7

Wounds: Biology, Pathology, and Management

H. Peter Lorenz and Michael T. Longaker

Wound Biology

The overlapping segments of the repair process are conceptually defined as inflammation, proliferation, and remodeling. During the inflammatory phase, hemostasis occurs and an acute inflammatory infiltrate ensues. The proliferative phase is characterized by fibroplasia, granulation, contraction, and epithelialization. The final phase is remodeling, which is commonly described as scar maturation (Fig. 7.1).

Inflammation

Inflammation is the first stage of wound healing. After tissue injury, the lacerated vessels immediately constrict and thromboplastic tissue products, predominantly from the subendothelium, are exposed. Platelets aggregate and form the initial hemostatic plug. The coagulation and complement cascades are initiated. The intrinsic and extrinsic coagulation pathways lead to activation of prothrombin to thrombin, which converts fibrinogen to fibrin, which is subsequently polymerized into a stable clot.

As thrombus is formed, hemostasis in the wound is achieved (Fig. 7.2). The aggregated platelets degranulate, releasing potent chemoattractants for inflammatory cells, activation factors for local fibroblasts and endothelial cells, and vasoconstrictors (Table 7.1). Platelet adhesiveness is mediated by integrin receptors such as GPIIb/IIIa (αIIbβ3 integrin).[1–3]

Within minutes, the repair processes are initiated. After the transient vasoconstriction induced by platelet factors, local small vessels dilate secondary to the effects of the coagulation and complement cascades. Bradykinin is a potent vasodilator and vascular permeability factor that is generated by activation of Hageman factor in the coagulation cascade.[2] The complement cascade generates the C3a and C5a anaphylatoxins, which directly increase blood vessel permeability and attract neutrophils and monocytes to the wound. These complement components also stimulate the release of histamine and leukotrienes C4 and D4 from mast cells. The local endothelial cells then break cell-to-cell contact, which enhances the margination of inflammatory cells into the wound site.[2]

An efflux of white blood cells (first neutrophils, later monocytes) and plasma proteins enter the wound site (Fig. 7.3). The early neutrophil infiltrate scavenges cellular debris, foreign bodies, and bacteria. Activated complement fragments aid in bacterial killing through opsonization. The primary role of the neutrophil is to sterilize the wound. Accordingly, the initial neutrophil infiltrate is decreased in clean surgical wounds when compared to contaminated or infected wounds.

Within 2 to 3 days, the inflammatory cell population begins to shift to one of monocyte predominance (Fig. 7.4). Circulating monocytes are attracted and infiltrate the wound site.[3,4] These elicited monocytes differentiate into macrophages, and in conjunction with resident macrophages, orchestrate the repair process. Macrophages not only continue to phagocytose tissue and bacterial debris, but also secrete multiple growth factors. These peptide growth factors activate and attract local endothelial cells, fibroblasts, and keratinocytes to begin their respective repair functions. More than 20 different cytokines and growth factors are known to be secreted by macrophages; the primary cells responsible for regulating repair. (Table 7.2).[5,6] Depletion of monocytes and macrophages causes a severe alteration in wound healing with poor debridement, delayed fibroblast proliferation, and inadequate angiogenesis.[7]

Proliferation

Fibroplasia

The proliferative phase begins with the deposition of the fibrin and fibrinogen matrix and the activation and turnover of local fibroblasts (fibroplasia). The initial fibrin–fibrinogen matrix is populated with platelets and macrophages. These

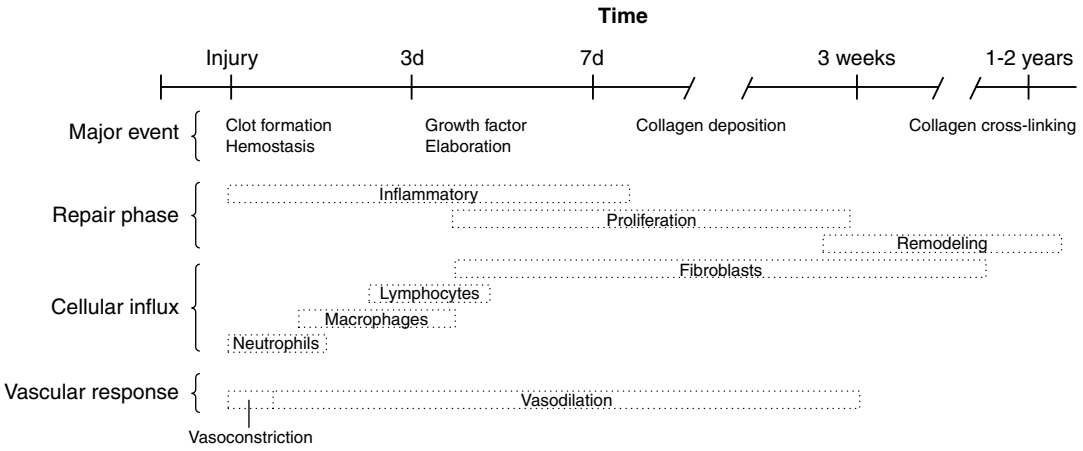

FIGURE 7.1. The temporal relationship of repair stages and cellular infiltrates into the wound. Overlap occurs between the stages, and the beginning and endpoints are approximate.

macrophages and the local extracellular matrix (ECM) release growth factors that initiate fibroblast activation. Fibroblasts migrate into the wound using the newly deposited fibrin and fibronectin matrix as a scaffold. Local fibroblasts become activated and increase protein synthesis in preparation for cell division. As fibroblasts proliferate, they become the prominent cell type by 3 to 5 days in clean, noninfected wounds (Fig. 7.5). After cell division and proliferation, fibroblasts begin synthesis and secretion of extracellular matrix products.

Integrins are transmembrane proteins with extracellular, membrane, and intracellular domains. They are heterodimeric and composed of alpha and beta subunits that interact to form the active protein receptor. Ligands include growth factors, ECM structural components such as collagen, elastin, and other cells. After ligands bind, a structural change occurs in the cytoplasmic domain of the integrin receptor and phosphorylation occurs. Signal transduction ultimately results in transcription factor synthesis and new gene expression resulting in new cellular function.

The initial wound matrix is provisional and is composed of fibrin and the glycosaminoglycan (GAG), hyaluronic acid.[4] Because of its large water of hydration, hyaluronic acid provides a matrix that enhances cell migration. As fibroblasts enter and populate the wound, they utilize hyaluronidase to digest the provisional hyaluronic acid–rich matrix, and larger, sulfated GAGs are subsequently deposited. Concomitantly, collagens are deposited by fibroblasts onto the fibronectin and GAG scaffold in a disorganized array.

Collagen types I and III are the major fibrillar collagens comprising the extracellular matrix and are the major structural proteins both in unwounded and wounded skin. There are now at least 19 different types of collagens described, each of which shares the right-handed triple helix as the basic structural unit.[8–12] Most collagen types are synthesized by fibroblasts; however, it is now known that some types are synthesized by epidermal cells.[13]

Granulation

Granulation tissue is present in wounds healing by secondary intention. This tissue is clinically characterized by its beefy-red appearance (i.e., "proud flesh"), which is a consequence of the rich bed of new capillary networks (neoangiogenesis) that have formed from endothelial cell division and migration. The directed growth of vascular endothelial cells is stimulated by platelet and activated macrophage and fibroblast products. One example is vascular endothelial growth factor, which is secreted by macrophages and acts to induce migra-

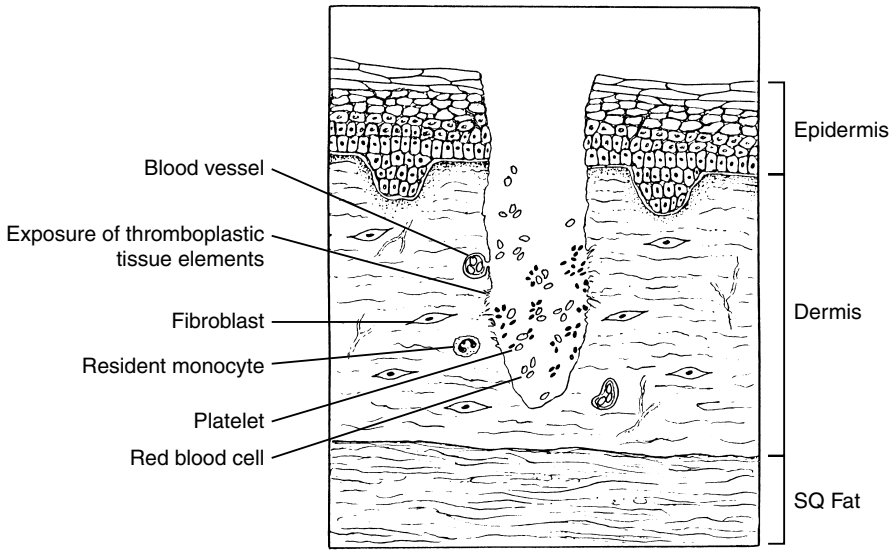

FIGURE 7.2. Immediately after tissue injury, hemostasis is stimulated by platelet degranulation and exposure of tissue thromboplastic agents.

TABLE 7.1. Platelets Contain Both Alpha and Dense Granules, Which Are Released at the Wound Site.

Substance	*Biological effect*
Alpha granules	
Platelet-derived growth factor	Matrix deposition
Transforming growth factor-β	Matrix deposition
Transforming growth factor-α	Epithelialization
Insulin-like growth factor-binding protein-3	Matrix deposition
Platelet factor-4	Activation of growth factors
β-Thromboglobulin	Activation of growth factors
Dense granules	
Adenosine diphosphate	Platelet aggregation
Calcium	Platelet aggregation
Serotonin	Vasoconstriction
Cytosol	
von Willebrand factor VIII	Mediator of platelet adhesion
Fibronectin	Ligand for platelet aggregation
Fibrinogen	Ligand for platelet aggregation
Thrombospondin	Ligand for platelet aggregation
Factor V	Hemostasis
Platelet-activating factor	Platelet activation
Thromboxane-A2	Vasoconstriction
12-Hydroxyeicosatetranoic acid (12-HETE)	Vasoconstriction

In addition, multiple activators and mediators of the hemostatic cascades are present on the platelet surface membrane (see Chapter 9). The substances released have myriad effects on the repair process. Vascular endothelial growth factor has recently been found to be present within platelets but its exact intracellular location is unknown.[29]

Source: Data were compiled from several sources (reviewed in References 1, 2, 3).

tion and proliferation of endothelial cells. Granulation tissue is a dense population of blood vessels, macrophages, and fibroblasts embedded within a loose provisional matrix of fibronectin, hyaluronic acid, and collagen.

The presence of granulation tissue is used as a clinical indicator that the wound is ready for skin graft treatment. Wounds that benefit from skin grafts are of sufficient size such that the healing time would be decreased. The high degree of vascularity enables granulation tissue to readily accept and support skin grafts.

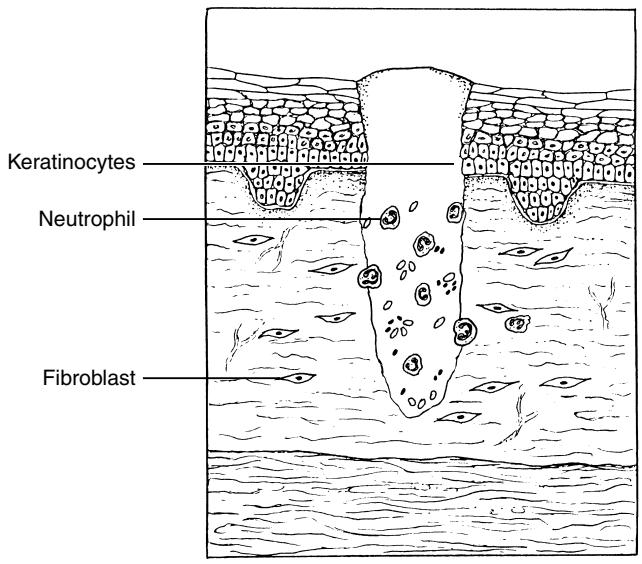

FIGURE 7.3. Within 24 hours, a neutrophil efflux into the wound occurs. The neutrophils scavenge debris, bacteria, and secrete cytokines for monocyte and lymphocyte attraction and activation. Keratinocytes begin migration when a provisional matrix is present.

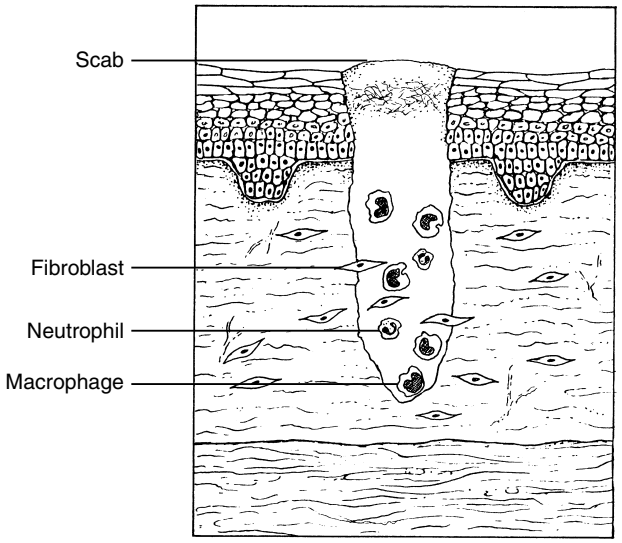

FIGURE 7.4. At 2 to 3 days after injury, the macrophage becomes the predominant inflammatory cell type in clean, noninfected wounds. These cells then regulate the repair process by secretion of a myriad of growth factors, including types that induce fibroblast and endothelial cell migration and proliferation.

Contraction

Open wounds are characterized by contraction, a phenomenon not present in closed surgical incisions. Open wounds occur after trauma, burns, and when previously closed wounds are secondarily opened because of infection. Contraction is the process in which the surrounding skin is pulled circumferentially toward the wound. Wound contraction decreases the size of the wound dramatically without new tissue formation. This repair component allows the wound to close, and thus heal much more rapidly than by epithelialization alone. In addition, the area of insensate scar is smaller.

The amount of contraction is related to the size of the wound and mobility of the skin. In humans, contraction is greatest in the trunk and perineum, least on the extremities, and intermediate on the head and neck. Up to 80% of wound closure can be caused by contraction in the trunk and perineum. These regional differences are thought to result from the relative differences in skin laxity in those areas.

Clinically, wound contraction can lead to contracture, which distorts tissue and leads to decreased function. For example, wound contraction across a joint can lead to a contracture in that joint. The range of motion, and thus function, of the joint is diminished. Contractures can develop in the extremities, eyelids, neck, spine, and fingers.

Epithelialization

Within hours after injury, morphological changes in keratinocytes at the wound margin are evident. In skin wounds, the epidermis thickens, and marginal basal cells enlarge and migrate over the wound defect (see Fig. 7.5). Once these epithelial cells begin migrating, they do not divide until epidermal continuity is restored. New epithelial cells for

TABLE 7.2. A Partial List of Growth Factors Present at the Wound Site.

Growth factor	*Cellular source*	*Target cells*	*Biological activity*
TGF-β_1, TGF-β_2	Macrophages, platelets, fibroblasts, keratinocytes	Inflammatory cells, keratinocytes, fibroblasts	Chemotaxis, proliferation, matrix production (fibrosis)
TGF-β_3	Macrophages	Fibroblasts	Anti-scarring?
TGF-α	Macrophages, platelets, keratinocytes	Keratinocytes, fibroblasts, endothelial cells	Proliferation
TNF-α	Neutrophils	Macrophages, keratinocytes, fibroblasts	Activation of growth factor expression
PDGF	Macrophage, platelets, fibroblasts, endothelial cells, vascular smooth muscle cells	Neutrophils, macrophages, fibroblasts, endothelial cells, vascular smooth muscle cells	Chemotaxis, proliferation, matrix production
FGF-1, FGF-2, FGF-4	Macrophage, fibroblasts, endothelial cells	Keratinocytes, fibroblasts, endothelial cells, chondrocytes	Angiogenesis, proliferation, chemotaxis
FGF-7 (KGF)	Fibroblasts	Keratinocytes	Proliferation, chemotaxis
EGF	Platelets, macrophages, keratinocytes	Keratinocytes, fibroblasts, endothelial cells	Proliferation, chemotaxis
IGF-1/Sm-C	Fibroblasts, macrophages, serum	Fibroblasts, endothelial cells	Proliferation, collagen synthesis
IL-1α and IL-1β	Macrophages, neutrophils	Macrophages, fibroblasts, keratinocytes	Proliferation, collagenase synthesis, chemotaxis
CTGF	Fibroblasts, endothelial cells	Fibroblasts	Downstream of TGF-β_1
VEGF	Macrophages, keratinocytes	Endothelial cells	Angiogenesis

Redundant biological effects through both autocrine and paracrine mechanisms are apparent.

TGF-β, transforming growth factor-beta; TGF-α, transforming growth factor-alpha; TNF-α, tumor necrosis factor-alpha; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; KGF, keratinocyte growth factor; EGF, epidermal growth factor; IGF-1, insulin-like growth factor-1; Sm-C, somatostatin-C; IL-1, interleukin-1; CTGF, connective tissue growth factor; VEGF, vascular endothelial cell growth factor.

wound closure are provided by fixed basal cells in a zone near the edge of the wound.[14] Their daughter cells flatten and migrate over the wound matrix as a sheet (epiboly). Cell adhesion glycoproteins, such as tenascin and fibronectin, provide the "railroad tracks" to facilitate epithelial cell migration over the wound matrix. Following the reestablishment of the epithelial layer, keratinocytes and fibroblasts secrete laminin and type IV collagen to form the basement membrane.[13] The keratinocytes then become columnar and divide as the layering of the epidermis is established, thus reforming a barrier to further contamination and moisture loss.

Interestingly, keratinocytes can respond to foreign-body stimulation with migration as well. Sutures in skin wounds provide tracts along which these cells can migrate. Subsequent epithelial thickening and keratinization produce fibrotic reactions, cysts, or sterile abscesses centered on the suture. These are treated by removal of the inciting suture and epithelial cell sinus tract or cyst.

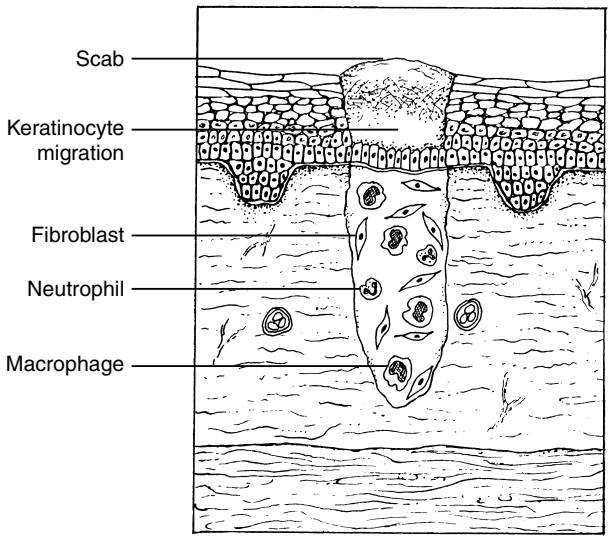

FIGURE 7.5. Fibroblasts are activated and present at the wound by 3 to 5 days after injury. These cells secrete matrix components and growth factors that continue to stimulate healing. Keratinocyte migration (epiboly) begins over the new matrix. Migration starts from the wound edges as well as from epidermal cell nests at sweat glands and hair follicles in the center of the wound.

Remodeling Phase

The ECM is the scaffold that supports cells in both the unwounded and wounded states. The ECM is dynamic and during repair is constantly undergoing remodeling. The regulation of the remodeling is poorly understood, but simplistically can be conceptualized as the balance between synthesis, deposition, and degradation. Lysyl oxidase is the major intermolecular collagen cross-linking enzyme.[15] Collagen cross-linking improves wound tensile strength. Collagenases, gelatinases, and stromelysins are matrix metalloproteinases (MMPs) that degrade ECM components.The balance of collagen deposition and degradation is in part determined by the regulation of MMP activity. Proteins called tissue inhibitor of matrix metalloproteinases (TIMPs) specifically inactivate the MMPs.[16] Current investigation is examining the regulation of MMP–TIMP balance during wound ECM remodeling.[17,18]

Ultimately, the outcome of mammalian wound healing is scar formation (Fig. 7.6). Scar is defined morphologically as the lack of tissue organization compared to surrounding normal tissue architecture and is characterized by disorganized collagen deposition. New collagen fibers secreted by fibrob-

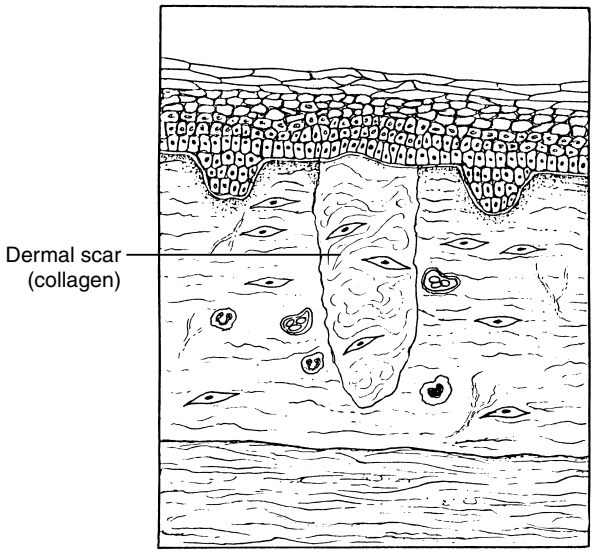

FIGURE 7.6. Scar formation is the outcome of healing in postnatal skin. Scar is composed of densely packed, disorganized collagen fiber bundles. Remodeling occurs up to 1 to 2 years after injury and consists of further collagen cross-linking and regression of capillaries, which account for the softening of scar and its color change from red to white.

lasts are present as early as 3 days after wounding. As the collagenous matrix forms, densely packed fibers fill the wound site. The ultimate pattern of collagen in scar is one of densely packed fibers and not the reticular pattern found in unwounded dermis.

During remodeling, wounds gradually become stronger with time. Wound tensile strength increases rapidly from 1 to 8 weeks post wounding. Thereafter, tensile strength increases at a slower pace and has been documented to increase up to 1 year after wounding in animal studies (Fig. 7.7). However, the tensile strength of wounded skin at best only reaches approximately 80% that of unwounded skin.[19] The final result of tissue repair is scar, which is brittle, less elastic than normal skin, and does not contain any skin appendages such as hair follicles or sweat glands. The major benefit of repair by scar is the relatively rapid reformation of tissue integrity.

Regulation of Wound Repair

Growth Factors

Growth factors play a prominent role in the regulation of chemotaxis and wound healing. These polypeptides are released by a variety of activated cells at the wound site (see Table 7.2). They act in either paracrine or autocrine fashion to stimulate or inhibit protein synthesis by cells in the wound, and many have overlapping functions.

Platelet-Derived Growth Factor

Platelet-derived growth factor (PDGF) is released from platelet alpha-granules immediately after injury. PDGF attracts neutrophils, macrophages, and fibroblasts to the wound and serves as a powerful mitogen. Macrophages, endothelial cells, and fibroblasts also synthesize and secrete PDGF. Platelet-derived growth factor stimulates fibroblasts to synthesize new extracellular matrix, predominantly noncollagenous components such as GAGs and adhesion proteins. PDGF also increases the amount of fibroblast-secreted collagenase, indicating a role for this cytokine in tissue remodeling.[20,21]

Transforming Growth Factor–Beta

Transforming growth factor–beta (TGF–β) directly stimulates collagen synthesis and decreases extracellular matrix degradation by fibroblasts.[22,23] It is released from platelets and macrophages at the wound. In addition, TGF-β is released from fibroblasts and keratinocytes. TGF-β acts in an autocrine fashion to further stimulate its own synthesis and secretion. TGF-β also chemoattracts fibroblasts and macrophages to the wound.

TGF-β is a profibrotic growth factor which accelerates wound repair at the expense of increased fibrosis. It has been implicated in pathological fibrosis in multiple different organ systems and is thought to be a factor in the formation of intestinal adhesions. TGF-β stimulates ECM synthesis and accumulation, increases integrin expression and therefore enhances cell–matrix interactions.

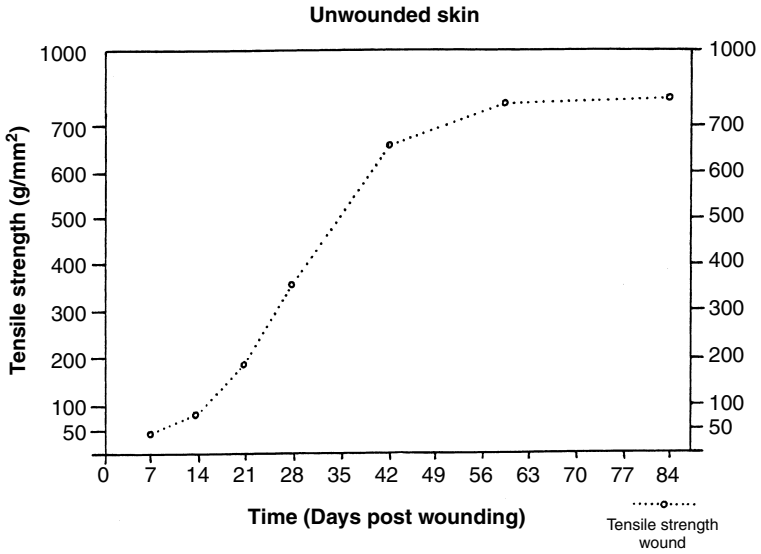

FIGURE 7.7. Wound tensile strength as a function of time. Maximal wound tensile strength is 75%–80% of unwounded skin. (Modified from Levenson SM, Geever EF, Crowley LV, Oaks JF, Berard CW, Rosen H. The healing of rat skin wounds. Ann Surg 1965;161:293–308, with permission.)

Fibroblast Growth Factors

Angiogenesis is stimulated by acidic and basic fibroblast growth factors (FGF-1 [or aFGF] and FGF-2 [or bFGF], respectively).[24] Endothelial cells, fibroblasts, and macrophages produce FGF-1 and FGF-2. The FGFs stimulate endothelial cells to divide and form new capillaries. They also chemoattract endothelial cells and fibroblasts.

Vascular Endothelial Growth Factor

Vascular endothelial growth factor is also a potent angiogenic stimulus.[25,26] It acts in a paracrine manner to stimulate proliferation by endothelial cells after release from platelets, macrophages, fibroblasts, and keratinocytes.[27–29] Its expression is also increased in hypoxic conditions, such as those found at the wound site.[28]

Other Growth Factors

Epithelialization is directly stimulated by at least two growth factors: epidermal growth factor (EGF) and keratinocyte growth factor (KGF [or FGF-7]).[24,30] EGF is released by keratinocytes to act in an autocrine fashion, whereas KGF is released by fibroblasts to act in a paracrine fashion to stimulate keratinocyte division and differentiation.

Multiple other growth factors affect wound repair. For example, insulin-like growth factor–I (IGF-I) stimulates collagen synthesis by fibroblasts, and IGF–I functions synergistically with PDGF and bFGF to facilitate fibroblast proliferation.[31] Interferon-gamma has been shown to downregulate collagen synthesis. The various interleukins mediate inflammatory cell functions at the wound site (see Chapter 4).[32]

The Extracellular Matrix

The ECM is a depository of growth factors in latent forms under normal, unwounded conditions. With injury and matrix destruction, the previously bound and inactive growth factors are released in active form and thereby assist in initiating and regulating the repair process.

Scarless Repair: The Fetal Paradigm

Unlike the adult, the early-gestation fetus can heal skin wounds with regenerative-type repair and not with scar formation.[33,34] The epidermis and dermis are restored to a normal architecture in which the collagen matrix pattern is reticular and unchanged from unwounded dermis. The wound hair follicle and sweat gland patterns are normal.

Interestingly, inflammation plays a prominent role in postnatal repair, but it is not present in significant amounts in fetal skin wounds. An understanding of the biology of scarless fetal wound repair will help surgeons develop therapeutic strategies to minimize scar and fibrosis.

Wound Pathology

Nonhealing Wounds

Chronic or nonhealing wounds are open wounds that fail to epithelialize and close in a reasonable amount of time. These wounds are clinically stagnant and without evidence of further closure. There are likely many reasons why these wounds do not heal, but no broad, unifying theory exists. Simply put, chronic wounds may be thought of as lacking appropriate "start" signals. These wounds can be broadly categorized into three groups: pressure sores, lower extremity ulcers, and radiation skin injury.

Pressure Sores

Pressure sores develop over a bony prominence, usually in the immobile patient. These are frequently called "decubitus ulcers" or "bed sores." The sacrum, ischium, and greater trochanter are the most common locations affected.[35]

Pressure necrosis is a function of the amount of pressure on the tissue and duration of pressure. Microcirculation is compromised when the tissue pressure is greater than 25 to 30 mmHg, which blocks capillary perfusion pressure.[36] Necrosis can occur with as little as 2 h of sustained pressures at this level.[36] Skin is more resistant to pressure necrosis than the underlying fat and muscle, which explains the common finding of a small area of skin ulceration overlying a large area of subcutaneous fat and muscle necrosis.

To begin treatment of these patients, efforts should be made to control the factors leading to increased pressure. Paralyzed patients require periodic rotation and air mattress or other types of low-pressure beds. In addition, behavior and contractures may need to be addressed. Tight-fitting casts should be removed and replaced by those with no excess pressure. Other contributing factors should be identified and controlled, such as malnutrition, infection, and diabetes control. Necrotic tissue requires debridement.

With avoidance of pressure over the involved area, most pressure sores heal. However, they heal with scar formation, which is less resistant to trauma than intact skin. Thus, a higher incidence of recurrence exists after spontaneous closure of these wounds than if they are closed surgically with flaps of normal skin and muscle over the bony prominence.[37,38]

Lower-Extremity Ulcers

Leg ulcers generally arise from either one of two different vascular diseases: arterial or venous insufficiency. Most (80%–90%) result from venous valvular disease (venous insufficiency).[35,39,40] Increased venous pressure in the dependent lower extremity leads to localized edema and tissue necrosis. Tissue edema is thought to be a major inhibitor of repair at the ulcer site, but the exact mechanism is not known. Oxygen delivery and diffusion are likely impaired. Postcapillary obstruction leads to an increased perfusion pressure and hypoxia. Protein and red blood cell extravasation occurs, which further limits diffusion and oxygen delivery.

Arterial insufficiency to the lower extremity greatly impairs healing. Minor trauma, resulting from scratches and abrasions that would otherwise heal quickly in a normal patient, can progress into large wounds and ultimately necrotizing infection that is not only limb- but also life-threatening. A reliable clinical sign of adequate arterial inflow is the simple presence of an arterial pulse. If a single palpable pulse is present in the foot, then most wounds will heal. A nonhealing wound in an ischemic extremity is generally regarded as an indication for revascularization of that extremity.

Radiation Injury

External beam radiation through skin to treat deep pathology has both acute and chronic effects on skin. Acutely, a self-limiting erythema may develop that spontaneously resolves. Its late effect can be a more significant injury to fibroblasts, keratinocytes, and endothelial cells. DNA damage to these cells propagates over time and impairs the ability of these cells to divide successfully. Ultimately, a skin ulcer may occur spontaneously, but usually it occurs after repeated mild trauma such as abrasions.[41] If a surgical incision needs to be placed through an area of irradiated skin, then that incision is not likely to heal. Currently the only treatment modalities for these wounds are hyperbaric oxygen therapy or coverage with vascularized tissue flaps.

Clinical Factors Affecting Repair

Infection

Wound infection is an imbalance between host resistance and bacterial growth.[42] Bacterial infection impairs healing through several mechanisms.[43] At the wound site, acute and chronic inflammatory infiltrates slow fibroblast proliferation and thus slow ECM synthesis and deposition. Although the exact mechanisms are not known, sepsis causes systemic effects that can impede the repair processes.

Open wounds invariably become colonized by bacteria. The wound has no protective barrier to prevent bacterial adherence in the exposed dermis or subcutaneous fat and muscle. Colonization does not preclude healing. However, if bacterial infection occurs, then healing can be not only delayed but also stopped.

A threshold number of bacteria in the wound appears to be necessary to overcome host resistance and cause clinical wound infection. Bacterial contamination results in clinical infection and delays healing if more than 10^5 organisms per gram of tissue are present in the wound.[43,44] Skin grafts on open wounds are likely to fail if quantitative culture shows more than 10^5 organisms/g tissue, which provides further evidence that bacterial load impacts repair.[45] Similarly, well-vascularized muscle flaps heal open wounds successfully if bacterial loads are not greater than 10^5 organisms/g tissue.[46] These studies demonstrate that high levels of bacteria inhibit the normal healing processes.

Treatment of the closed, infected wound depends on whether fluid or necrotic tissue is present. If no fluid is draining or loculated, then cellulitis can be successfully treated with appropriate antibiotics. The wound should be opened, sutures removed, irrigated, and debrided if pus or necrotic tissue is present. Appropriate antibiotic administration following wound cultures treats surrounding cellulitis. Signs of wound infection include fever, tenderness, erythema, edema, and drainage.

Nutrition

Wound healing is an anabolic event that requires additional caloric intake.[47] However, the precise calorie requirements for optimal wound healing have not been determined. Large injuries such as burns greatly increase metabolic rate and nutritional requirements.[48] Severely malnourished and catabolic patients clinically appear to have diminished healing; however, no studies have definitely proved this finding.[49]

Appropriate levels of vitamins C and A are necessary for proper fibroplasia, collagen synthesis and cross linking and epithelialization processes. Animal studies show that vitamin A also reverses the impaired healing that occurs with chronic steroid treatment.[50,51] While this is not proved conclusively in human studies, most surgeons administer vitamin A postoperatively to their patients on steroid therapy. Vitamin A is fat soluble and can be taken in toxic doses, so careful administration is essential. The oral dose is 25,000 U/day.[52]

Vitamin B_6 (pyridoxine) deficiency impairs collagen cross-linking.[53] Vitamin B_1 (thiamine) and vitamin B_2 (riboflavin) deficiencies cause syndromes associated with poor wound repair. Supplementation with these vitamins does not improve healing unless a preexisting deficiency condition is present.

Trace metal deficiencies such as zinc and copper have been implicated with poor wound repair because these divalent cations are cofactors in many important enzymatic reactions.[52] Zinc deficiency is associated with poor epithelialization and chronic, nonhealing wounds. Trace metal deficiency is now extremely rare in both enterally and parenterally fed patients.

Vitamin and mineral excess administration can be detrimental and cause toxicity, especially the fat-soluble vitamins. Adequate amounts are present in today's enteral feeding solutions and as supplemental additives to parenteral solutions. Supplemental administration is only necessary in deficiency states and certain unique clinical situations as described earlier.

Oxygen and Perfusion

Wounds require adequate oxygen delivery to heal. Ischemic wounds heal poorly and have a much greater risk of infection.[54,55] Wound ischemia occurs secondary to a variety of factors: occlusive vascular disease, vasoconstriction, and hypovolemia. Excessive suture tension during wound closure will cause local wound ischemia and result in wound healing complications. Conversely, increased oxygen delivery at the wound improves wound healing.[54–57] Experimentally, collagen synthesis by fibroblasts is increased with supplemental oxygen.[58,59]

Anemia in the normovolemic patient is not detrimental to wound repair so long as the hematocrit is greater than 15%, because oxygen content in blood does not affect wound collagen synthesis.[58]

Diabetes Mellitus and Obesity

Wound healing is impaired in diabetic patients by unknown mechanisms, although recent studies have implicated a lack of KGF and PDGF function in the wound.[60,61] Many of these patients have microvascular occlusive disease that may cause ischemia and impaired repair. Healing is enhanced if glucose levels are well controlled.[62] Obesity interferes with repair independently of diabetes.[63]

Corticosteroids

Both topically applied and pharmacological steroid use impairs healing, especially when given in the first 3 days after wounding.[64,65] Steroids reduce wound inflammation, collagen synthesis, and contraction.[52] The exact mechanisms un-

derlying how steroids impair healing are not fully understood. Because steroids decrease inflammation, they may decrease host bacterial resistance and thus increase wound infection complications. The entire repair process is slowed and risk of dehiscence and infection is increased. As stated previously, vitamin A administration can reverse this effect.[50,51]

Radiation Therapy

Both radiation and chemotherapeutic agents have their greatest effects on dividing cells. The division of endothelial cells, fibroblasts, and keratinocytes is impaired in irradiated tissue, which retards wound healing.

Chemotherapy

During the proliferative phase of repair, numerous cell types are active at the wound site. Antiproliferative chemotherapeutic agents act to slow this process and thus retard healing.[66] Following oncological surgical procedures, in most institutions chemotherapeutic agents are not administered until at least 5 to 7 days postoperatively to prevent impairment of the initial healing events.

Excessive Healing

Normal wounds have stop signals that halt the repair process when the dermal defect is closed and epithelialization is complete. When these signals are absent or ineffective, then the repair process may continue and result in a condition of excessive scar. The underlying regulatory mechanisms leading to excessive repair are not yet known.

To minimize visible scar on skin, elective incisions are least noticeable when placed parallel to the natural lines of skin tension (Langer's lines). This placement location has two advantages: the scar is parallel or within a natural skin crease, which camouflages the scar, and this location places the least amount of tension on the wound. Wound tension prevents thin scar formation. Sharply defined and aligned wound edges approximated without tension heal with the least amount of scar. Infection or separation of the wound edges with subsequent secondary intention repair also results in more scar formation.

Hypertrophic Scar

Hypertrophic scars and keloids are distinguished based on their clinical characteristics. Hypertrophic scars are defined as scars that have not overgrown the original wound boundaries, but are instead raised. They usually form secondary to excessive tensile forces across the wound and are most common in wounds across flexion surfaces, the extremities, breasts, sternum, and neck. Physical therapy with range-of-motion exercises is helpful in minimizing hypertrophic scar as well as joint contracture in the extremities.

Hypertrophic scar is a self-limited type of overhealing after tissue injury, and usually regresses with time. These scars generally fade in color as well as flatten to the surrounding skin level. There has never been a clear histological difference between hypertrophic scar and keloid. In general, both keloid and hypertrophic scar fibroblasts have an upregulation of collagen synthesis, deposition, and accumulation.[67]

Treatment options for hypertrophic scars include the application of pressure garments or topical silicone sheets, laser therapy, and reexcision with primary closure.[68–70] The latter option is most useful in cases of excess scar caused by infection or dehiscence. If the original wound was closed following the basic tenets described above, then reexcision with primary closure is not likely to result in an improved scar compared to the initial procedure. Recurrence of hypertrophic scar is quite high in these circumstances, and therefore most surgeons do not treat hypertrophic scar with excision and primary closure unless they plan adjuvant therapy.

Keloid

Scars that overgrow the original wound edges are called keloids. This clinical characteristic distinguishes keloid from hypertrophic scar. True keloid scar is not common and occurs mainly in darkly pigmented individuals with an incidence of 6% to 16% in African populations.[71] It has a genetic predisposition with autosomal dominant features. The keloid scar continues to enlarge past the original wound boundaries and behaves like a benign skin tumor with continued slow growth.

Keloids consist mainly of collagen. They are relatively acellular in their central portions with fibroblasts present along their enlarging borders. They do not contain a significant excess number of fibroblasts. Collagen scar deposition outpaces degradation, and the lesion continues to enlarge.

No uniformly successful treatment of keloid scar exists. Excision and primary closure invariably result in recurrence. Steroid injection directly into the keloid has the most benefit early in the course.[72]

Patients presenting with mature lesions of months to years duration that are slowly changing respond poorly to steroid injection. The only viable treatment options for mature keloids are to monitor and do nothing or to excise and administer adjuvant therapy.

Clinical Management of Wounds

Primary Intention

Primary intention healing occurs in closed wounds, which are wounds with the edges approximated. These wounds are usually closed in layers along tissue planes. Deep sutures are placed in collagen-rich layers such as fascia and dermis. These layers are strong and can hold sutures with a high degree of tension. Fatty tissue layers, such as subcutaneous fat, do not have significant collagen and cannot hold sutures under tension. For this reason, most surgeons do not close the subcutaneous fat layer, even in the morbidly obese patient.

To decrease scar formation and risk of infection, meticulous hemostasis should be performed. This step limits the amount of hematoma to be cleared, and thus decreases the inflammatory phase and likely decreases scar. Because hematoma is a culture media for bacteria, decreased bleeding also decreases the risk of infection. By limiting inflammation with sterile technique and tight hemostatic control, repair by activated fibroblasts can begin earlier and thus shorten the healing period.

Smaller surgical scars are achieved with no skin edge trauma and less resultant inflammation. Forceps crush-injury of the epidermis and dermis should be avoided by using fine forceps and skin hooks to retract and assist in dermal closure;

this decreases the amount of necrotic tissue at the wound edge and thereby reduces inflammation.

Uncomplicated wounds healing with primary intention epithelialize within 24 to 48 h. At this point, water barrier function has been restored and patients can be allowed to shower or wash. This stage has a psychological benefit during the postoperative recovery period. In addition, gentle cleansing removes old serum and blood, which reduces potential bacterial accumulation and infection risk.

Secondary Intention

Open wounds heal with the same basic processes of inflammation, proliferation, and remodeling as do closed wounds. The major difference is that each sequence is much longer, especially the proliferative phase. There is also much more granulation tissue formation and contraction. This type of healing process is referred to as secondary intention.

Open wound edges are not approximated but are instead separated, which necessitates epithelial cell migration across a longer distance. Before epiboly can occur, a provisional matrix must be present. Granulation tissue must form. There are variable amounts of bacteria, tissue debris, and inflammation present depending on wound location and etiology. Infection, with high protein exudative losses and acute and chronic inflammation, can disregulate repair and transform the healing wound into a clinically nonhealing wound.

During the proliferative phase of an uncomplicated course of secondary intention healing, a bed of granulation tissue is present. If no infection is present and the area is of sufficient size that healing will not be complete for at least 2 to 3 weeks, then placement of a partial or full-thickness skin graft should be considered. Grafts readily adhere to granulation tissue and will quickly speed the repair process. Partial thickness skin graft donor sites can heal in as little as 2 weeks, depending on graft thickness.

Topical Wound Treatment

Local Care

Closed Wounds

Closed wounds healing by primary intention require much less care than open wounds. Closed wounds should be kept

TABLE 7.3. Classes of Wound Dressings and Skin Replacements Currently Available.

Class	*Composition*	*Characteristics/function*	*Commercial examples*
Gauze	Woven cotton fibers	Permeable with desiccation; debridement; painful removal	Curity®
Calcium-alginates	Seaweed polymer that forms a gel when absorbs fluid	Absorbs exudate; nonadherent; nonirritating; requires a cover dressing (permeable)	Algisorb,® Sorbsan®
Impregnated gauzes	Fine mesh fabric (silicone, nylon) with dermal porcine collagens	Nonadherent; semipermeable	Biobrane II®
Films	Plastic (polyurethane); semipermeable	Allows water vapor permeation; adhesive	Opsite,® Tegaderm®
Foams	Hydrophilic (wound side) and hydrophobic (outer side); semipermeable	Necrotic/exudative wounds	Lyofoam,® Allevyn®
Hydrogels	Water (96%) and polymer (polyethyleneoxide)	Aqueous environment; requires secondary dressing; no adherence; not recommended if infection is present; semipermeable	Vigilon,® Aquasorb®
Hydrocolloids	Hydrophilic colloidal particles and adhesive	Absorbs fluid; necrotic tissue autolysis; little adherence; occlusive	Duoderm,® Intrasite®
Absorptive powders and pastes	Starch copolymers, hydrocolloidal particles	Absorbs exudate; used as a filler; good for deep wounds	Geliperm,® Duoderm granules®
Silicone	Silicone sheets	Sheet induces a localized electromagnetic field; decreases scar formation?	Sil-K®
Mechanical vacuum	Vacuum, sponge, plastic film	Sponge conforms to wound and vacuum removes edema fluid; stimulation of repair?	VAC® device
Dermal matrix replacements	Acellular matrix	Permeable; increased stimulation of repair?	Alloderm® (human, dermis), SIS® (porcine, small bowel submucosa), Integra® (bovine collagen, GAG, and silicone epidermal-type layer)
Dermal living replacements	Absorbable matrix populated with fibroblasts	Permeable; increased stimulation of repair?	Dermagraft®
Skin living replacement	Bovine collagen matrix populated with human fibroblasts with an outer layer of human keratinocytes	Impermeable; increased stimulation of repair?	Apligraf® (FDA approved 6/98)

A multitude of brands within each class are available, and only a few examples are listed. Recently, skin replacements have become available. Although expensive, they have great potential clinical usefulness. No particular brands are recommended in any class.

Source: Data partially taken from Feedar JA. Clinical management of chronic wounds. In: McCulloch JM, Kloth LC, Feedar JA, eds. Wound Healing Alternatives in Management. Philadelphia: Davis, 1995:137–185.

sterile for 24 to 48 h until epithelialization is complete. Tensile strength is only 20% of normal skin at 3 weeks when collagen cross-linking is becoming significant. At 6 weeks, wounds are at 70% of the tensile strength of normal skin, which is nearly the maximal tensile strength achieved by scar (75%–80% of normal; see Fig. 12.10).[19] Therefore, if absorbable suture is used to close deep structures that are under significant tension, such as abdominal fascia, the suture should retain significant tensile strength for at least 6 weeks before absorption severely weakens the suture. In addition, heavy activity should be limited for a minimum of 6 weeks while healing of deep fascial structures occurs.

Open Wounds

Necrotic material should be removed from open wounds on initial presentation and subsequently as it accumulates. Necrotic tissue serves only as a culture source for bacteria and does not aid healing. The only exception to immediate debridement is a dry, chronic, arterial insufficiency eschar without evidence of infection. These types of wounds may be best treated by revascularization before debridement.

Open wounds heal optimally in a moist, sterile environment. By keeping the wound covered and moist without infection, desiccation necrosis and healing delay are prevented.[73]

Wound Dressings

A plethora of wound dressings are available for all types of wounds. No type has conclusively been shown to accelerate healing more than the others. The optimal open wound dressing maintains a moist, clean environment that prevents pressure and mechanical trauma, reduces edema, stimulates repair, and is inexpensive. Less frequent dressing changes and prevention of skin irritation are also beneficial. At this time, no ideal dressing exists (see Table 7.3).

Engineered Skin Replacements

With the biotechnology revolution, several dermal and skin replacements are now available through tissue engineering technology (Table 12.4). These products have the additional potential benefit of accelerating or augmenting repair by means of their biocomponents.

Pharmacological Treatment

Antibiotic Ointments

Antibiotic ointments are commonly used in burn wound care as well as deep partial thickness injuries. They remain controversial in the nonburn patient because the risk of developing an invasive infection by resistant bacteria may be too high to justify their use. Open wounds are colonized by bacteria, and systemic antibiotics are only indicated if invasive infection is present. Nonburn wounds without infection will likely heal without topical antibiotic treatment so long as local care is adequate.

In burn patients, silver sulfadiazine is commonly used (see Chapter 20). It is inexpensive, has few side effects, and rarely induces bacterial resistance. It can be useful in chronic wound dressings as well. It is inexpensive and is well tolerated by pediatric and geriatric patients alike.

Collagenases

The collagenases are useful to treat wounds that require fine debridement of necrotic tissue that is not amenable to surgical debridement, such as a thin layer of adherent exudate or small amounts of necrotic tissue remaining after a bedside wound debridement.

Growth Factors

Experimentally, exogenous application of several growth factors has been shown to accelerate normal healing as well as improve healing rates and efficacy in impaired models of healing.[24,74–83] The best-studied growth factors with the most promise to improve healing are PDGF, TFG-β, and members of the FGF family (see Table 12.2). Several obstacles must be overcome before widespread use, including efficacious application, vehicle development, and cost.

References

1. Ginsberg MH, Du X, Plow EF. Inside-out integrin signaling. Curr Opin Cell Biol 1992;4:766–771.
2. Roberts HR, Tabares AH. Overview of the coagulation reactions. In: High KA, Roberts HR, eds. Molecular Basis of Thrombosis and Hemostasis. New York: Dekker, 1995:35–50.
3. Wahl LM, Wahl SM. Inflammation. In: Cohen IK, Diegelmann RF, Lindblad WJ, eds. Wound Healing, Biochemical and Clinical Aspects. Philadelphia: Saunders, 1992:40–62.
4. Clark RAF. Wound repair. Overview and general considerations. In: Clark RAF, ed. The Molecular and Cellular Biology of Wound Repair. New York: Plenum Press, 1996:3–50.
5. DiPietro LA. Wound healing: the role of the macrophage and other immune cells. Shock 1995;4:233–240.
6. Riches DWH. Macrophage involvement in wound repair, remodeling, and fibrosis. In: Clark RAF, ed. The Molecular and Cellular Biology of Wound Repair. New York: Plenum Press, 1996:95–141.
7. Leibovich SJ, Ross R. The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. Am J Pathol 1975;78:71–100.
8. Miller EJ, Gay S. Collagen structure and function. In: Cohen IK, Diegelmann RF, Lindblad WJ, eds. Wound Healing. Biochemical and Clinical Aspects. Philadelphia: Saunders, 1992:130–151.
9. Lodish H, Baltimore D, Berk A, Zipursky SL, Matsudaira P, Darnell J. Multicellularity: cell–cell and cell–matrix interactions. In: Molecular Cell Biology. New York: Scientific American, 1995: 1123–1200.
10. Walchli C, Koch M, Chiquet M, Odermatt BF, Trueb B. Tissue-specific expression of the fibril-associated collagens XII and XIV. J Cell Sci 1994;107:669–681.
11. Hagg P, Rehn M, Huhtala P, Vaisanen T, Tamminen M, Pihlajaniemi T. Type XIII collagen is identified as a plasma membrane protein. J Biol Chem 1998;273:15590–15597.
12. Berthod F, Germain L, Guignard R, et al. Differential expression of collagens XII and XIV in human skin and in reconstructed skin. J Invest Dermatol 1997;108:737–742.
13. Marinkovich MP, Keene DR, Rimberg CS, Burgeson RE. Cellular origin of the dermal-epidermal basement membrane. Dev Dyn 1993;197:255–267.
14. Woodley DT. Reepithelialization. In: Clark RAF, ed. The Molecular and Cellular Biology of Wound Repair. New York: Plenum Press, 1996:339–350.
15. Kobayashi H, Ishii M, Chanoki M, et al. Immunohistochemical localization of lysyl oxidase in normal human skin. Br J Dermatol 1994;131:325–330.

16. Talhouk RS, Bissell MJ, Werb Z. Coordinated expression of extracellular matrix-degrading proteinases and their inhibitors regulates mammary epithelial function during involution. J Cell Biol 1992;118:1271–1282.
17. Moses MA, Marikovsky M, Harper JW, et al. Temporal study of the activity of matrix metalloproteinases and their endogenous inhibitors during wound healing. J Cell Biochem 1996;60:379–386.
18. Witte MB, Thornton FJ, Kiyama T, et al. Metalloproteinase inhibitors and wound healing: a novel enhancer of wound strength. Surgery (St. Louis) 1998;124:464–470.
19. Levenson SM, Geever EF, Crowley LV, Oates JF, Berard CW, Rosen H. The healing of rat skin wounds. Ann Surg 1965;161: 293–308.
20. Pierce GF, Brown D, Mustoe TA. Quantitative analysis of inflammatory cell influx, procollagen type I synthesis, and collagen cross-linking in incisional wounds: influence of PDGF-BB and TGF-beta 1 therapy. J Lab Clin Med 1991;117:373–382.
21. Pierce GF, Mustoe TA, Altrock BW, Deuel TF, Thomason A. Role of platelet-derived growth factor in wound healing. J Cell Biochem 1991;45:319–326.
22. Pierce GF, Vande BJ, Rudolph R, Tarpley J, Mustoe TA. Platelet-derived growth factor-BB and transforming growth factor beta 1 selectively modulate glycosaminoglycans, collagen, and myofibroblasts in excisional wounds. Am J Pathol 1991;138:629–646.
23. Cromack DT, Pierce GF, Mustoe TA. TGF-beta and PDGF mediated tissue repair: identifying mechanisms of action using impaired and normal models of wound healing. Prog Clin Biol Res 1991;365:359–373.
24. Mustoe TA, Pierce GF, Morishima C, Deuel TF. Growth factor-induced acceleration of tissue repair through direct and inductive activities in a rabbit dermal ulcer model. J Clin Invest 1991;87:694–703.
25. Suh DY, Hunt TK, Spencer EM. Insulin-like growth factor-I reverses the impairment of wound healing induced by corticosteroids in rats. Endocrinology 1992;131:2399–2403.
26. Nissen NN, Polverini PJ, Koch AE, Volin MV, Gamelli RL, DiPietro LA. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. Am J Pathol 1998;152:1445–1452.
27. Banks RE, Forbes MA, Kinsey SE, et al. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. Br J Cancer 1998;77:956–964.
28. Frank S, Hubner G, Breier G, Longaker MT, Greenhalgh DG, Werner S. Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. J Biol Chem 1995;270:12607–12613.
29. Fukumura D, Xavier R, Sugiura T, et al. Tumor induction of VEGF promoter activity in stromal cells. Cell 1998;94:715–725.
30. Werner S, Smola H, Liao X, et al. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. Science 1994;266:819–822.
31. Robertson JG, Pickering KJ, Belford DA. Insulin-like growth factor I (IGF-I) and IGF-binding proteins in rat wound fluid. Endocrinology 1996;137:2774–2781.
32. Heino J, Heinonen T. Interleukin-1 beta prevents the stimulatory effect of transforming growth factor-beta on collagen gene expression in human skin fibroblasts. Biochem J 1990;271:827–830.
33. Ferguson MW, Whitby DJ, Shah M, Armstrong J, Siebert JW, Longaker MT. Scar formation: the spectral nature of fetal and adult wound repair. Plast Reconstr Surg 1996;97:854–860.
34. Adzick NS, Lorenz HP. Cells, matrix, growth factors, and the surgeon. The biology of scarless fetal wound repair. Ann Surg 1994;220:10–18.
35. Young T. Pressure sores: incidence, risk assessment and prevention. Br J Nurs 1997;6:319–322.
36. Schubert V, Perbeck L, Schubert PA. Skin microcirculatory and thermal changes in elderly subjects with early stage of pressure sores. Clin Physiol 1994;14:1–13.
37. Relander M, Palmer B. Recurrence of surgically treated pressure sores. Scand J Plast Reconstr Surg Hand Surg 1988;22:89–92.
38. Kierney PC, Engrav LH, Isik FF, Esselman PC, Cardenas DD, Rand RP. Results of 268 pressure sores in 158 patients managed jointly by plastic surgery and rehabilitation medicine. Plast Reconstr Surg 1998;102:765–772.
39. Burton CS. Venous ulcers. Am J Surg 1994;167:37S–40S; discussion 40S–41S.
40. Margolis DJ, Cohen JH. Management of chronic venous leg ulcers: a literature-guided approach. Clin Dermatol 1994;12:19–26.
41. Bernstein EF, Harisiadis L, Salomon GD, et al. Healing impairment of open wounds by skin irradiation. J Dermatol Surg Oncol 1994;20:757–760.
42. Robson MC. Infection in the surgical patient: an imbalance in the normal equilibrium. Clin Plast Surv 1979;6:493–503.
43. Robson MC. Wound infection. A failure of wound healing caused by an imbalance of bacteria. Surg Clin North Am 1997;77: 637–650.
44. Robson MC, Stenberg BD, Heggers JP. Wound healing alterations caused by infection. Clin Plast Surg 1990;17:485–492.
45. Robson MC, Krizek TJ. Predicting skin graft survival. J Trauma 1973;13:213–217.
46. Murphy RC, Robson MC, Heggers JP, Kadowaki M. The effect of microbial contamination on musculocutaneous and random flaps. J Surg Res 1986;41:75–80.
47. Barbul A, Purtill WA. Nutrition in wound healing. Clin Dermatol 1994;12:133–140.
48. Muller MJ, Herndon DN. The challenge of burns. Lancet 1994;343:216–220.
49. Albina JE. Nutrition and wound healing. J Parenter Enteral Nutrition 1994;18:367–376.
50. Ehrlich HP, Hunt TK. Effects of cortisone and vitamin A on wound healing. Ann Surg 1968;167:324–328.
51. Ehrlich HP, Tarver H, Hunt TK. Effects of vitamin A and glucocorticoids upon inflammation and collagen synthesis. Ann Surg 1973;177:222–227.
52. Levenson SM, Demetriou AA. Metabolic factors. In: Cohen IK, Diegelmann RF, Lindblad WJ, eds. Wound Healing, Biochemical and Clinical Aspects. Philadelphia: Saunders, 1992:248–273.
53. Masse PG, Pritzker KP, Mendes MG, Boskey AL, Weiser H. Vitamin B6 deficiency experimentally-induced bone and joint disorder: microscopic, radiographic and biochemical evidence. Br J Nutr 1994;71:919–932.
54. LaVan FB, Hunt TK. Oxygen and wound healing. Clin Plast Surg 1990;17:463–472.
55. Allen DB, Maguire JJ, Mahdavian M, et al. Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. Arch Surg 1997;132:991–996.
56. Hopf HW, Hunt TK, West JM, et al. Wound tissue oxygen tension predicts the risk of wound infection in surgical patients. Arch Surg 1997;132:997–1004; discussion 1005.
57. Knighton DR, Hunt TK, Scheuenstuhl H, Halliday BJ, Werb Z, Banda MJ. Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science 1983;221:1283–1285.
58. Jonsson K, Jensen JA, Goodson WH, et al. Tissue oxygenation, anemia, and perfusion in relation to wound healing in surgical patients. Ann Surg 1991;214:605–613.
59. Hunt TK, Pai MP. The effect of varying ambient oxygen tensions on wound metabolism and collagen synthesis. Surg Gynecol Obstet 1972;135:561–567.
60. Werner S, Breeden M, Hubner G, Greenhalgh DG, Longaker MT. Induction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. J Invest Dermatol 1994;103:469–473.
61. Beer HD, Longaker MT, Werner S. Reduced expression of PDGF

and PDGF receptors during impaired wound healing. J Invest Dermatol 1997;109:132–138.
62. Goodson WH, Hunt TK. Wound healing in experimental diabetes mellitus: importance of early insulin therapy. Surg Forum 1978;29:95–98.
63. Goodson WH, Hunt TK. Deficient collagen formation by obese mice in a standard wound model. Am J Surg 1979;138:692–694.
64. Ehrlich HP, Hunt TK. The effects of cortisone and anabolic steroids on the tensile strength of healing wounds. Ann Surg 1969;170:203–206.
65. Marks JG Jr, Cano C, Leitzel K, Lipton A. Inhibition of wound healing by topical steroids. J Dermatol Surg Oncol 1983;9: 819–821.
66. Drake DB, Oishi SN. Wound healing considerations in chemotherapy and radiation therapy. Clin Plast Surg 1995;22:31–37.
67. Bettinger DA, Yager DR, Diegelmann RF, Cohen IK. The effect of TGF-beta on keloid fibroblast proliferation and collagen synthesis. Plast Reconstr Surg 1996;98:827–833.
68. Alster TS, West TB. Treatment of scars: a review. Ann Plast Surg 1997;39:418–432.
69. Ellitsgaard V, Ellitsgaard N. Hypertrophic scars and keloids: a recurrent problem revisited. Acta Chir Plast 1997;39:69–77.
70. Lee SM, Ngim CK, Chan YY, Ho MJ. A comparison of Sil-K and Epiderm in scar management. Burns 1996;22:483–487.
71. Murray JC, Pinnell SR. Keloids and excessive dermal scarring. In: Cohen IK, Diegelmann RF, Lindblad WJ, eds. Wound Healing, Biochemical and Clinical Aspects. Philadelphia: Saunders, 1992:500–509.
72. Maguire HC. Treatment of keloids with triamcinolone acetonide injected intralesionally. JAMA 1965;192:325–327.
73. Svensjo T, Pomahac B, Yao F, Eriksson E. Healing of full-thickness porcine wounds in dry, moist, and wet environments. Surg Forum 1998;48:150–153.
74. Greenhalgh DG, Sprugel KH, Murray MJ, Ross R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. Am J Pathol 1990;136:1235–1246.
75. Slavin J, Nash JR, Kingsnorth AN. Effect of transforming growth factor beta and basic fibroblast growth factor on steroid-impaired healing intestinal wounds. Br J Surg 1992;79:69–72.
76. Richard JL, Parer RC, Daures JP, et al. Effect of topical basic fibroblast growth factor on the healing of chronic diabetic neuropathic ulcer of the foot. A pilot, randomized, double-blind, placebo-controlled study. Diabetes Care 1995;18:64–69.
77. Steed DL. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. Diabetic Ulcer Study Group. J Vasc Surg 1995;21: 71–78; discussion 79–81.
78. Steed DL, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group. J Am Coll Surg 1996; 183:61–64.
79. Pierce GF, Tarpley JE, Allman RM, et al. Tissue repair processes in healing chronic pressure ulcers treated with recombinant platelet-derived growth factor BB. Am J Pathol 1994;145:1399–1410.
80. Lynch SE, de Castilla GR, Williams RC, et al. The effects of short-term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing. J Periodontol 1991;62:458–467.
81. Antoniades HN, Galanopoulos T, Neville-Golden J, Kiritsy CP, Lynch SE. Injury induces in vivo expression of platelet-derived growth factor (PDGF) and PDGF receptor mRNAs in skin epithelial cells and PDGF mRNA in connective tissue fibroblasts. Proc Natl Acad Sci USA 1991;88:565–569.
82. Cromack DT, Porras-Reyes B, Purdy JA, Pierce GF, Mustoe TA. Acceleration of tissue repair by transforming growth factor beta 1: identification of in vivo mechanism of action with radiotherapy-induced specific healing deficits. Surgery (St. Louis) 1993;113:36–42.
83. Jones SC, Curtsinger LJ, Whalen JD, et al. Effect of topical recombinant TGF-beta on healing of partial thickness injuries. J Surg Res 1991;51:344–352.