What Makes Wounds Chronic

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KEYWORDS

• Chronic wound • Ulcer • Biofilm • Bacteria • Inflammation • Proteases • Cytokines • Wound healing

KEY POINTS

- Ischemia is a common denominator in chronic ulcers. The result of ischemia is tissue injury, necrosis, and the development of open wounds that are quickly colonized by bacteria.
- The infection sets the stage for chronic and uncontrolled inflammation. As the host's inflammatory cells try to remove the damaged tissue, reactive oxygen species and proteases are released, causing further tissue damage.
- The proinflammatory response is perpetuated by the formation of a biofilm that walls off and protects the bacteria and the inflamed ulcer site.
- Because of the raging inflammatory environment, residual connective tissue cells have decreased mitogenic activity and become senescent.
- This vicious cycle of inflammation and tissue destruction persists until aggressive clinical strategies are used to remove bacteria, damaged and necrotic tissue and reduce inflammation.

INTRODUCTION

The wound healing process consists of a carefully coordinated sequence of events after a cutaneous injury leading to regeneration of the skin protective barrier.[1] After an initial insult, wounds that fail to progress through the stages of wound healing within a 3-month period of time are deemed chronic wounds.[2] Chronic wounds are characterized by a prolonged and sustained inflammatory phase that prevents dermal and epidermal cells from responding to chemical signals.[3] Most chronic wounds begin with small tissue insults, including minor trauma or skin tears and insect bites. In the setting of comorbidities including diabetes and arterial insufficiency that inhibit blood flow, these wound often evolve into chronic nonhealing wounds. With the increasing elderly population combined with increased worldwide risk of diabetes, chronic wounds represent a significant contributor to health care costs and morbidity.

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TYPES OF CHRONIC WOUNDS

Chronic wounds are classified into vascular ulcers (venous and arterial), diabetic ulcers, and pressure ulcers, all of which have different causes but can lead to nonhealing wounds. Most chronic wounds fail to progress beyond the inflammatory phase of wound healing and are often impacted by the presence of infection[4] drug-resistant biofilms (see Steven R. Evelhoch's article, "Biofilm and Chronic Non-Healing Wound Infections," in this issue),[5] and a loss of response to chemotactic stimuli[6] that preclude them from healing. Although there are similarities among these nonhealing wounds, they differ in terms of the mechanism underlying their inability to heal.[7]

Vascular Ulcers

Vascular ulcers can arise from either venous or arterial insufficiency. Venous ulcers are preceded by symptoms of heaviness and pain in the legs and often associated with swelling, varicose veins, and areas of hyperpigmentation owing to hemosiderin, a breakdown product of hemoglobin. Lipodermatosclerosis, an inflammation of the layer of fat under the epidermis of the limb, occurs when skin and subcutaneous tissue are replaced by fibrinous scar before ulcer formation. Venous ulcers then occur when incompetent valves or obstruction in the superficial and deep veins result in a backflow of blood, leading to increased venous pressure, changes in blood vessel permeability with fibrin, plasma, and red blood cell leakage into the interstitial space. These entities serve as chemoattractants for leukocytes infiltration into the area.[3] Fibrin accumulation downregulates collagen synthesis and accumulates in the form of pericapillary fibrin cuffs.[8] There are 3 main theories regarding the development of ulcers in venous insufficiency.[9] The fibrin cuff theory supports the trapping of various factors that stimulate prolonged inflammation and interfere with oxygen tissue diffusion, further impacting the normal wound healing cascade. The leukocyte entrapment theory suggests that venous hypertension leads to a decrease in the pressure gradient in the capillaries such that blood moves sluggishly and increases the adherence of blood cells to the endothelium resulting in the release of inflammatory mediators such as tumor necrosis factor α (TNFα) that upregulates the adhesion molecules, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and reactive oxygen species causing ischemia and ulceration.[10] Last, the microangiopathy theory suggests there is occlusion of capillaries by microthrombi leading to poor oxygenation. These associated venous skin changes predispose patients to developing venous stasis ulcers in the setting of minor trauma.

Arterial ulcers result from arterial insufficiency from atherosclerosis that prevents adequate blood flow perfusion leading to tissue ischemia and necrosis.[11,12] These ulcers may be associated with advanced age, smoking, diabetes mellitus, hypertension, dyslipidemia, family history, obesity, and a sedentary lifestyle. There are multiple theories regarding the pathogenesis of ischemic leg ulcers, but all coalesce into decreased tissue oxygenation secondary to poor blood flow.

Pressure Ulcers

Pressure ulcers occur from tissue ischemia from sustained direct pressure and shearing forces applied to skin. These ulcers are most common in patients with poor mobility and neuropathies, although they can be worsened by a patient with concomitant venous or arterial insufficiency.

Although friction and shear stress cause direct epidermal and dermal skin changes and subepidermal breaks in skin, the mechanical load causes changes in interstitial fluid content, ultimately leading to tissue necrosis. There is a significant upregulation

of inflammatory markers in the adipose tissue in response to reperfusion injury related hypoxia and reoxygenation, suggesting that the adipocytes may drive this inflammatory response. Pressure ulcers are characterized by an excessive density of neutrophils.[13]

Diabetic Ulcers

Diabetic ulcers occur in the setting of peripheral neuropathy in which patients are unable to recognize repeated minor trauma to the legs. The mechanism underlying the pathogenesis of diabetic ulcers includes elevated glucose levels causing increased levels of reactive oxygen species, nitric oxide blockade, DNA alternation, elevation protein kinase C, ischemia, and inflammation.[14] Peripheral arterial disease further contributes to disease pathogenesis owing to decreased capillary size, thickening of the basement membrane, and arteriolar hyalinosis.[15] Persistent hyperglycemia also causes endothelial dysfunction and smooth muscle abnormalities, with resulting vasoconstriction[16] The chronicity and poor wound healing associated with diabetic ulcers is multifactorial and includes abnormalities in growth factor production, angiogenesis, cell migration and proliferation, collagen deposition, and extracellular matrix remodeling by proteases.[17–19]

NORMAL WOUND HEALING

The process of normal wound healing consists of 4 key phases in which cells and an extracellular matrix provide a framework for collagen growth and deposition.[1] The 4 phases of wound healing include hemostasis, inflammation, proliferation, and remodeling (**Fig. 1**) and relies on chemical mediators including growth factors, chemokines, and inhibitors. In the hemostasis phase, the process begins with vasoconstriction followed by platelet activation by collagen binding to an extracellular matrix. The platelets release chemical factors, including fibronectin, thrombospondin, sphingosine-1-

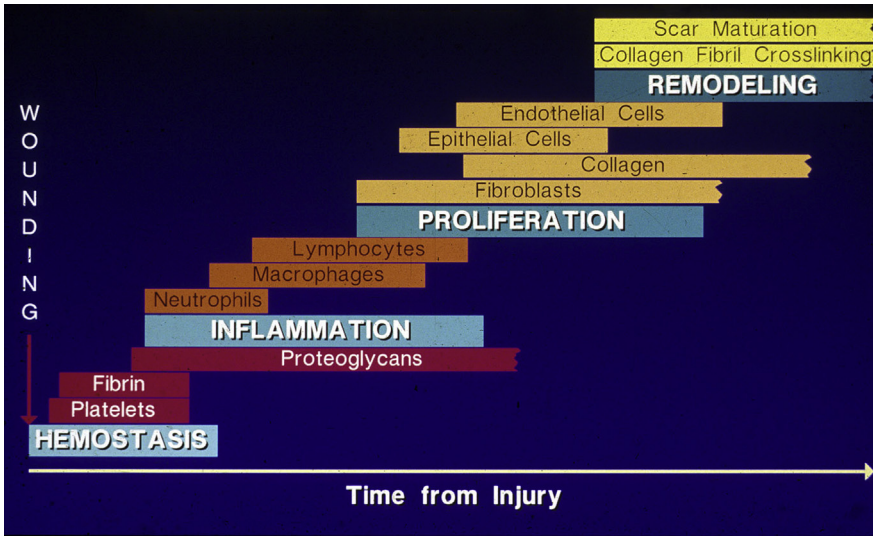

Fig. 1. Phases of normal wound healing. Cellular and molecular events during normal wound healing progress through 4 major, integrated, phases: hemostasis, inflammation, proliferation, and remodeling. (*From* Cohen IK, Diegelmann RF, Linblad WJ. Wound Healing: Biochemical & Clinical Aspects. Philadelphia, PA: W.B. Saunders Co.; 1992; with permission.)

phosphate, and von Willebrand's factor, which aid in ongoing control of bleeding in the wound.[20–22] Insoluble fibrin forms a provisional matrix to which platelets adhere and form a plug or clotlike structure[23] (Fig. 2).

Wounds then progress within 24 hours after injury to the next phase of wound healing called the inflammatory phase lasting until postinjury day 4. This phase is characterized by cell-mediated removal of bacteria and devitalized tissue through the migration of neutrophils and macrophages to allow for subsequent collagen deposition by fibroblasts and neovascularization[24] (Fig. 3). Neutrophils bind to specialized cell adhesion molecules on endothelial cells to then marginate and squeeze through leaky cell junctions into the interstitial space through a process of pavementing and diapedesis. Neutrophil migration is termed chemotaxis and is mediated by both chemokines and bacterial presence within a wound. Neutrophils generate reactive oxygen species via the enzyme myeloperoxidase, phagocytize foreign debris, and release matrix metalloproteinases (MMPs), which further digest surrounding necrotic tissue. Activated macrophages also function as phagocytic cells and release proteases to further digest injured tissues.

The transition to the next phase of wound healing occurs when neutrophils release IL-1 and TNFα to activate fibroblasts and epithelial cells and macrophages release multiple growth factors including platelet-derived growth factor, transforming growth factor-β, TNFα, fibroblast growth factor, insulin-like growth factor-1, and IL-6. The critical phase of wound healing, called the proliferative phase, occurs between postinjury days 4 and 21 and heavily relies on fibroblast proliferation and migration. This phase is characterized by collagen synthesis, deposition and cross-linking, and formation of a reconstituted extracellular matrix by the addition of proteoglycans (Fig. 4). Open wounds begin to contract through specialized cells called myofibroblasts.[25] The presence of MMPs from inflammatory cells and fibroblasts allows for ongoing collagen remodeling but they are regulated by specific inhibitors, called tissue inhibitors of MMPs. The net result is that there is more collagen deposition than destruction.[26,27]

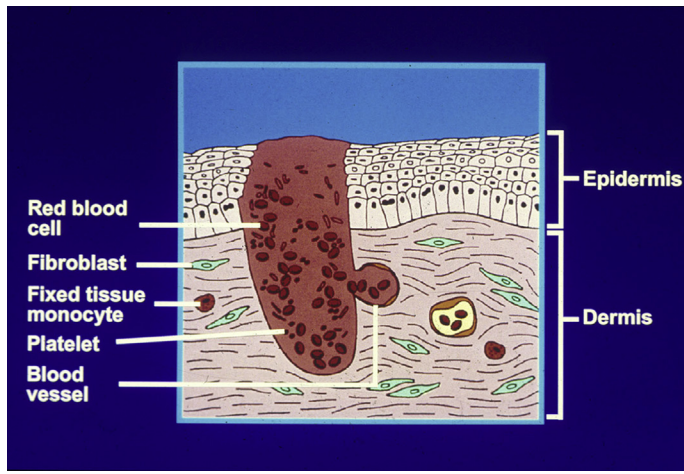

Fig. 2. Hemostasis phase. At the time of injury, the fibrin clot forms the provisional wound matrix and platelets release multiple growth factors that initiate the repair process. (*From* Chandawarkar R, Miller MJ. Wound Healing. In: Mulholland MW, Lillemoe KD, Doherty GM et al. Greenfield's Surgery: Scientific Principles & Practice. 6th ed. Philadelphia, PA: J. B. Lippincott & Co.; 1993; with permission.)

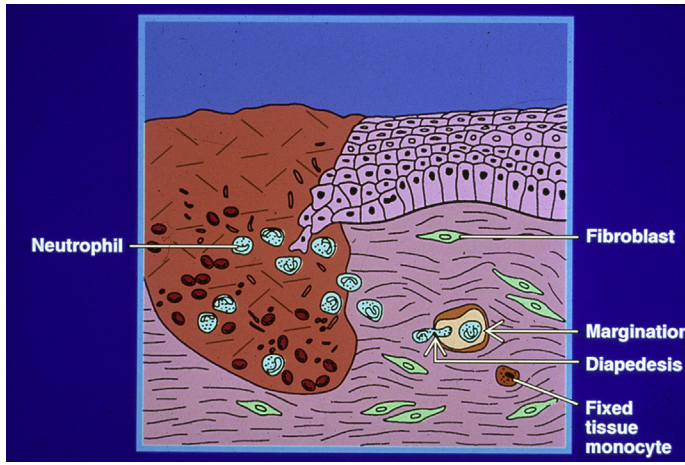

Fig. 3. Inflammatory phase. Within 1 day after an injury, the inflammatory phase is initiated by neutrophils that attach to endothelial cells in the vessel walls surrounding the wound (margination), change shape and move through the cell junctions (diapedesis), and migrate to the wound site (chemotaxis). (*From* Chandawarkar R, Miller MJ. Wound Healing. In: Mulholland MW, Lillemoe KD, Doherty GM et al. Greenfield's Surgery: Scientific Principles & Practice. 6th ed. Philadelphia, PA: J. B. Lippincott & Co.; 1993; with permission.)

The last phase of wound healing is called the remodeling phase, goes on for years, and occurs once all extracellular matrix components have been deposited in the wound site (Fig. 5). The final wound appears as a scar with approximately 80% of the original tensile strength of normal tissue. In this phase, the initial type III collagen that was deposited, is replaced by type 1 collagen and cross-linked.[28]

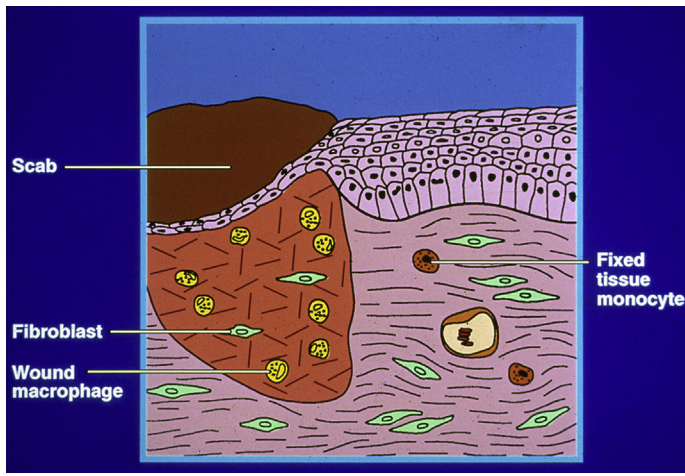

Fig. 4. Proliferation phase. Fixed tissue monocytes activate, move into the site of injury, transform into activated wound macrophages that kill bacteria, release proteases that remove denatured extracellular matrix, and secrete growth factors that stimulate fibroblast, epidermal cells, and endothelial cells to proliferate and produce scar tissue. (*From* Chandawarkar R, Miller MJ. Wound Healing. In: Mulholland MW, Lillemoe KD, Doherty GM et al. Greenfield's Surgery: Scientific Principles & Practice. 6th ed. Philadelphia, PA: J. B. Lippincott & Co.; 1993; with permission.)

CHRONIC WOUND HEALING

Chronic wounds fail to progress through the organized phases of wound healing in a timely manner.[2] Although they differ in etiology, chronic wounds are typically characterized by excessive levels of proinflammatory cytokines, proteases, reactive oxygen species, senescent cells, bioburen, and a deficiency of functional stem cells.[29]

Inflammation: Bacteria and Biofilms in Chronic Wounds

Bacteria have the ability to both colonize and infect wounds, causing major problems in the chronic wound setting. Most chronic wounds are polymicrobial in nature with a preponderance of *Staphylococcus* and *Pseudomonas* species.[30] Anaerobic bacteria are found in a relative abundance in chronic wounds, which are continually exposed to high levels of oxygen.[31] Interestingly, there is a paucity of *Corynebacterium*, a commensal bacteria, in these wounds. Commensal bacteria have long been shown to benefit the host organism by educating the host adaptive response and inhibiting the growth of pathogenic bacteria[32,33] (recent data have shown coryneform bacteria to be pathogenic in wounds). The polymicrobial nature of wound allows for microbial diversity and heterogeneity with in a wound, further challenging the wound's ability to heal.

To strengthen their antimicrobial resistance, planktonic bacteria evolved to create biofilms. Biofilms are formed when bacterial cells attach to a surface and use quorum-sensing molecules to induce changes in gene expression, which ultimately creates a barrier consisting of predominately exopolymers, with some residual bacterial cells. The biofilm consists of 85% exopolymers, including polysaccharides, proteins, and nucleic acids, combined with 15% bacteria and form the mature biofilm. Biofilms are consistently polymicrobial with planktonic cells leaving the area to find additional areas to colonize. Steven R. Evelhoch's article, "Biofilm and Chronic Non-Healing Wound Infections," in this issue focuses on role of biofilms in wound healing.

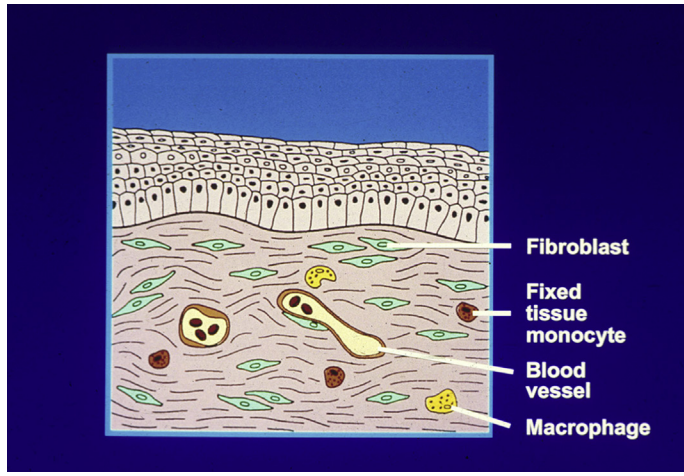

Fig. 5. Remodeling phase. The initial, disorganized scar tissue is slowly replaced by a matrix that more closely resembles the organized extracellular matrix of normal skin. (*From* Chandawarkar R, Miller MJ. Wound Healing. In: Mulholland MW, Lillemoe KD, Doherty GM et al. Greenfield's Surgery: Scientific Principles & Practice. 6th ed. Philadelphia, PA: J. B. Lippincott & Co.; 1993; with permission.)

Biofilms stimulate the host immune response

Biofilms have been shown to be even more recalcitrant than bacteria to the host immune response, making them an even greater challenge for chronic wounds than bacteria alone. Leukocytes within the wound have difficulty penetrating and maneuvering through the biofilm and have an impaired ability to produce reactive oxygen species.[34] This property also prevents phagocytosis of bacteria through normal wound healing pathways. The structural exopolymer of the biofilm has been suggested to evade aspects of the host inflammatory response by further blocking complement activation, suppressing the lymphoproliferative response, and impairing the ability of opsonins on bacterial walls to be detected by phagocytes. There seems to be heterogeneity in biofilms, likely depending on the specific pathogenic micro-organisms.

Biofilms directly resist antimicrobial therapy

By creating and incorporating into biofilms, bacterial cells create an environment where they have decreased metabolic activity, thus rendering themselves less effective against antimicrobial agents that target metabolically active cells.[35] An additional mechanism of resistance is that the exopolysaccharide in the biofilm functions as a mechanical barrier to protect bacteria from antimicrobials and the host immune cells.[36] Biofilms allow for the transfer of plasmid-mediated antimicrobial resistance genes among bacteria within a biofilm that not only adds to the heterogeneity of the wound, but also provides added resistance. Some biofilms are thought to have concentration gradients to minimize the impact of antibiotics and antiseptics, whereas some biofilms may be eradicated after antimicrobial therapy only to have persister cells stimulate regrowth of biofilm once these agents have been removed. Biofilms may possess an additional evolutionary response to antimicrobial therapy by developing thicker mucoid-like phenotypes in response to some antimicrobial therapies.

Biofilms stimulate chronic inflammation

Biofilms are present in nearly 60% of chronic wound but only 10% of acute wounds, and notably stimulate chronic inflammation in the chronic setting. Stimulation of the immune system when unable to effectively eradicate infection can lead to worsening of chronic inflammation and perpetuate the cycle of the chronic wound. This phenomenon occurs through gene expression, which induces inflammation to promote plasma leakage from local capillaries for nutrition.[37] Additionally, biofilms contribute to wound bed senescence cause by oxidative stress and protease-mediated degradation of receptors and cytokines. This leads to alterations in host cell cytoskeleton, inhibition of mitosis, and apoptosis.

PROTEASES

Wounds produce MMPs, calcium-dependent zinc-containing enzymes, that, together with their inhibitors, play key role in the regulation of extracellular matrix deposition and degradation.[38] MMPs can be divided into 7 groups based on the substrate preference and domain organization: (1) collagenases, (2) gelatinases, (3) stromelysins, (4) matrilysins, (5) metalloelastases, (6) membrane-type MMPs, and (7) other MMPs.

Overexpression of MMPs causes damage to the extracellular matrix and drives the underlying pathology of chronic, nonhealing wounds. Overproduction of MMPs also destroys vital growth factors such as platelet-derived growth factor and transforming growth factor-β necessary for wound healing. This overproduction results in an unregulated, continuous inflammatory phase for chronic nonhealing wounds. In normal tissue, there are very low levels of MMPs. In injured tissue, fibroblasts, keratinocytes, endothelial, and inflammatory cells secrete MMPs in response to cytokines, hormones, and

other cell types in the extracellular matrix. Cytokines and growth factors known to transcriptionally activate MMPs include transforming growth factor-β, vascular endothelial growth factor, epidermal growth factor, interleukins and interferons, all of which are important in wound healing.[39] Overexpression of MMP-1 delays re-epithelization and is known to be elevated in chronic wounds associated with diabetic foot ulcers.[19,40]

Neutrophil-derived MMP-8 has been associated with chronic wounds.[41] Circulating neutrophils respond to a site of injury and secrete proinflammatory mediators to recruit other inflammatory cells. Neutrophils subsequently undergo apoptosis with phagocytosis by macrophages, which ends the inflammatory phase of wound healing in normal wounds. However, in chronic wounds, neutrophils continue to recruit additional inflammatory cells to the wound bed, leading to ongoing inflammation. Extracellular release of reactive oxygen species and proteases also cause ongoing tissue damage, which further impairs wound healing through defective collagen deposition, decreased wound strength, and delayed re-epithelialization. Protease function is also regulated by multiple protease inhibitors stored in neutrophils. α1-Antitrypsin has been shown to be degraded in chronic wounds, which is thought to contribute to excess serine protease activity in chronic wounds. Fibronectin degradation in chronic wounds depends on the relative levels of elastase, α-1-proteinase inhibitor, and α-2-macroglobulin.[42,43]

PROINFLAMMATORY MEDIATORS (IL-8, IL-6, AND TUMOR NECROSIS FACTOR-α)

Although there are a number of proinflammatory mediators and factors responsible for the chronicity and prolonged inflammation in chronic ulcers, there are several that are major players.[7] IL-8 is a well-known chemoattractant and activator of neutrophils and has a prominent role in chronic ulcers.[44] One of the hallmarks of inflammation is the excessive presence of neutrophils that release several damaging proteases such as MMP 8 and elastase.[13] IL-6 is an interesting and a multifunctional cytokine that elicits a spectrum of responses.[45] It is a potent proinflammatory signal and contributes to the chronicity of chronic ulcers.[46,47] TNFα is another well-known proinflammatory cytokine that has been shown to cause tissue damage in sites of infection.[48] As with IL-6 and IL-8, TNFα also has a critical role in the chronicity of nonhealing ulcers.[49]

TREATMENT OF CHRONIC WOUNDS: HOW TO BREAK THE INFLAMMATORY CYCLE

Debridement, Infection/Inflammation, Moisture Management, Edge/Environment, Support Products and Services: A Holistic Approach to the Management of Chronic Wounds

The debridement, infection/inflammation, moisture management, edge/environment, support products and services wound care guideline was developed as an overall approach to managing patient's wounds and addressing underlying comorbidities.[50] The infection/inflammation, moisture management, edge/environment, support products and services process consists of a comprehensive approach to wound bed preparation, control of infection and inflammation, and maintaining an appropriate moisture balance within the wound (**Fig. 6**).

Specifically, wounds are assessed for the presence of devitalized, infected, and/or inflamed tissue that may inhibit the wound healing process. Ongoing within the chronic wounds are then addressed by physical examination for heat, pain, redness, and swelling. Pain is a reliable marker of infection. Debridement of this devitalized tissue removes areas with high bacterial loads, biofilm, and helps to reinvigorate the wound healing process in tissue with a higher oxygen tension.

Removal of this tissue also removes the bacteria, proteases, inflammatory mediators, and hyperproliferative wound edges that stall a wound in a prolonged

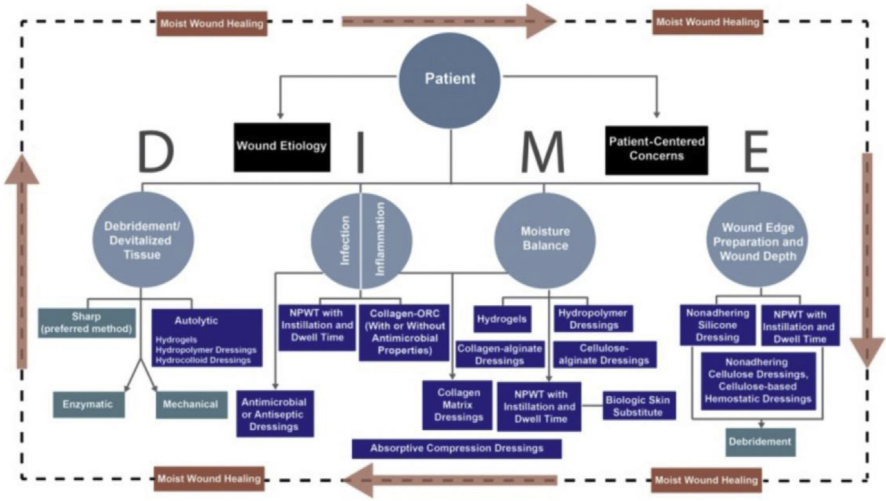

Fig. 6. Schematic of devitalized tissue, infection/inflammation, moisture balance, and edge preparation wound treatment strategy. NPWT, negative pressure wound therapy; ORC, oxidized regenerated cellulose. (*From* Snyder RJ, Fife C, Moore Z. Components and Quality Measures of DIME (Devitalized Tissue, Infection/Inflammation, Moisture Balance, and Edge Preparation) in Wound Care. *Adv Skin Wound Care*. 2016;29(5):205-215; with permission.)

inflammatory phase. Removal of chronic hyper granulation tissue is necessary; it has been shown to decrease the amount of antibiotics that can reach a wound infection and prolong the wound healing process. Wound debridement can be in the form of sharp debridement, mechanical debridement with negative pressure wound therapy with instillation and dwell time, or autolytic debridement through hydrogels and hydrocolloid dressings, depending on the extent of devitalized tissue burden. The presence of inflammation is addressed by looking for underlying causes including malignancy, vasculitis, vasculopathy, and pathergy in the form of pyoderma gangrenosum and biopsy when necessary. Collagen matrix dressings may be used to facilitate a decrease in wound inflammation. Maintaining wound moisture balance is also an important aspect of chronic wound healing. The wound bed is assessed through an evaluation of the quality, odor, and consistency of drainage both within the wound and the surrounding periwound tissue. A moist environment is necessary to promote growth factors, cytokines, and chemokine function in a wound. Too much moisture, however, can lead to periwound maceration and stall the wound care process within the wound bed. A dry wound bed, resulting from exposure of the wound to air, leads to desiccation and necrosis and perpetuates the cycle of poor wound healing.

ADJUNCTIVE THERAPIES

Many adjunctive wound care therapies have been developed to facilitate the healing of chronic wounds. Topical antibiotics may be used to minimize bacterial infection with bacterial threshold of 10^5. Silver- and iodine-impregnated dressings may also be used to control bacterial load on an ongoing basis. Moist occlusive dressings help to create an environment with low oxygen tension and facilitate re-epithelization through activation of hypoxia-inducible factor 1.[51]

Negative pressure wound therapy has been widely used to facilitate chronic wound healing by 4 primary mechanisms (macrodeformation, microdeformation, fluid

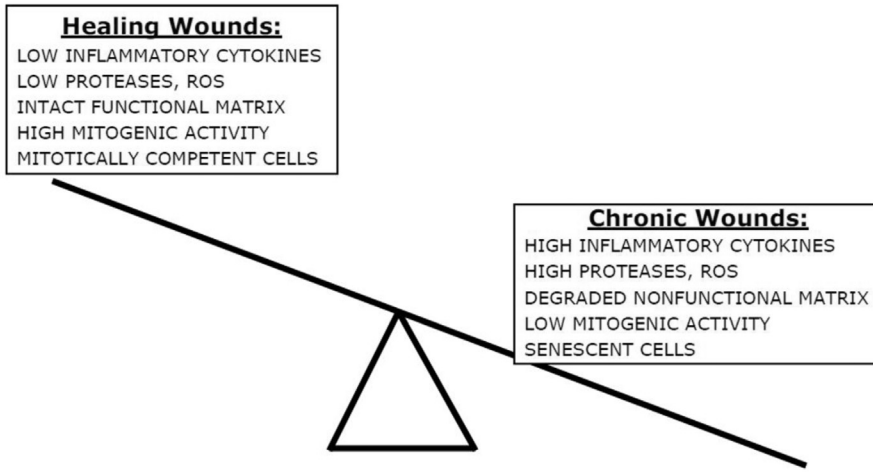

Fig. 7. Comparison of the molecular and cellular environments of normal healing compared with chronic wounds. Elevated levels of cytokines and proteases in chronic wounds reduce mitogenic activities and response of wound cells thus impairing healing. ROS, reactive oxygen species. (*From* Schultz GS, Chin GA, Moldawer L, et al. Principles of Wound Healing. In: Fitridge R, Thompson M, eds. Mechanims of Vascular Disease: A Reference Book for Vascular Specialists [Internet]. Adelaide, Australia: University of Adelaide Press; 2011; with permission.)

removal, and alteration of the wound environment) and various secondary mechanisms (including neurogenesis, angiogenesis, modulation of inflammation, and alterations in bioburden).[52,53]

SUMMARY

Fig. 7 summarizes this article by showing the comparison of the molecular and cellular environments of normal healing compared with chronic wounds. Increased levels of cytokines and proteases in chronic wounds decrease mitogenic activities and the response of wound cells, thus impairing healing.[54]

DISCLOSURE

S.R. Goldberg: Investigative PI for Pfizer and UCB studies. R.F. Diegelmann: No Disclosures.

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