Chapter 2

Wound Healing

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2.1 Introduction

Understanding wound healing today involves much more than simply stating there are three phases: inflammation, proliferation, and maturation. Wound healing is a complex series of reactions and interactions among cells and "mediators." Each year, new mediators are discovered and our understanding of inflammatory mediators and cellular interactions grows. Many intrinsic and extrinsic factors affect wound healing, and an enormous industry provides the clinician with a huge and complex armamentarium to battle wound-healing problems.

2.2 Wound Healing

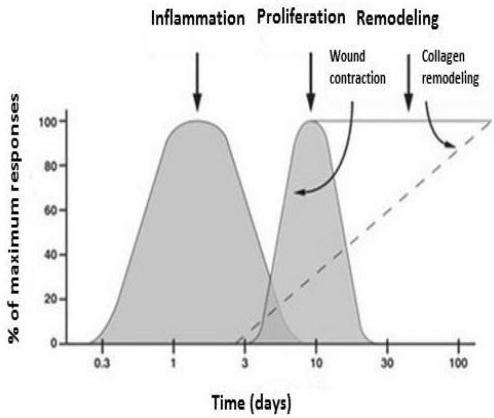

Figure 2. *Phases of wound healing.*

Wound healing has traditionally been divided into three distinct phases:

inflammation, proliferation, and remodeling[13, 14]. A detailed review of the basic science of wound healing can be found in this Supplement. This discussion will serve as a broad overview of clinical wound healing (see figure 2).

2.2.1 Hemostasis and Inflammation (from Immediately upon Injury through Days 4 to 6)

The inflammatory phase is characterized by hemostasis and inflammation. Collagen exposed during wound formation activates the clotting cascade (both the intrinsic and extrinsic pathways), initiating the inflammatory phase. After injury occurs, the cell membranes release the potent vasoconstrictors thromboxane A2 and prostaglandin 2-α. The clot that forms is made of collagen, platelets, thrombin, and fibronectin, and these factors release cytokines and growth factors that initiate the inflammatory response[15]. The fibrin clot serves as scaffolding for arriving cells, such as neutrophils, monocytes, fibroblasts, and endothelial cells[16]. It also serves to concentrate the cytokines and growth factors[17].

2.2.2 Chemotaxis and Activation

Immediately after the clot is formed, a cellular distress signal is sent out and neutrophils are the first responders. As the inflammatory mediators accumulate, and prostaglandins are elaborated, the nearby blood vessels vasodilate to allow for the increase cellular traffic as neutrophils are drawn into the injured area by interleukin (IL)-1, tumor necrosis factor (TNF)-α, transforming growth factor (TGF), platelet factor-4 (PF4), and bacterial "products."[18, 19] Monocytes in the nearby tissue and in the blood will be attracted to the area and transform into macrophages, usually around 48 to 96 hours after injury. Activation of the inflammatory cells is critical, especially for the macrophage. An activated

macrophage is important for the transition into the proliferative phase. An activated macrophage will mediate angiogenesis, fibroplasia, and synthesize nitric oxide[20].

Neutrophils will enter into the wound site and begin clearing it of invading bacteria and cellular debris. The neutrophil releases caustic proteolytic enzymes that will digest bacteria and nonviable tissue. The next cells present in the wound are the leukocytes and the macrophages (monocytes). The macrophage is essential for wound healing. Numerous enzymes and cytokines are secreted by the macrophage, including collagenases, which debride the wound; ILs and TNF, which stimulate fibroblasts (produce collagen) and promote angiogenesis; and TGF, which stimulates keratinocytes.

2.2.3 Proliferative Phase (Epithelization, Angiogenesis, and Provisional Matrix Formation; Day 4 through 14)

Epithelialization, angiogenesis, granulation tissue formation, and collagen deposition are the principal steps in this building portion of wound healing. Epithelialization occurs early in wound repair. If the basement membrane remains intact, the epithelial cells migrate upward in the normal pattern. The epithelial progenitor cells remain intact below the wound (in skin appendages), and the normal layers of epidermis are restored in 2 to 3 days. If the basement membrane has been destroyed, then epithelial cells located on the skin edge begin proliferating and sending out projections to re-establish a protective barrier[21]. Angiogenesis, stimulated by TNF-α, is marked by endothelial cell migration and capillary formation. The migration of capillaries into the wound bed is critical for proper wound healing. The granulation phase and tissue deposition require nutrients supplied by the capillaries, and failure of this to occur results in a chronically unhealed wound.

Epithelial cells located on the skin edge begin proliferating and sending out projections to reestablish a protective barrier against fluid losses and further bacterial invasion. The stimulus for epithelial proliferation and chemotaxis is epidermal growth factor (EGF) and TGF-α produced by activated platelets and macrophages (Fibroblasts don't appear to synthesize TGF-α)[22]. Epithelization begins shortly after wounding and is first stimulated by inflammatory cytokines. IL-1 and TNF upregulate keratinocyte growth factor (KGF) gene expression in fibroblasts. In turn, fibroblasts synthesize and secrete KGF-1, KGF-2, and IL-6, which simulate neighboring keratinocytes to migrate in the wound area, proliferate, and differentiate in the epidermis[23, 24]. It has been shown that, for humans, KGF-2 is most important for directing this process[25].

The final part of the proliferative phase is granulation tissue formation. Fibroblasts migrate into the wound site from the surrounding tissue, become activated, and begin synthesizing collagen and proliferate. Platelet-derived growth factor (PDGF) and EGF are the main signals to fibroblasts and are derived from platelets and macrophages. PDGF expression by fibroblasts is amplified by autocrine and paracrine signaling. Fibroblasts already located in the wound site (termed "wound fibroblasts") will begin synthesizing collagen and transform into myofibroblasts for wound contraction (induced by macrophage-secreted TGF-β1); they have less proliferation compared with the fibroblasts coming in from the wound periphery[26–28]. In response to PDGF, fibroblasts begin synthesizing a provisional matrix composed of collagen type III, glycosaminoglycans, and fibronectin[29].

2.2.4 Maturation and Remodeling (Day 8 through Year 1)

Clinically, the maturation and remodeling phase is perhaps the most important. The main feature of this phase is the deposition of collagen in an organized and

well-mannered network. If patients have matrix deposition problems (from diet or disease), then the wound's strength will be greatly compromised; if there is excessive collagen synthesis, then a hypertrophic scar or keloid can result.

Net collagen synthesis will continue for at least 4 to 5 weeks after wounding. The increased rate of collagen synthesis during wound healing is not only from an increase in the number of fibroblasts but also from a net increase in the collagen production per cell[30]. The collagen that is initially laid down is thinner than collagen in uninjured skin and is orientated parallel to the skin. Over time, the initial collagen threads are reabsorbed and deposited thicker and organized along the stress lines. These changes are also accompanied by a wound with an increased tensile strength, indicating a positive correlation between collagen fiber thickness/orientation and tensile strength[15].

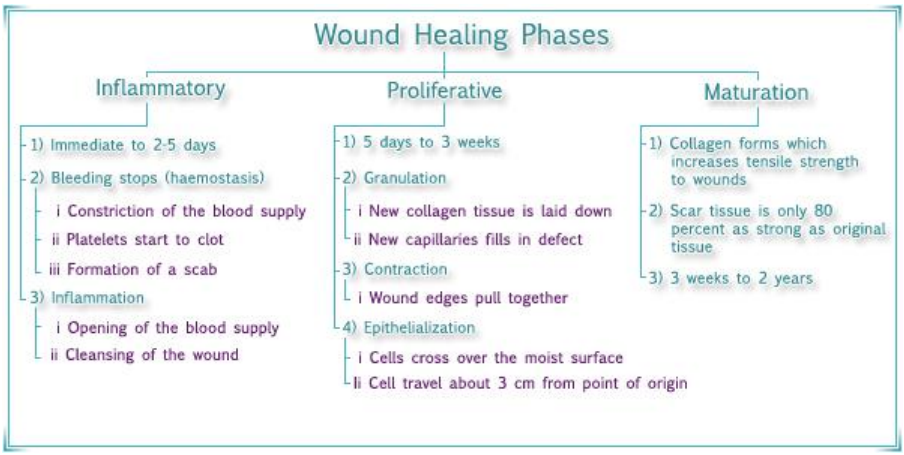

Figure 3. *Summary of events for wound healing phases.*

The collagen found in granulation tissue is biochemically different from collagen from uninjured skin. Granulation tissue collagen has a greater hydroxylation and glycosylation of lysine residues, and this increase of glycosylation correlates with the thinner fiber size[31]. The collagen in the scar

(even after a year of maturing) will never become as organized the collagen found in uninjured skin. Wound strength also never returns to 100 percent. At 1 week, the wound has only 3 percent of its final strength; at 3 weeks, 30 percent; and at 3 months (and beyond), approximately 80 percent[32]. Finally the events of wound healing can be summarized in figure (3).

2.3 Skin Metabolism and Physiology

The blood supply of the skin is far greater than it requires metabolically. Blood vessels in the skin are capable of carrying 20–100X the amounts of oxygen and nutrients that are needed for cellular survival and function. (Cells above the basal layer of the epidermis have largely lost their mitochondria and respire mainly through glycolysis, contributing little to the metabolic needs of the skin.) Despite the abundant blood supply, skin perfusion is insufficient to support wound healing, which requires granulation tissue.

Ryan[33] summarizes this paradox as follows: Although the skin can resist many hours of compression and obliteration of its blood supply but non-healing of the skin is one of the most common of problems and is often blamed on impairment of blood supply. The dilemma is explained by the fact that exchange between blood vessels and the supplied tissue services the functions of that tissue, and, although it is often stated that richness of the skin vasculature exceeds nutritional need, this statement is a misconception that is why the frequent stimuli of scratching, stretching, compressing, heating, or cooling of the skin require restoration of skin stiffness to a status quo. In restoring itself to the status quo, the mechanical properties of the skin must be instantly repaired and this repair requires a luxurious blood supply to maintain not merely cell metabolism but the physical properties of the interstitium.

2.3.1 Collagen

Collagen is the principal building block of connective tissue, accounting for one third of the total protein content of the body. Collagen is an unusual protein in that it is almost devoid of the sulfur containing amino acids cysteine and tryptophan. In their stead, collagen contains hydroxyproline and hydroxylysine, two amino acids with very limited distribution otherwise - only in collagen, elastin, the C1q subcomponent of the complement system, and the tail structure of acetylcholinesterase[34, 35]. Collagen has a very complex tertiary and quaternary molecular structure consisting of three polypeptide chains, each chain wound upon itself in a left handed helix and the three chains together wound in a right-handed coil to form the basic collagen unit. The polypeptide chains are held in their relative configurations by covalent bonds. Each triple helical structure is a tropocollagen molecule. Tropocollagen units associate in a regular fashion to form collagen filaments; collagen filaments in turn aggregate as collagen fibrils, and collagen fibrils unite to form collagen fibers, which are visible under the light microscope (Fig 4).

Five types of collagen have been identified in humans on the basis of amino acid sequences. Their relative distribution in connective tissues varies, hinting at individual properties valuable for specific functions (Table 1). Type I collagen is abundant in skin, tendon, and bone. These tissues account for more than 90% of all collagen in the body. Normal skin contains Type I and Type III collagen in a 4:1 ratio, the latter mainly in the papillary dermis. In hypertrophic and immature scars the percentage of Type III collagen may be as high as 33% (a 2:1 Type I:III ratio)[36].

Collagen synthesis takes place extracellularly as well as intracellularly. Certain substances inhibit the formation of collagen either by interfering with its

synthesis or activating its degradation (Fig 5). Normal connective tissue is in a state of dynamic equilibrium balanced between synthesis and degradation, and this makes it vulnerable to local stimuli such as mechanical forces on the tissue. While excessive collagen degradation results from unchecked collagenase synthesis, not enough collagenase gives rise to tissue fibrosis. Homeostasis is achieved through *activation* of collagenase by parathyroid hormone, adrenal corticosteroids, and colchicine; and *inhibition* of collagenase synthesis by serum alpha-2 macroglobulin, cysteine and progesterone[37].

Table 1. *Types and distribution of collagen.*

Type	Structure	Distribution
I	Hyprid of two chains low in hydroxylysine and glycosylated hydroxylysine.	Bone, tendon, skin, dentin, ligament, fascia, arteries, and uterus
II	Relatively high in hydroxylysine and glycosylated hydroxylysine.	Hyaline cartilage, eye tissues
III	High in hydroxylysine and low in hydroxylysine contain interchains disulfide bonds.	Skin, arteries, uterus, and bowel wall.
IV	High in hydroxylysine and glycosylated hydroxylysine. May contain large globular regions.	Basement membrane.
V	Similar to Type IV	Basement membrane and perhaps other tissues

(Annotated from Prockop DJ et al.: The biosynthesis of collagen and its disorders. N Engl J Med 301:13, 1979.)

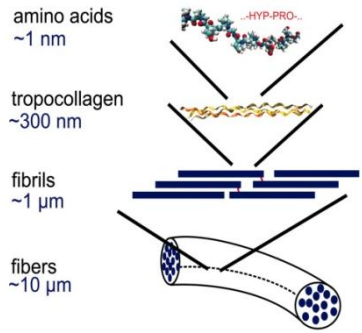

Figure 4. *Molecular and fibrillar structure of collagen.*

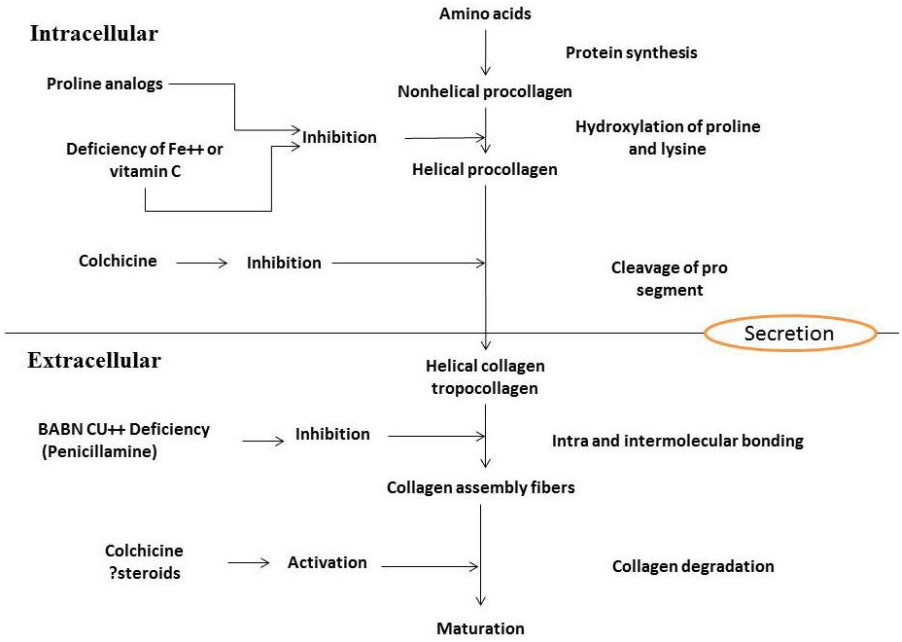

Figure 5. *Collagen synthesis and site of action of common inhibitors.*

2.3.2 The Myofibroblast and Wound Contraction

Contraction is an essential part of the repair process by which the organism closes a gap in the soft tissues. *Contracture*, on the other hand, is an undesirable result of healing, at times due to the process of contraction and at other times due to fibrosis or other tissue damage[38].

In 1971 Gabbiani, Ryan, and Majno[39] first noted a type of fibroblast in granulation tissue that bore some structural similarities to smooth muscle cells. Myofibroblasts differ from ordinary fibroblasts by having cytoplasmic microfilaments similar to those of smooth muscle cells. Within the filamentous system are areas of "dense bodies" that serve as attachments for contraction. The nuclei demonstrate numerous surface irregularities such as those of smooth

muscle cells but unlike those of ordinary fibroblasts. Myofibroblasts are also different from normal fibroblasts in that they have well-formed intercellular attachments such as desmosomes and maculae adherens. Myofibroblasts are the source of contraction within a wound[40–43].

Rudolph[44, 45] found a direct relationship between the rate of wound contraction and the number of myofibroblasts within a wound. Rudolph[45]also demonstrated the presence of myofibroblasts throughout the wound, not just adjacent to the wound margins. McGrath and Hundahl[46] confirmed the parallel paths of wound contraction and number of myofibroblasts in the wound and the relatively even distribution of myofibroblasts in granulation tissue except at the wound bed (fewer) and adjacent to foci of inflammation (more). Their findings support the "pull theory" of wound contraction, which holds that the entire granulating surface of the wound acts as a contractile organ. This concept implies contraction of individual myofibroblasts to shorten the wound, followed by collagen deposition and crosslinking to maintain the shortening, in a lock-step mechanism. Prostaglandin inhibitors do not inhibit myofibroblast production, therefore wound contraction is not altered[47]. Although present in a number of contracture disorders like Dupuytren's disease[48], Peyronie's, and lederhosen disease[41], myofibroblasts have not been implicated in their etiology.

2.3.3 Tensile Strength

The *tensile strength* of a wound is a measurement of its load capacity per unit area. A wound's *breaking strength* is defined as the force required to break it regardless of its dimensions. Depending solely on different skin thicknesses, breaking strength can vary several folds; tensile strength, on the other hand, is constant for wounds of similar size.

Experimental studies give evidence that collagen fibers are largely responsible for the tensile strength of wounds[49]. The rate at which a healing wound regains strength varies not only among species, but also among individuals and even among different tissues in the same individual[50]. The healing pattern of the various tissues, however, is remarkably similar within a phylogenetic family.

All wounds gain strength at approximately the same rate during the first 14–21 days, but thereafter the curves may diverge significantly according to the tissue involved. In skin, the peak tensile strength is achieved at approximately 60 days after injury[51] (Fig 6). Given optimal healing conditions, the tensile strength of a wound never reaches that of the original, unwounded skin, leveling off at about 80%.

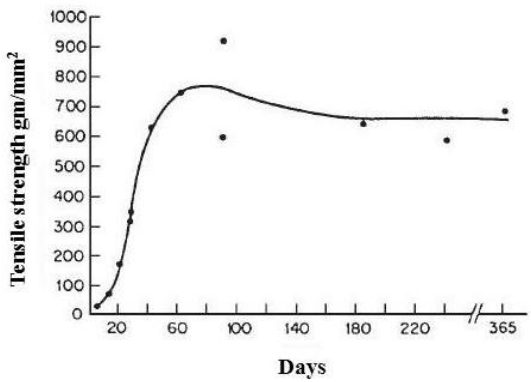

Figure 6. *Tensile strength of a healing skin incision as a function of time. (Reprinted with permission from Levenson SM et al.: The healing of rat skin wounds. Ann Surg 161:293, 1965.)*

2.4 Factors Affecting Wound Healing

Impaired wound healing can be caused by multiple factors. This can be categorized into local and systemic factors.

2.4.1 Local Factors That Influence Healing

(a) Oxygenation

Oxygen is important for cell metabolism, especially energy production by means of ATP, and is critical for nearly all wound-healing processes. It prevents wounds from infection, induces angiogenesis, increases keratinocyte differentiation, migration, and re-epithelialization, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction[52]. In addition, the level of superoxide production (a key factor for oxidative killing pathogens) by polymorphonuclear leukocytes is critically dependent on oxygen levels.

The microenvironment of the early wound is depleted of oxygen and is quite hypoxic. Several systemic conditions, including advancing age and diabetes, can create impaired vascular flow, thus setting the stage for poor tissue oxygenation. In the context of healing, this overlay of poor perfusion creates a hypoxic wound. Chronic wounds are notably hypoxic; tissue oxygen tensions have been measured transcutaneously in chronic wounds from 5 to 20 mm Hg, in contrast to control tissue values of 30 to 50 mm Hg[53].

Healing is impaired in wounds where oxygenation is not restored,. Temporary hypoxia after injury triggers wound healing, but prolonged or chronic hypoxia delays wound healing[52]. In acute wounds, hypoxia serves as a signal that stimulates many aspects of the wound-healing process. Hypoxia can induce cytokine and growth factor production from macrophages, keratinocytes, and fibroblasts. Cytokines that are produced in response to hypoxia include PDGF, TGF-β, VEGF, and tumor necrosis factor-α (TNF-α), are crucial promoters of cell proliferation, migration and chemotaxis, and angiogenesis in wound healing[54].

In normally healing wounds, ROS such as hydrogen peroxide (H_2O_2) and superoxide (O^2) are thought to act as cellular messengers to stimulate key processes associated with wound healing, including cell motility, cytokine action (including PDGF signal transduction), and angiogenesis. Both hypoxia and hyperoxia increase ROS production, but an increased level of ROS transcends the beneficial effect and causes additional tissue damage[54].

In summary, the proper oxygen level is crucial for optimum wound healing. Hypoxia stimulates wound healing such as the release of growth factors and angiogenesis, while oxygen is needed to sustain the healing process[52]. One therapeutic option that can sometimes overcome the influence of tissue hypoxia is hyperbaric oxygen therapy (HBOT)[54]. While HBOT can be an effective treatment for hypoxic wounds, its availability is limited.

(b) Infections

After skin injury, micro-organisms that are normally sequestered at the skin surface obtain access to the underlying tissues. The state of infection and replication status of the micro-organisms determines whether the wound is classified as having contamination, colonization, local infection/critical colonization, and/or spreading invasive infection. Contamination is the presence of non-replicating organisms on a wound, while colonization is defined as the presence of replicating micro-organisms on the wound without tissue damage. Local infection/critical colonization are an intermediate stage, with micro-organism replication and the beginning of local tissue responses. Invasive infection is defined as the presence of replicating organisms within a wound with subsequent host injury[55].

Normal part of the wound-healing process is Inflammation, and is important to the removal of contaminating micro-organisms. In the absence of effective

decontamination, however, inflammation may be prolonged, since microbial clearance is incomplete. Both bacteria and endotoxins can lead to the prolonged elevation of pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α and elongate the inflammatory phase. If this continues, the wound may enter a chronic state and fail to heal. This prolonged inflammation also leads to an increased level of matrix metalloproteases (MMPs), a family of proteases that can degrade the ECM. In tandem with the increased protease content, a decreased level of the naturally occurring protease inhibitors occurs. This shift in protease balance can cause growth factors that appears in chronic wounds to be rapidly degraded[56]. Similar to other infective processes, the bacteria in infected wounds occur in the form of biofilms, which are complex communities of aggregated bacteria embedded in a self-secreted extracellular polysaccharide matrix (EPS)[55]. Mature biofilms develop protected microenvironments and are more resistant to conventional antibiotic treatment. *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and β-hemolytic *streptococci* are common bacteria in infected and clinically non-infected wounds[57].

Many chronic ulcers probably do not heal because of the presence of biofilms containing *P. aeruginosa*, thus shielding the bacteria from the phagocytic activity of invading polymorphonuclear neutrophils (PMNs). This mechanism may explain the failure of antibiotics as a remedy for chronic wounds[58].

2.4.2 Systemic Factors That Influence Healing

(a) Age

Increased age is a major risk factor for impaired wound healing. Many clinical and animal studies at the cellular and molecular level have examined age-related changes and delays in wound healing. It is commonly recognized that, in healthy

older adults, the effect of aging causes a temporal delay in wound healing, but not an actual impairment in terms of the quality of healing[59]. Delayed wound healing in the aged is associated with an altered inflammatory response, such as delayed T-cell infiltration into the wound area with alterations in chemokine production and reduced macrophage phagocytic capacity[60]. Delayed re-epithelialization, collagen synthesis, and angiogenesis have also been observed in aged mice as compared with young mice[60]. Overall, there are global differences in wound healing between young and aged individuals. A review of the age-related changes in healing capacity demonstrates that every phase of healing undergoes characteristic age-related changes, including enhanced platelet aggregation, increased secretion of inflammatory mediators, delayed infiltration of macrophages and lymphocytes, impaired macrophage function, decreased secretion of growth factors, delayed re-epithelialization, delayed angiogenesis and collagen deposition, reduced collagen turnover and remodeling, and decreased wound strength[59].

Age-related impairment of healing can be reduced by several treatments. Interestingly, exercise has been reported to improve cutaneous wound healing in older adults as well as aged mice, and the improvement is associated with decreased levels of pro-inflammatory cytokines in the wound tissue. The improved healing response may be due to an exercise-induced anti-inflammatory response in the wound[61].

(b) Sex Hormones in Aged Individuals

Aged males have been shown to have delayed healing of acute wounds when compared with females. A partial explanation for this is that the female estrogens (estrone and 17β-estradiol), male androgens (testosterone and 5α-dihydrotestosterone), and their steroid precursor dehydroepiandrosterone

(DHEA) appear to have significant effects on the wound-healing process[62]. It was recently found that the differences in gene expression between elderly male and young human wounds are almost exclusively estrogen-regulated[63]. Estrogen affects wound healing by regulating a variety of genes associated with regeneration, matrix production, protease inhibition, epidermal function, and the genes primarily associated with inflammation[63]. Studies indicate that estrogen can improve the age-related impairment in healing in both men and women, while androgens regulate cutaneous wound healing negatively[62].

(c) Stress

Stress has a great impact on human health and social behavior. Many diseases - such as cardiovascular disease, cancer, compromised wound healing, and diabetes - are associated with stress. Numerous studies have confirmed that stress-induced disruption of neuroendocrine immune equilibrium is consequential to health[64]. The pathophysiology of stress results in the deregulation of the immune system, mediated primarily through the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal medullary axes or sympathetic nervous system (SNS)[65].

Studies in both humans and animals have demonstrated that psychological stress causes a substantial delay in wound healing. Caregivers of persons with Alzheimer's and students undergoing academic stress during examinations demonstrated delayed wound healing[66]. The hypothalamic-pituitary-adrenal and the sympathetic-adrenal medullary axes regulate the release of pituitary and adrenal hormones. These hormones include the adrenocorticotrophic hormones, cortisol and prolactin, and catecholamines (epinephrine and norepinephrine). Stress up-regulates glucocorticoids (GCs) and reduces the levels of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α at the wound site. Stress also

reduces the expression of IL-1α and IL-8 at wound sites - both chemoattractants that are necessary for the initial inflammatory phase of wound healing[65]. Furthermore, GCs influence immune cells by suppressing differentiation and proliferation, regulating gene transcription, and reducing expression of cell adhesion molecules that are involved in immune cell trafficking[67]. The GC cortisol functions as an anti-inflammatory agent and modulates the Th1-mediated immune responses that are essential for the initial phase of healing. Thus, psychological stress impairs normal cell-mediated immunity at the wound site, causing a significant delay in the healing process[65].

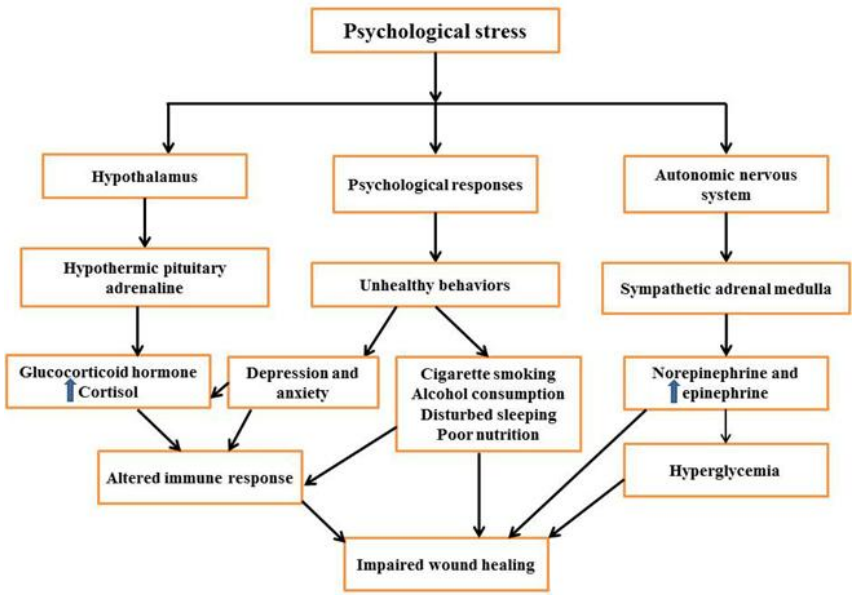

Figure 7. *The effects of stress on wound healing.*

Stressors can lead to negative emotional states, such as anxiety and depression, which may in turn have an impact on physiologic processes and/or behavioral patterns that influence health outcomes. In addition to the direct influences of anxiety and depression on endocrine and immune function, stressed individuals are more likely to have unhealthy habits, which include poor sleep patterns,

inadequate nutrition, less exercise, and a greater propensity for abuse of alcohol, cigarettes, and other drugs. All of these factors may come into play in negatively modulating the healing response. The effects of stress on wound healing are summarized in Fig. 7.

(d) Diabetes

Diabetes affects hundreds of millions of people worldwide. Diabetic individuals exhibit a documented impairment in the healing of acute wounds. Moreover, this population is prone to develop chronic non-healing diabetic foot ulcers (DFUs), which are estimated to occur in 15% of all persons with diabetes. DFUs are a serious complication of diabetes, and precede 84% of all diabetes-related lower leg amputations[68]. The impaired healing of both DFUs and acute cutaneous wounds in persons with diabetes involves multiple complex pathophysiological mechanisms. DFUs, like venous stasis disease and pressure-related chronic non-healing wounds, are always accompanied by hypoxia[53]. A situation of prolonged hypoxia, which may be derived from both insufficient perfusion and insufficient angiogenesis, is detrimental for wound healing. Hypoxia can amplify the early inflammatory response, thereby prolonging injury by increasing the levels of oxygen radicals[69]. Hyperglycemia can also add to the oxidative stress when the production of ROS exceeds the anti-oxidant capacity[70]. The formation of advanced glycation end-products (AGEs) under hyperglycemia and the interaction with their receptors (RAGE) are associated with impaired wound healing in diabetic mice as well[71]. High levels of metalloproteases are a feature of diabetic foot ulcers, and the MMP levels in chronic wound fluid are almost 60 times higher than those in acute wounds. This increased protease activity supports tissue destruction and inhibits normal repair processes[69].

Several dysregulated cellular functions are involved in diabetic wounds, such

as defective T-cell immunity, defects in leukocyte chemotaxis, phagocytosis, and bactericidal capacity, and dysfunctions of fibroblasts and epidermal cells. These defects are responsible for inadequate bacterial clearance and delayed or impaired repair in individuals with diabetes[72].

As mentioned above, hypoxia contributes to the compromised healing of DFUs, and diabetic wounds exhibit inadequate angiogenesis. Several studies that have investigated the mechanisms behind the decreased restoration of vasculature in diabetic wounds have implied that EPC mobilization and homing are impaired, and that the level of VEGF, the primary pro-angiogenic factor in wounds, is decreased in the diabetic state[68]. Stem-cell-based therapies aimed at inducing EPCs or BM-MSCs have shown a promising outcome in diabetic non-healing wounds, both in animals and in clinical trials[73]. In animal studies, therapeutic restoration of VEGF has been shown to improve repair outcomes significantly[74].

The neuropathy that occurs in diabetic individuals probably also contributes to impaired wound healing. Neuropeptides such as nerve growth factor, substance P, and calcitonin gene-related peptide are relevant to wound healing, because they promote cell chemotaxis, induce growth factor production, and stimulate the proliferation of cells. A decrease in neuropeptides has been associated with DFU formation. In addition, sensory nerves play a role in modulating immune defense mechanisms, with denervated skin exhibiting reduced leukocyte infiltration[75].

In summary, the impaired healing that occurs in individuals with diabetes involves hypoxia, dysfunction in fibroblasts and epidermal cells, impaired angiogenesis and neovascularization, high levels of metalloproteases, damage from ROS and AGEs, decreased host immune resistance, and neuropathy. The influence of these factors on wound healing is summarized in Fig. 8.

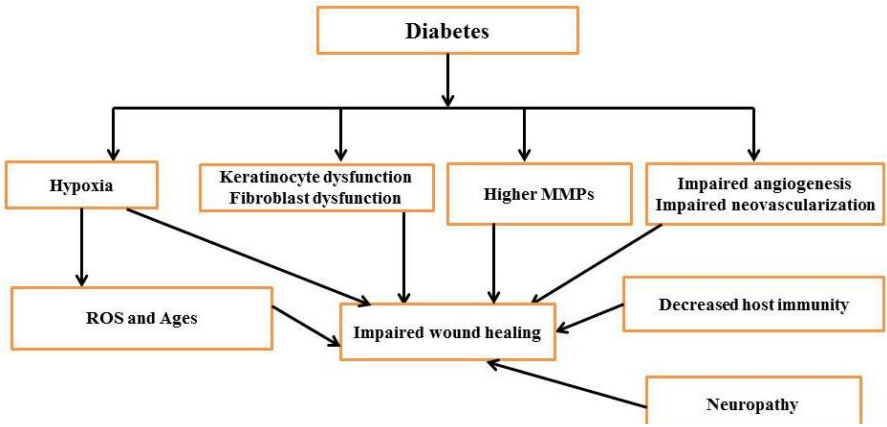

Figure 8. *The potential effects of diabetes on wound healing.*

(e) Medications

Many medications, such as those which interfere with clot formation or platelet function, or inflammatory responses and cell proliferation have the capacity to affect wound healing. Here we review only the commonly used medications that have a significant impact on healing, including glucocorticoid steroids, non-steroidal anti-inflammatory drugs, and chemotherapeutic drugs.

Glucocorticoid Steroids

Systemic glucocorticoids (GC), which are frequently used as anti-inflammatory agents, are well-known to inhibit wound repair *via* global anti-inflammatory effects and suppression of cellular wound responses, including fibroblast proliferation and collagen synthesis. Systemic steroids cause wounds to heal with incomplete granulation tissue and reduced wound contraction[76]. Glucocorticoids also inhibit production of hypoxia-inducible factor-1 (HIF-1), a key transcriptional factor in healing wounds[77]. Beyond effects on repair itself, systemic corticosteroids may increase the risk of wound infection. While systemic corticosteroids inhibit wound repair, topical application produces quite

different effects. Topical low-dosage corticosteroid treatment of chronic wounds has been found to accelerate wound healing, reduce pain and exudate, and suppress hypergranulation tissue formation in 79% of cases. While these positive effects are striking, careful monitoring is necessary to avoid a potential increased risk of infection with prolonged use[78].

Non-steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen are widely used for the treatment of inflammation and rheumatoid arthritis and for pain management. Low-dosage aspirin, due to its anti-platelet function, is commonly used as a preventive therapeutic for cardiovascular disease, but not as an anti-inflammatory drug[79]. There are few data to suggest that short-term NSAIDs have a negative impact on healing. However, the question of whether long-term NSAIDs interfere with wound healing remains open. In animal models, systemic use of ibuprofen has demonstrated an anti-proliferative effect on wound healing, resulting in decreased numbers of fibroblasts, weakened breaking strength, reduced wound contraction, delayed epithelialization[80], and impaired angiogenesis[81]. The effects of low-dose aspirin on healing are not completely clear. Clinical recommendations suggest that, to avoid anti-platelet effects, individuals should discontinue NSAIDs for a time period equal to 4 to 5 times the half-life of drugs before surgery. Thus, the majority of surgical patients do not have significant NSAID activity at the time of wound repair. The exception may be those cardiac patients who must be maintained on low-dose aspirin due to severe risk of cardiovascular events[79]. In terms of the topical application of NSAIDs on the surfaces of chronic wounds, the local use of ibuprofen-foam provides moist wound healing, reduces persistent and temporary wound pain, and benefits chronic venous leg ulcer healing[82].

Chemotherapeutic Drugs

Most chemotherapeutic drugs are designed to inhibit cellular metabolism, rapid cell division, and angiogenesis and thus inhibit many of the pathways that are critical to appropriate wound repair. These medications inhibit DNA, RNA, or protein synthesis, resulting in decreased fibroplasia and neovascularization of wounds[76]. Chemotherapeutic drugs delay cell migration into the wound, decrease early wound matrix formation, lower collagen production, impair proliferation of fibroblasts, and inhibit contraction of wounds[76]. In addition, these agents weaken the immune functions of the patients, and thereby impede the inflammatory phase of healing and increase the risk of wound infection. Chemotherapy induces neutropenia, anemia, and thrombocytopenia, thus leaving wounds vulnerable to infection, causing less oxygen delivery to the wound, and also making patients vulnerable to excessive bleeding at the wound site.

Impaired wound healing due to chemotherapeutic drugs such as adriamycin is most common when the drugs are administered pre-operatively or within 3 weeks post-operatively[83]. Additionally, low post-operative albumin levels, low post-operative hemoglobin, advanced stage of disease, and electrocautery use have all been reported as risk factors for the development of wound complications[84].

A newer generation of tumor chemotherapeutics is the angiogenesis inhibitors, such as bevacizumab, which is an antibody fragment that neutralizes VEGF. These therapies work in conjunction with traditional chemotherapeutics to limit the blood supply to tumors, reducing their ability to grow. Wound-healing complications, including an increase in wound dehiscence, have been described in patients on angiogenesis inhibitors[85]. A caveat is that most patients on angiogenesis inhibitors are also on traditional chemotherapeutics, making it

difficult to sort out whether angiogenesis inhibitors alone would perturb repair[86]. Nevertheless, current recommendations include discontinuation of angiogenesis inhibitors well in advance of any surgical procedures.

(f) Obesity

The prevalence of obesity continues to increase among adults, children, and adolescents in the United States, with more than 30% of adults and 15% of children and adolescents classified as obese in a recent survey (Centers for Disease Control and Prevention, CDC). Obesity is well-known to increase the risk of many diseases and health conditions, which include coronary heart disease, type 2 diabetes, cancer, hypertension, dyslipidemia, stroke, sleep apnea, respiratory problems, and impaired wound healing. Obese individuals frequently face wound complications, including skin wound infection, dehiscence, hematoma and seroma formation, pressure ulcers, and venous ulcers[87]. An increased frequency of wound complications has been reported for obese individuals undergoing both bariatric and non-bariatric operations[88]. In particular, a higher rate of surgical site infection occurs in obese patients. Many of these complications may be a result of a relative hypoperfusion and ischemia that occurs in subcutaneous adipose tissue. This situation may be caused by a decreased delivery of antibiotics as well. In surgical wounds, the increased tension on the wound edges that is frequently seen in obese patients also contributes to wound dehiscence. Wound tension increases tissue pressure, reducing microperfusion and the availability of oxygen to the wound[87].

The increase in pressure ulcers or pressure-related injuries in obese individuals is also influenced by hypovascularity, since poor perfusion makes tissue more susceptible to this type of injury. In addition, the difficulty or inability of obese individuals to reposition themselves further increases the risk of pressure-related

injuries. Moreover, skin folds harbor micro-organisms that thrive in moist areas and contribute to infection and tissue breakdown. The friction caused by skin-on-skin contact invites ulceration. Together, these factors predispose obese individuals to the development of impaired wound healing[89].

In addition to local conditions, systemic factors also play an important role in impaired wound healing and wound complications in obese patients. Obesity can be connected to stress, anxiety, and depression, all situations which can cause an impaired immune response[87].

The function of adipose tissue used to be considered as primarily caloric storage. However, more recent findings have documented that adipose tissue secretes a large variety of bioactive substances that are collectively named adipokines. Both adipocytes themselves as well as macrophages inside the adipose tissue are known to produce bioactive molecules including cytokines, chemokines, and hormone-like factors such as leptin, adiponectin, and resistin. Adipokines have a profound impact on the immune and inflammatory response[90]. The negative influence of adipokines on the systemic immune response seems likely to influence the healing process, although direct proof for this is lacking. Impaired peripheral blood mononuclear cell function, decreased lymphocyte proliferation, and altered peripheral cytokine levels have been reported in obesity. Importantly, though, many of the obesity-related changes in peripheral immune function are improved by weight loss[91].

(g) Alcohol Consumption

Clinical evidence and animal experiments have shown that exposure to alcohol impairs wound healing and increases the incidence of infection[92]. The effect of alcohol on repair is quite clinically relevant, since over half of all emergency room trauma cases involve either acute or chronic alcohol exposure[93]. Alcohol

exposure diminishes host resistance, and ethanol intoxication at the time of injury is a risk factor for increased susceptibility to infection in the wound[94]. Studies have demonstrated profound effects of alcohol on host-defense mechanisms, although the precise effects are dependent upon the pattern of alcohol exposure (*i.e.*, chronic *vs*. acute alcohol exposure, amount consumed, duration of consumption, time from alcohol exposure, and alcohol withdrawal). A recent review on alcohol-induced alterations on host defense after traumatic injury suggested that, in general, short-term acute alcohol exposure results in suppressed pro-inflammatory cytokine release in response to an inflammatory challenge. The higher rate of post-injury infection correlates with decreased neutrophil recruitment and phagocytic function in acute alcohol exposure[95].

Beyond the increased incidence of infection, exposure to ethanol also seems to influence the proliferative phase of healing. In murine models, exposure to a single dose of alcohol that caused a blood alcohol level of 100 mg/dL (just above the legal limit in most states in the US) perturbed re-epithelialization, angiogenesis, collagen production, and wound closure[96]. The most significant impairment seems to be in wound angiogenesis, which is reduced by up to 61% following a single ethanol exposure. This decrease in angiogenic capacity involves both decreased expression of VEGF receptors and reduced nuclear expression of HIF-1alpha in endothelial cells. The ethanol-mediated decrease in wound vascularity causes increased wound hypoxia and oxidative stress. Connective tissue restoration is also influenced by acute ethanol exposure, and results in decreased collagen production and alterations in the protease balance at the wound site. In summary, acute ethanol exposure can lead to impaired wound healing by impairing the early inflammatory response, inhibiting wound closure, angiogenesis, and collagen production, and altering the protease balance at the wound site[97].

As mentioned previously, the host response to chronic alcohol exposure

appears to be different from that of acute alcohol exposure. Analysis of clinical data indicates that chronic alcohol exposure causes impaired wound healing and enhanced host susceptibility to infections, but the detailed mechanisms that explain this effect need more investigation.

(h) Smoking

Post-operatively, patients who smoke show a delay in wound healing and an increase in a variety of complications such as infection, wound rupture, anastomotic leakage, wound and flap necrosis, epidermolysis, and a decrease in the tensile strength of wounds[98]. Approximately over 4000 substances in tobacco smoke have been identified, and some have been shown to have a negative impact on healing[99]. Most studies have focused on the effects of nicotine, carbon monoxide, and hydrogen cyanide from smoke. Nicotine probably interferes with oxygen supply by inducing tissue ischemia, since nicotine can cause decreased tissue blood flow *via* vasoconstrictive effects[100].

Several cell types and processes that are important to healing have been shown to be adversely affected by tobacco smoke. In the inflammatory phase, smoking causes impaired white blood cell migration, resulting in lower numbers of monocytes and macrophages in the wound site, and reduces neutrophil bactericidal activity. Lymphocyte function, cytotoxicity of natural killer cells, and production of IL-1 are all depressed, and macrophage-sensing of Gram-negative bacteria is inhibited. These effects result in poor wound healing and an increased risk of opportunistic wound infection[99].

During the proliferative phase of wound healing, exposure to smoke yields decreased fibroblast migration and proliferation, reduced wound contraction, hindered epithelial regeneration, decreased extracellular matrix production, and upset in the balance of proteases[99].

Despite the overall negative effects of smoking, some recent studies have suggested that low doses of nicotine enhance angiogenesis and actually improve healing[101, 102].

(i) Nutrition

Carbohydrates, Protein, and Amino Acids

Fats and carbohydrates are the primary source of energy in the wound-healing process. Glucose is the major source of fuel used to create the cellular ATP that provides energy for angiogenesis and deposition of the new tissues[103]. The use of glucose as a source for ATP synthesis is essential in preventing the depletion of other amino acid and protein substrates[104].

Collagen is the major protein component of connective tissue and is composed primarily of glycine, proline, and hydroxyproline. Collagen synthesis requires hydroxylation of lysine and proline, and co-factors such as ferrous iron and vitamin C. Impaired wound healing results from deficiencies in any of these co-factors[105].

Arginine is a semi-essential amino acid that is required during periods of maximal growth, severe stress, and injury. Arginine has many effects in the body, including modulation of immune function, wound healing, hormone secretion, vascular tone, and endothelial function. Arginine is also a precursor to proline, and, as such, sufficient arginine levels are needed to support collagen deposition, angiogenesis, and wound contraction[103]. Arginine improves immune function, and stimulates wound healing in healthy and ill individuals[106]. Under psychological stress situations, the metabolic demand of arginine increases, and its supplementation has been shown to be an effective adjuvant therapy in wound healing[105].

Glutamine is the most abundant amino acid in plasma and is a major source of metabolic energy for rapidly proliferating cells such as fibroblasts, lymphocytes, epithelial cells, and macrophages[104]. The serum concentration of glutamine is reduced after major surgery, trauma, and sepsis, and supplementation of this amino acid improves nitrogen balance and diminishes immunosuppression[105].

Fatty Acids

In surgical or critically ill patients lipids are used as nutritional support for to help meet energy demands and provide essential building blocks for wound healing and tissue repair. They have been reported to affect pro-inflammatory cytokine production, cell metabolism, gene expression, and angiogenesis in wound sites[107]. The true benefit of omega-3 fatty acids may be in their ability to improve the systemic immune function of the host, thus reducing infectious complications and improving survival[104].

Vitamins, Micronutrients, and Trace Elements

Vitamin C has many roles in wound healing, and a deficiency in this vitamin has multiple effects on tissue repair. Vitamin C deficiencies result in impaired healing, and have been linked to decreased collagen synthesis and fibroblast proliferation, decreased angiogenesis, and increased capillary fragility. Also, vitamin C deficiency leads to an impaired immune response and increased susceptibility to wound infection[104].

Similarly, vitamin A deficiency leads to impaired wound healing. The biological properties of vitamin A include anti-oxidant activity, increased fibroblast proliferation, modulation of cellular differentiation and proliferation, increased collagen and hyaluronate synthesis, and decreased MMP-mediated extracellular matrix degradation[108].

Vitamin E, an anti-oxidant, maintains and stabilizes cellular membrane integrity by providing protection against destruction by oxidation. Vitamin E also has anti-inflammatory properties and has been suggested to have a role in decreasing excess scar formation in chronic wounds. Animal experiments have indicated that vitamin E supplementation is beneficial to wound healing[104], and topical vitamin E has been widely promoted as an anti-scarring agent. However, clinical studies have not yet proved a role for topical vitamin E treatment in improving healing outcomes[109].