The Physiology of Wound Healing: Injury Through Maturation

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KEYWORDS

- Wound healing
- Skin physiology
- Soft tissue injury
- Coagulation cascade
- Fibroplasias

The physiology of wound healing is repeatedly described in medical literature. Most classic descriptions of wound healing consist of three phases: inflammation, proliferation, and maturation. However, the three phases of wound healing are not discrete events. The true complexity of healing evolves with increasing knowledge of cellular interactions and inflammatory mediators. The stages of wound healing occur both sequentially and simultaneously. Several variations exist in the recent literature, trying to create a framework for the molecular biology and cellular physiology of the healing process. The following description of wound healing provides a general summary of the events, cellular components, and influential mediators of wound healing over time.

INJURY

The initiation of healing starts with the creation of a wound. A wound is defined as an injury to the body that typically involves laceration or breaking of a membrane and damage to the underlying tissues. Injury can occur from any number of mechanical or thermal forces that lead to disruption of the skin and damage to the connective tissue and vasculature. Bleeding ensues along with exposure of collagen, endothelium, and intravascular and extravascular proteins. This environment serves as a stimulus for hemostasis.

HEMOSTASIS

The resolution of injury begins with hemostasis. Vasoconstriction and clot formation lead to cessation of bleeding. Hemostasis is achieved through the activation of platelets and the coagulation cascade.
**Vasoconstriction**

Contraction of the smooth muscle within the endothelium is the first response to vessel injury. Reflexive vasoconstriction occurs before activation of platelets and coagulation. The endothelium of damaged vessels produces its own vasoconstrictor, endothelin. Other mediators for vasoconstriction are derived from circulating catecholamines (epinephrine), the sympathetic nervous system (norepinephrine), and the release of prostaglandins from injured cells. Coagulation and platelet activation contribute additional stimuli for vasoconstriction through the following mediators: bradykinin, fibrinopeptides, serotonin, and thromboxane A2.

**Coagulation Cascade**

The coagulation cascade is made up of two converging pathways: extrinsic and intrinsic. The extrinsic coagulation pathway is an essential pathway for normal thrombus formation. It is initiated by exposed tissue factor on the subendothelial surface. Tissue factor binds to factor VII and leads to the subsequent activation of factors IX and X. The intrinsic pathway is not essential to coagulation. As suggested by name, all components of the pathway are intrinsic to the circulating plasma. Initiation of the intrinsic pathway is through the autoactivation of factor XII. Factor XII has the unique ability to change shape in the presence of negatively charged surfaces. Factor XII, in its active form, is a stimulus for the activation of factors XI, IX, VIII, and X. Although each pathway has a distinct trigger, both lead to the activation of factor X and the production of thrombin. Thrombin serves two important roles in clot formation: a catalyst for the conversion of fibrinogen to fibrin and an initiator for platelet activation (Fig. 1).

**Platelets Adherence, Aggregation, and Degranulation**

Platelets are the first cells to respond in wound healing. Activated platelets contribute to hemostasis through the process of adherence, aggregation, and degranulation. The presence of platelets at the site of injury is stimulated by exposed collagen and thrombin. Collagen within the subendothelial matrix comes in contact with blood flow, leading to the adhesion of circulating platelets. Platelet adherence is achieved
through interactions between platelet glycoproteins VI and collagen. Additional interactions occur between platelet glycoprotein Ib-V-IX complex and collagen-bound von Willebrand’s factor. Platelet integrins play a supportive role in the adherence of platelets to collagen, von Willebrand’s factor, fibrinogen, and other platelets. As mentioned above, tissue factor activates the extrinsic coagulation pathway leading to the production of thrombin. Thrombin is an independent initiator of platelet activation. Thrombin interacts with a receptor on the platelet surface (Par1) and leads to the release of ADP, serotonin, and thromboxane A2. These substances enhance platelet aggregation. Thromboxane A2 and serotonin also act as potent mediators of vasoconstriction.

Platelet aggregation in the environment of the fibrin matrix forms a clot. Thrombus prevents ongoing bleeding, establishes a protective barrier, and provides a reservoir for substances released by platelet degranulation. Degranulation involves the release of numerous cytokines, growth factors, and matrix proteins stored within platelet alpha granules. These substances promote a variety of cellular and extracellular mechanisms important to hemostasis as well as several other stages of wound healing: matrix deposition, chemotaxis, cell proliferation, angiogenesis, and remodeling (Table 1).

INFLAMMATION

Achievement of hemostasis leads to the immediate onset of inflammation. Inflammation is evident through the physical signs of erythema, heat, edema, and pain. On a cellular level, inflammation represents vessel dilation, increased vascular permeability, and leukocyte recruitment to the site of injury. Two leukocyte populations sequentially dominate the inflammatory events of wound healing: neutrophils and macrophages. Both provide the critical function of wound debridement, whereas the latter also promotes ongoing cellular recruitment and activation necessary for subsequent steps in wound healing (Fig. 2).

Vasodilation and Increased Permeability

The establishment of vasoconstriction for hemostasis lasts only minutes before several factors stimulate the reverse response of vasodilation. Vasodilation is mediated by the presence of kinins, histamine, prostaglandins, and leukotrienes. Vascular dilation increases blood flow to the wound, resulting in the characteristic inflammatory signs of erythema and heat. Increased flow also hastens the delivery of circulating cells and mediators to the site of injury. As vessels dilate, gaps form between the endothelial cells, increasing vascular permeability. Many of the same mediators of vasodilation (prostaglandins and histamine) also stimulate increased vascular permeability. Vasodilation in conjunction with increased permeability allows the transport of intravascular fluid, protein, and cellular components into the extravascular space. The extravasation fluid and migration of cells result in wound edema.

Leukocyte Migration and Chemotaxis

Although plasma passively leaks between endothelial gaps and proteins adhere to the wound matrix, leukocytes undergo the active process of diapedesis to enter the wound. Selectins provide weak adherence between leukocytes and the endothelium of capillaries. Stronger bonds are created between leukocytes, surface integrins, and intercellular adhesion molecules on the endothelial surface. Cell migration from the endothelial surface into the extravascular space of the wound is mediated by numerous chemical factors and is known as chemotaxis. Chemotactic agents can include complement factors, histamine, bacterial products, prostaglandins,
### Table 1
Platelet alpha granule components and their role in wound healing

<table>
<thead>
<tr>
<th>Adhesion</th>
<th>Glycoproteins</th>
<th>Proteoglycans</th>
<th>Hemostasis Factors &amp; Cofactors</th>
<th>Cellular Mitogens</th>
<th>Protease Inhibitors</th>
<th>Miscellaneous</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>• Fibronectin</td>
<td>• PF4</td>
<td>• Fibrinogen</td>
<td>• PDGF</td>
<td>• α2-Macroglobulin</td>
<td>• IgG, IgA, IgM</td>
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<td></td>
<td>• Vitronecin</td>
<td>• βTG</td>
<td>• Factor V, VII, XI, XII</td>
<td>• TGF-β</td>
<td>• α2-Antitrypsin</td>
<td>• Albumin</td>
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<td></td>
<td>• Thrombospondin</td>
<td>• Serglycin</td>
<td>• Kininogens</td>
<td>• EGF</td>
<td>• α2-Antiplasmin</td>
<td>• GPIa/multimerin</td>
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<td>• vWF</td>
<td>• HRGP</td>
<td>• Protein S</td>
<td>• VEGF/VPF</td>
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<td>• Plasminogen</td>
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<td>• Interleukin-β</td>
<td>• α2-PI</td>
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<td>• PN-2/APP</td>
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<td>• C1 inhibitor</td>
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</table>

Neutrophils

Neutrophils are the first subset of leukocytes to enter the wound. Stimulated by prostaglandins, complement, IL-1, tumor necrosis factor alpha (TNF-α), transforming growth factor-beta TGF-β, PF4, and bacterial products, neutrophils arrive at the injury site in large numbers within 24 to 48 hours after wounding.3–5 At this time point, neutrophils can make up 50% of all cells present within the wound. The primary functions of neutrophils are to defend the wound from bacteria and remove tissue debris. Neutrophils release several types of proteolytic enzymes, breaking down bacteria and extracellular matrix within the site of injury. Protease inhibitors protect tissue not involved in the inflammatory process. Degraded bacterial and matrix debris are removed from the wound by neutrophil phagocytosis. In addition to proteases, neutrophils produce reactive oxygen free radicals that combine with chlorine to make the wound less hospitable to bacteria.7 The secondary role of neutrophils is to perpetuate the early phase of the inflammatory process through the excretion of cytokines.3 One cytokine of particular importance is TNF-α. TNF-α amplifies neutrophil chemotaxis and stimulates macrophage, keratinocyte, and fibroblast expression of growth factors needed in angiogenesis and collagen synthesis. Neutrophils do not directly contribute to collagen deposition or wound strength.3 In time, neutrophils are eliminated from the wound by either apoptosis or macrophage phagocytosis.

Macrophages

At 48 to 96 hours after wounding, the predominant leukocyte within a wound is the macrophage. Derived from extravasated monocytes, macrophages are essential to wound healing. They perform diverse tasks throughout both the inflammatory and proliferative phases of wound healing. Macrophages, like neutrophils, remove wound debris through the continuation of phagocytosis, proteases secretion, and bacterial...
sterilization. Serving as a primary source of numerous cytokines and growth factors, macrophages are necessary to support cellular recruitment and activation, matrix synthesis, angiogenesis, and remodeling. Unlike neutrophils, macrophages remain within a wound until healing is complete (Table 2).

**T Lymphocytes**
Attracted to the site of injury by interleukin-2 (IL-2) and other factors, T lymphocytes populate the wound to a lesser degree than macrophages. By week 2, lymphocytes represent the predominant leukocyte cell type within the wound. Lymphocytes are thought to be critical to the inflammatory and proliferative phases of repair. In addition to providing cellular immunity and antibody production, lymphocytes act as mediators within the wound environment through the secretion of lymphokines and direct cell-to-cell contact between lymphocytes and fibroblasts. The details of how lymphocytes contribute to healing are not fully understood.

**Mast Cells**
Another leukocyte recruited during inflammation is the mast cell. Mast cells can achieve a five-fold increase in number at the site of injury. Granules within the mast cells contain histamine, cytokine (TNF-α), prostaglandins, and protease. Degranulation leads to enhanced vascular permeability, cellular activation, collagen deposition, and remodeling (Fig. 3).

**PROLIFERATION**
The events of inflammation lead to wound debridement. Once debrided, wound healing enters a constructive phase of repair. This stage of wound healing is referred to as the proliferative phase. Proliferation takes place around postinjury days 4 through 12. During this time period, fibroblasts, smooth muscle cells, and endothelial cells infiltrate the wound as epithelial cells begin to cover the site of injury. In concert, these cells reestablish tissue continuity through matrix deposition, angiogenesis, and epithelialization.³

**Fibroplasia and Myofibroblasts**
Fibroblasts are one of the last cell populations to enter the wound. They are mobilized to the site of injury by products of the cell lines that came before them. The first signals for fibroblast recruitment comes from platelet-derived products: platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-1), and TGF-β. The maintenance of fibroblasts within the wound is achieved through paracrine and autocrine signals. Macrophages and fibroblasts release numerous growth factors and cytokines that contribute to fibroblast migration: fibroblast growth factor (FGF), IGF-1, Vascular endothelial growth factor (VEGF), IL-1, IL-2, IL-8, PDGF, TGF-α, TGF-β, and TNF-α.⁸,⁹ Of these substances, PDGF is the most potent chemotactic and mitogenic factor for fibroblasts and their progenitor smooth muscle cells.³ Fibroblasts that migrate from surrounding tissue to the wound edge are activated by PDGF and endothelial growth factor (EGF) to proliferate and begin synthesizing collagen. Additionally, these fibroblasts are capable of producing matrix metalloproteinases (MMP). Secretion of MMPs allows for the degradation of the matrix obstructive to fibroblast migration.² There is a second population of fibroblasts that reside within the wound. Mediated by TGF-β, these “wound fibroblasts” differ from the fibroblasts that migrate from the surrounding tissue. They proliferate less, synthesize more collagen, and transform into myofibroblasts involved in matrix contraction. Fibroplasia is regulated by
<table>
<thead>
<tr>
<th>Phagocytosis &amp; Bacterial Stasis</th>
<th>Debridement</th>
<th>Cellular Recruitment &amp; Activation</th>
<th>Matrix Synthesis</th>
<th>Angiogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Oxygen free radicals</td>
<td>• Collagenase</td>
<td>• Growth factors: PDGF, TGF-β, EGF</td>
<td>• Growth factors: PDGF, TGF-β, EGF</td>
<td>• Growth factors: PDGF, TGF-β, EGF</td>
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<tr>
<td>• Nitric Oxide</td>
<td>• Elastase</td>
<td>• Cytokines: TNF-α, IL-1, IL-6</td>
<td>• Cytokines: TNF-α, IL-1, IFN-γ</td>
<td>• Cytokines: TNF-α</td>
</tr>
<tr>
<td></td>
<td>• Matrix metalloproteinase</td>
<td>• Fibronectin</td>
<td>• Enzymes: arginase, collagenase</td>
<td>• Nitric oxide</td>
</tr>
</tbody>
</table>

substances that inhibit fibroblast recruitment and mitogenesis: interferon-inducible protein (IP-10), interferons, and PF4.10

Matrix Deposition

In addition to mediating fibroplasia, PDGF and TGF-β play important roles in matrix deposition. Both of these growth factors stimulate the fibroblast production of a provisional matrix. The matrix consists of fibroblast-derived collagen monomer, proteoglycans, and fibronectin. Together these substances reestablish the continuity of connective tissue between the wound edges. As the matrix is created, TGF-β also functions to provide structural stability though decreasing protease activity, increasing tissue inhibitors of metalloproteinase, and augmenting production of cell adhesion proteins.10,11

Collagen and Proteoglycan Synthesis

Collagen, the most abundant protein in the body, exists in at least 20 subtypes.12 Two subtypes are important to wound repair. Type I collagen predominates the extracellular matrix of intact skin. Type III collagen, present in lesser amounts in undamaged skin, becomes more principal in the process of wound healing. Collagen synthesis begins hours after wounding, but it does not become significant until roughly 1 week postinjury. The activation of fibroblast to synthesize collagen is derived from growth factors and the metabolic environment within the wound. Collagen gene expression is mediated by promoter-binding sites for corticoids, TGF-β, and retinoids. Increasing concentrations of lactate or the hypoxic environment within the wound can also stimulate collagen gene transcription and processing.7 Lactate converts NAD+ to nicotinamide adenine
dinucleotide (NADH). This depletes the availability of NAD+ to be converted into adenosine diPhosphate ribosome (ADPR). ADPR is an inhibitor of collagen mRNA transcription and other steps of collagen transport. Thus, a reduction in ADPR leads to increase in collagen mRNA synthesis. Collagen transcription occurs within the nucleus of the fibroblast. The transcribed mRNA is processed and translated by ribosomes. The resultant polypeptide chain has a repeated triplet pattern with a praline or lysine in the second position and a glycine in every third position. This protocollagen is roughly 1000 amino acids in size. On entering the endoplasmic reticulum, the protocollagen undergoes hydroxylation and glycosylation. The process of hydroxylation requires the presence of cofactors (oxygen and iron), cosubstrate (a-ketogultarate), and an electron donor (ascorbic acid). Hydrogen bond formation is altered in the hydroxylated and glycosylated protocollagen chain, resulting in an α-helix. Protocollagen becomes procollagen as three α-helical chains wrap together in a right-handed superhelix. Procollagen is packaged within the Golgi apparatus and exported into the extracellular matrix. Within the extracellular space, a procollagen peptidase cleaves the ends of the chains, allowing for further cross-linking and polymerization. The covalent bond formation increases the strength of the resulting collagen monomer.

In addition to collagen, fibroblasts produce and secrete glycosaminoglycans. Typically, glycosaminoglycans couple with protein to become sulfated, polysaccharide chains known as proteoglycans. Proteoglycans are thought to be a primary constituent of the “ground substance” of granulation tissue. As the collagen matrix replaces the fibrin clot, proteoglycans may provide a supportive role for the assembly of collagen fibrils.

Angiogenesis

Vascular damage incurred through wounding undergoes the restorative process of angiogenesis. Angiogenesis begins within the first 1 to 2 days after vessel disruption and can become visibly evident by approximately 4 days postinjury. Endothelial cells from intact venules migrate from the periphery to the edge of the wound. Replication follows migration and new capillary tubules form. Integrins (αv, β3) upregulate on the endothelial cell surface, allowing for enhanced adhesion. Proteolytic degradation of the surrounding wound matrix facilitates the advancement of new vessels across the wound. In closed wounds, tubules from opposing edges quickly coalesce to revascularize the wound. Unlike closed wounds, the new capillary tubules of an unclosed wound merge with the adjacent vessel growing in the same direction, which contributes to the formation of granulation tissue. The events of angiogenesis are regulated by a milieu of growth hormones (TNF-α, TGF-β, VEGF, FGF, PDGF) derived from platelets, macrophages, and damaged endothelial cells. In addition to these mediators, the metabolic environment of the wound influences angiogenesis. Increased lactate along with decreased pH and oxygen tension contribute to a reduction in NAD+, an inhibitor of angiogenesis (Fig. 4).

Epithelialization

Much like angiogenesis, restoration of the epithelium begins early in healing, but it is not readily apparent until several days after wounding. Epithelialization reestablishes the external barrier that minimizes fluid losses and bacterial invasion. The process of epithelialization begins with epidermal thickening along wound edges. Basal cells at the margins of the wound elongate. Attachments between hemidesmosomes of the basal cells and the laminin of the basal lamina are broken down, allowing the cells to migrate. Migratory movements are facilitated by the expression of new integrins at the cell surface. Intracellular production and contraction of actinomycin also contribute to the forward progression of cells across the wound.
are capable of secreting MMP to breakdown fibrin in the course of their migration. The movement of basal cells parallels the direction of collagen fiber orientation within the wound, a process termed "contact guidance." Epithelial cells will continue to migrate and proliferate until they come in contact with epithelial cells traveling from other directions. Contact inhibition signals the epithelial cells to cease their migratory effort. A new monolayer of epithelium is created over the site of injury. Cells in this layer differentiate to take on a less elongated and more cuboidal or basal cell appearance. Hemidesmosomes bind once again to the basement membrane, reattaching these basal-like cells. Subsequent cellular proliferation leads to reestablishment of a multilayer epidermis. The events of epithelialization are influenced by intercellular signals, growth factors, and the metabolic environment within the wound. Low oxygen tension within the wound leads to increased production of TGF-β. TGF-β helps keep epithelial cells from differentiating, allowing for ongoing migration and mitogenesis. TGF-α and keratinocyte growth factor (KGF) more directly stimulate cellular replication. Conversely, moisture and higher oxygen tension support the differentiation of epithelial cells to complete the later events of epithelialization.

**MATURATION AND REMODELING**

In summary, the events of repair began with hemostasis and creation of a fibrin-fibronectin clot. Thrombus degradation followed with the arrival of inflammatory neutrophils and macrophages. Fibroplasia provided the ground substance made up of glycosaminoglycans, proteoglycans, and other proteins to support collagen deposition. New vessels navigated through this matrix as the new epithelium traversed the wound. The final events of repair remain collagen remodeling and strengthening.

**Collagen Maturation**

The last and longest event of wound healing is collagen maturation, starting 1 week postinjury and continuing for any where from 12 to 18 months. During this time period,
the collagen matrix continually undergoes reabsorption and deposition to remodel and strengthen the wound. The initial collagen matrix differs in content and organization from that of uninjured connective tissue. Intact tissue is composed of 80% to 90% type I collagen and 10% to 20% type III collagen. In contrast, the collagen matrix of an early wound consists of 30% type III collagen. The higher proportion of type III collagen contributes to a weaker matrix. Additionally, collagen fibrils within the matrix are more heavily glycosylated and thinner. These fibers are in a parallel orientation and do not interlace. At 1 week, the matrix strength is 3% of unwounded tissue. Collagenases and proteases cleave and degrade these early collagen fibrils.11 This process is countered ongoing by collagen deposition. Newly deposited collagen increases in thickness, strength, and organization. Lysyl oxidase promotes cross-linking between fibrils.11 With time, the ratio of type I to type II collagen approximates that of intact connective tissue.7 By 3 weeks, the tissue strength increases to 30%. After 3 months, the tissue achieves a maximum of 80% its original strength (Fig. 5).10

Healed wounds are not capable of completely restoring the quality structure of intact tissue. The ability to closely approximate uninjured tissue is heavily dependent on size, depth, location and type of wound, as well as the nutritional status, wound care, and overall health of the patient.

An understanding of the basic science of wound healing is crucial to the clinician. Limitless intrinsic and extrinsic patient factors affect each step of this complex process. By understanding the underlying biology, we can significantly influence our patients’ ability to heal.

REFERENCES