cytokines (interleukins), represent additional
sources of chemotactic signals for the recruitment of
leukocytes. Growth factors of PDGF and TGF-β are also
potent chemotactic factors for leukocytes. Once in the
wound site, integrin receptors found on the cell surface of
neutrophils enhance cell-matrix interactions. This allows
neutrophils to perform their function of killing and
phagocytosing bacteria and damaged matrix proteins within
the wound bed. Neutrophil infiltration normally lasts for
only a few days, but the presence of wound contamination
prolongs the presence of neutrophils within the wound and
may delay healing.

Monocytes emigrate into tissue spaces and transform into
larger phagocytic macrophages that soon become the
predominant cell type during the latter part of the
inflammatory phase. Monocytes are initially attracted to
the wound site by some of the same chemoattractants
that attract neutrophils, and their recruitment continues
through signals released by monocyte-specific chemoattrac-
tants, such as monocyte chemoattractant protein 1 and

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| Hageman factor’ indicates Hageman factor fragments; PAF, platelet-activating factor; ILs, interleukins; TNF, tumor necrosis factor. “Pathophysiology of acute wound healing” 11
Chemical mediators of inflammation

Macrophage inflammatory protein 1. The extracellular matrix degradation products collagen fragments, fibronectin fragments, and thrombin are also specific chemoattractants for monocytes. Macrophages are considered to be the most important regulatory cell in the inflammatory reaction. Macrophages phagocytize, digest, and kill pathogenic organisms; scavenge tissue debris; and destroy any remaining neutrophil. After binding to the extracellular membrane, bacterial, cellular, and tissue phagocytoses and subsequent destruction are accomplished through the release of biologically active oxygen intermediates and enzymatic proteins. These all-important processes performed by the monocyte/macrophage allow for induction of angiogenesis and formation of granulation tissue.

Macrophages release chemotactic factors (eg, fibronectin) that attract fibroblasts to the wound area. New blood vessel growth follows a gradient of angiogenic factors produced by hypoxic macrophages because macrophages do not produce these angiogenic factors when either fully oxygenated or anoxic. Macrophages can be considered as factories for growth factor production, including PDGF, fibroblast growth factor, vascular endothelial growth factor, TGF-β, and TGF-α. These cytokines are important in inducing cell migration as well as proliferation and matrix production. Macrophages thus appear to play a pivotal role in the transition between inflammation and repair.

**Chemical mediators of inflammation**

A number of chemical substances are involved in the initiation and control of inflammation. These chemicals work in concert: some are protagonists and others are antagonists of inflammation. They can be grouped as vasoactive amines of histamine and serotonin, plasma proteases of kinins and complements, plasma proteins of the coagulation system, arachidonic acid metabolites of prostaglandins (PGs) and LTs, cytokines and growth factors, and free radicals of nitric oxide and oxygen-derived free radicals (Table 1).

**Mast cell mediators**

One of the major substances released from mast cell granules is histamine. Histamine acts on histamine receptor 1 and causes the dilatation of arterials and increased permeability of venules. In addition to histamine, mast cell granules contain a number of other active materials, including serotonin and heparin, which lead in part to the initial short-lived increase in permeability of venules. Heparin is also an anticoagulant and serves to prevent coagulation of the excess tissue fluid and blood components during the early phase of the inflammatory response.

The kinins are biologically active and nearly indistinguishable peptides that are found in areas of tissue destruction. The most familiar kinin, bradykinin, is a potent inflammatory substance released from plasma proteins in injured tissue by the plasma enzyme kallikrein. The action of the kinins on the microvasculature is similar to that of histamine.

**Prostaglandins and LTs**

Prostaglandins and LTs are 2 major classes of potent biologic substances from arachidonic acid that are released from cell membrane phospholipids. Prostaglandins and LTs are produced by nearly all cells of the body in response to cell membrane injury. Prostaglandin I2, PGD2, PGE2, and PGF2α are potent substances for vasodilatation, whereas PGD2, PGE2, and PGF2α also increase vascular permeability, which can cause edema. Prostaglandin E2 has chemotactic activity and attracts leukocytes to the wound area as well. Prostaglandin E2 seems to synergize with other inflammatory substances such as bradykinin and is thought to be responsible for sensitizing pain receptors, causing a state of hyperalgesia. The other group of arachidonic acid metabolites refers to LTs. Leukotriene B4 is a potent chemotactic agent and induces aggregation of neutrophils, whereas LTC4, LTD4, and LTE4 cause vasoconstriction and increased vascular permeability.

**Complement system**

The complement system of more than 30 proteins is another major class of proteins critical to inflammation. These proteins may be present among the plasma proteins that leak from capillaries into tissue spaces. When an antibody binds, specific proteins of the complement system trigger a cascade of sequential reactions that produce multiple end-products that help prevent damage by the invading organism or toxin. With regard to wound healing, some of the end-products activate phagocytosis by both neutrophils and macrophages, whereas others enhance the lysis and agglutination of invading organisms. Still others activate mast cells and basophils to release histamine.

**Growth factors**

Growth factors have been shown to play multiple and critical roles in wound repair processes. For example, PDGF is a potent and important growth factor, especially in the early inflammatory phase of wound healing. It is chemo tactic for monocytes, macrophages, and neutrophils and is mitogenic for fibroblasts and smooth muscle cells in vitro. Many growth factors secreted by macrophages are pleiotropic and influence cell proliferation, angiogenesis, and extracellular matrix synthesis. For example, TGF-β plays an important role in keratinocyte migration and reepithelialization; TGF-β1, TGF-β2, and TGF-β3 strongly promote the migration of fibroblasts and endothelial cells and the deposition of extracellular matrices by fibroblasts during granulation tissue formation. Whereas increased TGF-β1 promotes scar formation, TGF-β3 exhibits an antiscarring effect.

**Pathologic outcomes of acute inflammation**

Most of the symptoms associated with acute inflammatory response last for approximately 2 weeks. If inflammation persists for months or years, it is called chronic inflammation. Chronic inflammation associated with
wounds often occurs when a wound is sealed by necrotic tissue, is contaminated with pathogens, or contains foreign material that cannot be phagocytized or solubilized during the acute inflammatory phase. Granulocytes disappear through lysis and migration with the resolution of the acute inflammatory phase, whereas mononuclear cells—specifically, lymphocytes, monocytes, and macrophages—persist at the site of inflammation. The chronic inflammatory response may not be characterized by the cardinal signs of inflammation. At times, the body responds to the presence of persistent foreign material and/or infection by local proliferation of mononuclear cells. In particular, macrophages that have ingested foreign particulate material will remain in the tissue if they are unable to solubilize the ingested material. Macrophages attract fibroblasts and over time may produce increased quantities of collagen, leading to a slowly forming encapsulated mass of fibrous tissue, a granuloma.

**Proliferative phase**

The initial inflammatory responses to injury provide the necessary framework to the subsequent production of a new functional barrier. In this phase of healing, cellular activity predominates. The major events during this phase are the creation of a permeability barrier (ie, reepithelialization), the establishment of appropriate blood supply (ie, angiogenesis), and reinforcement of the injured dermal tissue (ie, fibroplasia).

**Reepithelialization**

Reepithelialization is the process of restoring an intact epidermis after cutaneous injury. It generally involves several processes, including the migration of adjacent epidermal keratinocytes into the wound, the proliferation of keratinocytes used for the supplement of the advancing epidermal keratinocytes into the wound, the proliferation of mononuclear cells, the establishment of appropriate blood supply (ie, angiogenesis), and reinforcement of the injured dermal tissue (ie, fibroplasia).

**Keratinocyte migration**

Keratinocyte migration is an early event in wound reepithelialization. The keratinocytes initially respond to an epidermal defect by migrating from the free edges of the wound within 24 hours. The keratinocyte migration in partial-thickness wounds also occurs from remaining skin appendages and wound edges. Epidermal stem cells from the hair follicle are now thought to originate from the hair bulge that is believed to be the germinative portion of the hair and serve as a reservoir for keratinocytes in wound healing. A number of processes must occur within keratinocytes in preparation for migration. Approximately 12 hours after wounding, a series of events, such as flattening and elongation of keratinocytes, development of pseudopodlike projections of lamellipodia, loss of cell-cell and cell-matrix contacts, retraction of intracellular tonofilaments, and formation of actin filaments at the edge of their cytoplasm, occurs. Although keratinocytes are migrating, their proliferative potential is inhibited. Migrating basal keratinocytes may express selective cell surface markers such as CD44 and some markers usually expressed by squamous cells.

Several elements have been implicated in keratinocyte migration, including the extracellular matrix, integrin receptors, matrix metalloproteinases (MMPs), and growth factors. An early provisional matrix formed by fibrin, fibronectin, and type V collagen enables keratinocytes to migrate and dissect under eschar and debris that may be covering the wound. Keratinocytes use their surface integrin receptors to interact with a fibronectin-rich provisional matrix. The direction of migration is also regulated by the binding of keratinocytes to integrin receptors on the newly formed collagen molecules in the wound bed. Dissociation of this binding allows the keratinocytes to migrate forward. Matrix metalloproteinases also play an important role in keratinocyte migration by their involvement in this dissociation. Migrating keratinocytes produce MMPs, such as MMP-9, which specifically degrades type IV collagen and laminins in the basement membrane. This allows cells to leave the basement membrane and migrate into the wound. Matrix metalloproteinase 1 disrupts any attachment to a fibrillar collagen and facilitates the continued migration of keratinocytes in the wound.

**Keratinocyte proliferation**

Reepithelialization also involves increased proliferation of keratinocytes located near the cells of the migrating front tongue. This proliferating source of keratinocytes ensures an adequate supply of cells to migrate and cover the wound. When migration ceases, possibly as a result of contact inhibition, keratinocytes reattach themselves to the underlying substratum, reconstitute the basement membrane, and then resume the process of terminal differentiation to generate a stratified epidermis. One can observe that there are single-layered keratinocytes toward the wound center whereas there are multi-layered or stratified keratinocytes near the wound edges. Differentiation then follows proliferation as the proliferative index is found significantly increased at the wound center, whereas the differentiation of the stratum corneum (keratins 1/10, filaggrin, and loricrin) and differentiation of the epidermal junction (laminin 5 and collagen IV) are more advanced toward the wound margin. Epidermal growth factor, keratinocyte growth factor, and TGF-β have been shown to be among the important stimuli for keratinocyte migration, proliferation, and reepithelialization.

**Restoration of the BMZ**

The formation of an intact BMZ between the epidermis and the dermis is essential for the reestablishment of skin
integrity and function. Within 7 to 9 days after reepithelialization, the BMZ reforms. The BMZ forms an adhesion structure because its superior aspects serve as an attachment site for basal keratinocytes through the formation of a hemidesmosome-anchoring filament complex whereas the inferior or lower portion stabilizes the attachment to the underlying dermis by anchoring fibrils. The importance of BMZ is evidenced by a group of inherited blistering diseases known as epidermolysis bullosa. In these conditions, mutations of one of a variety of BMZ proteins, such as hemidesmosome collagen XVII in atrophic epidermolysis bullosa, defects of laminin 5 of a major anchoring filament in junctional epidermolysis bullosa, and deficiency of collagen VII anchoring fibrils in dystrophic epidermolysis bullosa lead to blistering and ulcer formation.

The skin BMZ consists of many extracellular matrix proteins; among these, collagens and laminins are the major components. Collagen IV is the most abundant component and forms a 3-dimensional lattice network within the electron-dense, or lamina densa, portion of skin BMZ. Collagen VII proteins, also called anchoring fibrils, span from the lamina densa to the upper papillary dermis, where they form a structure known as an anchoring plaque. Another important collagen is collagen XVII, also known as bullous pemphigoid antigen (bullous pemphigoid antigen 2 or bullous pemphigoid antigen 180), which is a 180-kDa transmembrane protein located on the hemidesmosome complex of basal keratinocytes.

Laminins are the major noncollagenous extracellular matrix components in a wide range of BMZs within human tissues. Several laminins are present in the BMZ of the dermoepidermal junction. Composed of α, β, and γ subunits, laminin 5 (α3β3γ2) and laminin 10 (α5β1γ1) are the major laminins in skin BMZ and found to be actively involved in wound repair.\(^{21}\) In response to wounding, keratinocytes in the migrating front edge deposit laminin 5, which serves as a track to allow subsequent keratinocytes to migrate.\(^{22}\) Recently, a new laminin member, laminin 10, has been described; it is located within the lamina densa.\(^{23}\) Laminin 10 was also found to be a major laminin of dermal microvascular blood vessels.\(^{24}\) Laminin 10 knockout mouse skin was found to exhibit discontinuity in BMZ lamina densa and defects in hair development.\(^{25}\)

Reconstitution of the dermis

Dermal reconstitution begins approximately 3 to 4 days after injury, characterized clinically by granulation tissue formation, which includes new blood vessel formation, or angiogenesis, and the accumulation of fibroblasts and ground matrices, named fibroplasia. The provisional extracellular matrix that is formed in part by the fibrin clot, which is rich in fibronectin, promotes granulation tissue formation by providing scaffolding and contact guidance for cells to migrate into wound spaces and for angiogenesis and fibroplasia to occur in an effort to replace the wounded dermal tissue.

Fibroplasia

Fibroplasia describes a process of fibroblast proliferation, migration into wound fibrin clot, and production of new collagen and other matrix proteins, which contribute to the formation of granulation tissue. As an early response to injury, fibroblasts in the wound edges begin to proliferate and by approximately day 4 start to migrate into the provisional matrix of the wound clot, where they lay down a collagen-rich matrix, including collagens, proteoglycans, and elastin.\(^{27,28}\) Once the fibroblasts have migrated into the wound, they gradually change to profibrotic phenotypes and switch their major function to protein synthesis.\(^{29}\) Fibroblasts are also modulated into phenotypes of myofibroblasts and participate in wound contraction.\(^{30}\) It is also possible that other subpopulations of fibroblasts exist and that these individual subpopulations may perform different roles during wound healing.

Structural molecules of the early extracellular matrix, such as fibronectin and collagen, contribute to granulation tissue formation by providing a scaffold for contact guidance and a reservoir for cytokines and growth factors. Fibronectin, a glycoprotein, is a major component of the gel-like substance initially secreted and provides for enhanced fibroblast activity. Fibronectin allows fibroblasts to bind to the extracellular matrix and provides an adherent base for cell migration.\(^{31}\) The fibronectin matrix also provides a scaffold for collagen fibrils and mediates wound contraction. Major fibroblastic chemotactic factors are in part derived from macrophages present in the wound. Both PDGF and TGF-β can stimulate fibroblast migration and up-regulate the expression of integrin receptors.\(^{32}\) Epidermal growth factor and fibroblast growth factor, among others, modulate fibroblast proliferation and migration.\(^{33,34}\) Fibroblast proliferation is also stimulated by an acidic low-oxygen condition found in the center of the wound. As angiogenesis proceeds with the formation of new vessels and increased oxygen carrying capacity, this stimulus diminishes.

Angiogenesis

Angiogenesis refers to new vessel growth by the sprouting of preexisting vessels adjacent to the wound. As in most normal adult tissues, dermal blood vasculatures remain quiescent. In response to the injury, microvascular endothelial cells initiate an angiogenic process consisting of activation of endothelial cells, local degradation of their basement membrane, sprouting into the wound clot, cell proliferation, tubule structure formation, reconstruction of the basement membrane, and, eventually, regression and involution of the newly formed vasculature as tissue remodeling.\(^{35}\) Cytoplasmic pseudopodia extend from endothelial cells on the second wound day, and, augmented by MMP secretion, migration into the perivascular space
occurs. Similar to the migration of the epithelium tongue, endothelial cells at the tip of capillaries migrate into the wound but do not undergo active proliferation. Newly formed vessels participate in granulation tissue formation and provide nutrition and oxygen to growing tissues. In addition, inflammatory cells require the interaction with blood vessels to enter the site of injury. During angiogenesis, endothelial cells also produce and secrete biologically active substances or cytokines.

Angiogenesis involves a phenotypic alteration of endothelial cells, directed migration, and various mitogenic stimuli. Cytokines released by macrophages stimulate angiogenesis during wound healing, as does low-oxygen tension, lactic acid, and biogenic amines produced in the wound. Several growth factors have been shown to play critical roles in wound angiogenesis, including vascular endothelial growth factor, angiopoietins, fibroblast growth factor, and TGF-β. Vascular endothelial growth factor, also known as vascular permeability factor, which exerts its biologic activity predominantly on endothelial cells, is a key mediator of angiogenesis. The vascular endothelial growth factor performs multiple functions on endothelial cells through 2 specific receptors: vascular endothelial growth factor receptor 1 or Flt1 and vascular endothelial growth factor receptor 2 or Flk1/KDR. Vascular endothelial growth factor is a potent mitogen for endothelial cells and induces endothelial cell migration and sprouting by up-regulation of several integrin receptors. Vascular endothelial growth factor acts as a survival factor for endothelial cells through the induction of the expression of the antiapoptotic protein Bcl2. Many cell types, such as keratinocytes, fibroblasts, and endothelial cells, are able to produce the vascular endothelial growth factor. Vascular endothelial growth factor is expressed at low levels in normal human skin, whereas its expression is highly up-regulated during wound healing. Low-oxygen tension, as that which occurs in tissue hypoxia during tissue injury, is a major inducer of this growth factor.

Development of new capillary vessels is dependent on not only the cells and cytokines present but also the production and organization of extracellular matrix components. The extracellular matrix is critical for blood vessel growth and maintenance by acting as scaffold support, through which endothelial cells may migrate, and as a reservoir and modulator for growth factors. Recent developments showed that laminins are one of the major extracellular matrix proteins important in wound angiogenesis. A study found that antibody to the laminin α4 chain inhibited endothelial cell branching. Two newly identified laminins, laminin 8 (α4β1γ1) and laminin 10 (α5β1γ1), are the laminins produced by human skin dermal microvascular endothelial cells (HDMECs) and found to have profound effects on HDMEC functions. The laminin α4G domain was found to support HDMEC attachment and spreading. The overexpression of laminin 8 trimeric molecule promoted endothelial cell migration and capillary structure formation. These studies supported the role of laminin 8 in angiogenesis and found that its functions are mediated by integrin receptors of β1 and αvβ3. Laminin 10 is another major laminin produced by HDMECs. Monoclonal antibody 4C7, specifically directed against the α5 chain of laminin 10, detected a high expression of laminin 10 in HDMECs and stained strongly positive in newly formed microvascular blood vessels of human skin wound granulation tissue, also supporting the role of laminin 10 in wound angiogenesis.

**Wound contraction**

Contraction of the wound begins soon after wounding and peaks at 2 weeks. The degree of wound contraction varies with the depth of the wound. For full-thickness wounds, contraction is an important part of healing and accounts for up to a 40% decrease in the size of the wound. In partial-thickness wounds, contraction is less as compared with that in full-thickness wounds and in direct proportion to their depth. Myofibroblasts are the predominant mediator of this contractile process because of their ability to extend and retract. During granulation tissue formation, fibroblasts are gradually modulated into myofibroblasts, which are characterized with actin microfilament bundles (not seen in networks of normal skin fibroblasts), similar to those seen in smooth muscle cells, along their plasma membrane. There is increased expression of smooth muscle differentiation markers of α-smooth muscle actin, smooth muscle myosin, and desmin starting on day 6 and reaching a maximum on day 15, after which these regress progressively.

Myofibroblasts contain one of the highest concentrations of actinomyosin of any cell. The cells within the wound align along the lines of contraction, and contraction of the wound occurs in directions of skin tension lines. This musculike contraction of myofibroblasts is mediated by PGF2α, 5-hydroxytryptamine, angiotensin, vasopressin, bradykinins, epinephrine, and norepinephrine. This contraction is unified and requires cell-cell and cell-matrix communication. Fibronectin not only provides the multiple functions described previously but also assists in wound contraction. Myofibroblast pseudopodia extend, and cytoplasmic actin binds to extracellular fibronectin, attaches to collagen fibers, and retracts, drawing the collagen fibers to the cell, thereby producing wound contraction. The rate of contraction is proportional to the cell number and inversely proportional to the lattice collagen concentration.

**Integrin receptors in wound healing**

The extracellular matrix is critical for wound healing by acting as scaffold support, through which keratinocytes, fibroblasts, and endothelial cells may migrate, and as a reservoir and modulator for growth factors that mediate healing through intercellular signaling pathways. The extracellular matrix binds cells through specific cell surface receptors, of which integrins are the major receptors for the extracellular matrix (Table 2). The sequence RGD (Arg-
Gly-Asp) has been found frequently to be the major recognition sequence for integrin receptors. Integrins are a family of heterodimeric transmembrane proteins, each consisting of one α chain and one β chain. Integrins mediate interactions between cells as well as between the cell and the matrix and transduce the signals between them. Many signaling pathways activated by integrins are also activated after growth factor stimulation, suggesting that cellular responses mediated by integrins and growth factors may act synergistically or coordinate cellular biochemical changes.

Integrin receptors are involved in all phases of wound repair. Immediately after injury, integrin αIIbβ3 conducts the interaction of platelets with the extracellular matrix, including fibrin, fibronectin, and thrombospondin, for stable clot formation. During subsequent phases of wound healing, migration of cells, leukocytes, keratinocytes, fibroblasts, and endothelial cells into the wound requires rapid binding and dissociation with extracellular molecules to permit cell movement. After fibroblasts cease migration and begin wound contraction, they need to bind tightly to collagens as well as fibronectin and organize a contractile cytoskeleton. Cells express and use different integrins for their migration and attachment. For example, in the normal epidermis, α3β1 integrins mediate interactions between keratinocytes and α6β4 integrins connect basal keratinocytes to the BMZ laminins. The α2β1 and α5β1 integrins mediate keratinocyte migration on collagen and fibronectin during wound repair.

### Remodeling phase

Remodeling consists of the deposition of the matrix and its subsequent changes over time. It occurs throughout the entire wound repair process as fibrin clot formed in the early inflammatory phase is replaced by the granulation tissue that is rich in type III collagen and blood vessels during the proliferative phase and subsequently replaced by a collagenous scar predominantly of type I collagen predominant with much less mature blood vessels. One of the characteristics of wound remodeling is the change of extracellular matrix composition. Collagen fibers constitute approximately 80% of the dry weight of normal human dermis and are the principal proteins providing structure, strength, and stiffness to dermal tissue. In healthy adults, type I collagen accounts for approximately 80% of collagens and type III collagen constitutes 10% of collagens in the dermis. During early wound healing, however, similar to the case in the fetal dermis, type III collagen is the predominant collagen synthesized by fibroblasts in granulation tissue. Type III collagen first appears after 48 to 72 hours and is maximally secreted between 5 and 7 days. The total amount of collagen increases early in repair, reaching a maximum between 2 and 3 weeks after injury. Over the period of 1 year or longer, the dermis gradually returns to the stable preinjury phenotype, consisting largely of type I collagen. Tensile strength, a functional assessment of collagen, increases to 40% of strength before the injury at 1 month and may continue to increase for 1 year, reaching up to 70% of its preinjury strength.

With wound closure, a gradual turnover of collagen occurs as type III collagen undergoes degradation and type I collagen synthesis increases. The process of this conversion of the dermis is accomplished through a tightly controlled synthesis of new collagen and lysis of old collagen, mainly carried out by the actions of MMPs. The stimulus for this conversion may be the biomechanical stress and strain placed across a closed wound. Matrix metalloproteinases are usually not detectable or at very low levels in healthy resting skin cells and major extracellular ligands

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<td>αvβ6</td>
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<td>αIIbβ3</td>
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KC indicates keratinocyte; Fbr, fibroblast; EC, endothelial cell; Coll, collagen; Fg, fibrinogen; Fn, fibronectin; Ln, laminin; Tn, tenasin; Vn, vitronectin.
tissue and are instead induced during wound repair, in response to cytokines, growth factors, and/or cell contact with the extracellular matrix. The catalytic activity of MMPs is also controlled in part by a family of tissue inhibitors of metalloproteinases. Tissue inhibitors of metalloproteinases specifically bind MMPs and are their natural inhibitors. The balance between the activities of MMPs and tissue inhibitors of metalloproteinases is also critical to wound repair and remodeling.57

Pathologic outcomes of wound proliferation and remodeling

The regulation of collagen synthesis is controlled by several growth factors, including TGF-β and fibroblast growth factor, both of which have strong effects on collagen gene expression. Transforming growth factor-β stimulates types I and III collagen production. Excess TGF-β1 has been found in the dermis of chronic venous ulcers and may play a role in fibrosis.58 Matrix metalloproteinases play an important role in wound remodeling. Unbalanced expression of MMPs and tissue inhibitors of metalloproteinases may also contribute to delayed healing or excessive fibrosis. Many pathobiologic states, such as diabetes, infection, and poor nutrition, lead to chronic or nonhealing wounds (ulcers) or excessive fibrosis (hypertrophic scars and keloids) that results in an altered structure and loss of function.

Whereas acute wounds go through the linear and overlapping events of the 3 wound healing phases, healing-impaired chronic wounds do not progress through the orderly process. Some areas of the wound are found in different phases, having lost the ideal synchrony of events that leads to normal (rapid) healing. More importantly, some cells in chronic wounds are phenotypically altered.59 Keratinocytes on the edge of chronic wounds are unable to migrate properly; therefore, the wound cannot be closed.60,61 One reason for the inability of nonhealing keratinocytes to migrate is because they are, for one reason or another, unresponsive to activation signals that promote cell migration. Fibroblasts of diabetic ulcers showed a decreased response to TGF-β1 and other growth factors59,62 as well as decreased expression of the TGF-β receptor and impaired signal transduction.63 It is thus critical to understand the normal repair process to better understand the mechanisms of delayed and/or nonhealing wounds or, alternatively, excessive fibrosis.

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