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Authors
Falanga, Vincent
Isseroff, Roslyn Rivkah
Soulika, Athena M
et al.

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CHRONIC WOUNDS PRIMER

Vincent Falanga[1,2], **Roslyn Rivkah Isseroff**[3,4], **Athena M. Soulika**[3,5], **Marco Romanelli**[6], **David Margolis**[7,8], **Suzanne Kapp**[9], **Mark Granick**[10], **Keith Harding**[11,12]

[1]Dept. of Dermatology, Boston University, Boston, MA, USA.

[2]Dept. of Biochemistry, Boston University, Boston, MA, USA.

[3]Dept. of Dermatology, University of California at Davis, Davis, CA, USA.

[4]Dermatology Section, VA Northern California Health Care System, Mather, CA, USA.

[5]Shriners Hospital for Children, Sacramento, CA, USA

[6]Dept. of Dermatology, University of Pisa, Pisa, Italy.

[7]Dept. of Dermatology, University of Pennsylvania, Philadelphia, PA, USA.

[8]Dept. of Biostatistics, Epidemiology and Informatics, University of Pennsylvania, Philadelphia, MA, USA.

[9]Dept. of Nursing, University of Melbourne, Melbourne, Australia

[10]Dept. of Surgery, Rutgers New Jersey Medical School, Newark, NJ, USA

[11]Clinical Innovation Hub Cardiff University, Cardiff, Wales, UK.

[12]Skin Research Institute Singapore (SRIS)

Abstract

Chronic wounds are characterized by their inability to heal within an expected timeframe and have emerged as an increasingly important clinical problem over the last several decades, due to their higher incidence and greater recognition of associated morbidity and socio-economic burden. Even up to a few years ago, the management of chronic wounds relied on standards of care that were outdated. However, our approach to these chronic conditions has recently enjoyed a renaissance in better prevention, diagnosis, and treatment. Such improvements are due to major advances in cellular and molecular aspects of basic science, in innovative and technological breakthroughs from biomedical engineering, and in our ability to conduct well controlled and reliable clinical research. The evidence-based approaches resulting from these recent advances have become the new standard of care. At the same time, satisfaction with these improvements is tempered by the recognition that persistent gaps exist in our scientific knowledge of impaired healing and our ability to lessen morbidity, loss of limb, and mortality. Therefore, taking stock

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of what we know and what is needed to improve our understanding of chronic wounds and their associated failure to heal is critical to ensuring better treatments and outcomes.

INTRODUCTION

Chronic wounds are characterized by their inability to heal within an expected timeframe, The definition of chronic wounds is itself challenging. Some authors have proposed guidance based on how long it might take for the wound to heal. This approach is artificial, but unfortunately a better definition that is scientifically acceptable is not available. A major part of the definitional challenge is that chronic wounds are quite heterogeneous in terms of etiology, pathogenesis, size, body location, morbidity, loss of affected limb, host factors, and several other variables. Figure 1 shows examples of the major and typical types of chronic wounds (V.F.). These wounds generally affect the adult population, and are the result of complications from venous insufficiency, diabetes and neuropathies, inability to move and/or spinal cord injury (pressure ulcers), and arterial insufficiency. There are several other clinical settings where the initial injury is the result of genetic factors (for example, the spectrum of epidermolysis bullosa in children) or radiation (accidental or therapeutic). Also, some chronic ulcers have a more predominant immunological basis. Pyoderma gangrenosum and atypical ulcers (such as those due to cryofibrinogenemia or cryoglobulinemia, are examples of that group of chronic wounds.[1] It is estimated that in developed countries and worldwide, about 1–2% of the population will experience a chronic wound sometime in their lives. In less developed countries the etiologies may also be quite different; examples include nutritional deficiencies, parasitic and chronic fungal infestation, leprosy. Still, a common denominator does exist, regardless of the underlying cause. That common denominator is “impaired healing” or what some authors and clinicians call “failure to heal.”

In the last few years, a renaissance has taken place in the understanding and treatment of chronic wounds and impaired healing. Basic science, mostly cellular and molecular, has uncovered many of the processes underlying extracellular matrix deposition, cell migration, and inflammatory responses. A direct result of advances in the basic sciences has been the development of technological breakthroughs for treatment, particularly in the areas of tissue engineering, stem cells, and growth factors. Devices and surgical approaches have been developed or improved for better therapeutic approaches. Therefore, the outlook for better understanding of impaired healing and therapies is brighter than it was even a few years ago.[2,3] Yet, critical gaps in our knowledge of chronic wounds in general and their characteristic feature of impaired healing remain. In this Primer, we will address the epidemiology (D.M.) and pathophysiologic mechanisms (R.R.I., A.M.S.) underlying the difficulties to overcome in the treatment of chronic wounds and how these difficulties also create opportunities for new discoveries. We will explore how these wounds can best be diagnosed (M.R., V.F, M.G.) and characterized to focus on targeted treatment modalities (K.H.), and how they affect patients’ quality of life (S.K). We will then discuss the outlook (V.F.) and initiate a discussion about what the needs are in these various areas of research clinical approaches to render chronic wounds less healing impaired.

EPIDEMIOLOGY

Chronic wounds are a worldwide problem. Epidemiologic discussions of chronic wounds are complicated by variations in terminology, underlying diseases, and regional distribution, as well as healing or effects of management. First, the term **chronic wound** includes many human aliments for which etiologies, outcomes, treatment, and prognosis vary widely, hampering a unified definition of what is a chronic wound.[4 5] In some instances, wounds are described as **complex wounds**, which are defined as superficial, partial or full thickness skin loss wounds healing by secondary intention. These have a point prevalence of 1.47 per 1000 in the UK. [6] Second, some wounds are defined as a co-morbidity of another illness. For example, in order to have a diabetic foot ulcer, the patient must have diabetes (e.g., population at risk); however, those with diabetes are also more prone to have other chronic wounds like venous leg ulcers. [7] Most chronic wounds are more common in the elderly; therefore epidemiologic discussions of chronic wounds will vary based on population age. Third, the rate of chronic wounds can vary by geographic location and community or facility under study.[8–10] Finally, chronic wounds can heal, be treated with limb altering interventions and recur, altering estimates of prevalence and incidence.

PUs are a worldwide problem especially among patients who are infirm. PU rates are highly dependent on residence (hospital, nursing home, etc.), patient characteristics, and age. [11–13] In a worldwide review from 2019, prevalence rates from cross-sectional studies of PU ranged from 3% to 31% in long-term care facilities.[13] Prevalence varied by country and many of reports reviewed were more than a decade old.[13] A 2019 study in Portugal emphasized the variation by residence and noted a prevalence rate of 5.8% in the hospital, 4.0% in nursing homes, and 0.02% in the community.[14] A similar community rate was noted in a study from Spain and a similar nursing home rate was noted in a study from China. [15,16] An assessment of fifty-one nursing homes in Switzerland revealed a prevalence of 0% to 19%. [12] This rate was highly dependent on patient age and Braden score (a risk score based on patient factors such as mobility, activity, nutrition, patent's sensory/perception, etc.). [12,17,18]

VLUs are the most common chronic wound. It is believed that between 1.5 and 3 per one thousand persons have a venous leg ulcer and that these wounds are more common in women. [15,19–21] The prevalence of VLU has been somewhat stable over time and by country. [22] VLU Prevalence also increases by the patient's age. [19,20] The prevalence also varies by location (full population or primary care setting). [23] The prevalence for those over 65 years of age is about 1.5% and a yearly incidence of 1.2%. [20] However, epidemiologic studies for VLU are also generally more than a decade old.

Probably the best studied chronic wound is the DFU. [9] This is likely because of the public health importance of diabetes as well as the association of this wound with an important complication of diabetes, lower extremity amputation (LEA), as well as death. [24,25] Those with diabetes and a DFU are more than 10 times more likely to have a LEA than those with diabetes and no DFU. [26] Those with a DFU or an LEA are also at a 2- or 3-times increased risk of death, respectively. [24,25] The prevalence of DFU varies from 1.2% to 20% for patients with diabetes in the hospital and from 0.02% to 10% for patients with diabetes in the community. [9,23,27] Among those with diabetes, the worldwide incidence of DFUs has been reported to vary from five per one-hundred-person years to 41 per 100 person years. [9,28] However, DFU prevalence also varies by age, other complications of diabetes, and region. [8,9,26,29] For example, in a study of all US Medicare beneficiaries, the yearly prevalence of DFU was about 8.0% but varied by age from a low of 6.1% for those with diabetes between 65 and 74 years of age to a high of 15.0% for those over 95. [30] Similar variation was noted for the incidence, with an overall rate of 6 per hundred person-years, a lower rate of 4.6 for those between 65 and 74 and a high of 11.5 per hundred person-years for those over 95. [30] All rates varied 3 to 5-fold by US hospital referral region (5,25,26). Regional variation has also been noted in the United Kingdom. [31]

It is expected that epidemiological work will continue to play a key role in prevention of chronic wounds and in identifying targeted therapies, both established and novel. For diabetic ulcers, the focus may necessarily shift to a concerted effort to prevent leg and foot amputation. Information on that topic will continue to accumulate within the United States Diabetes Surveillance System (US CDC: https://gis.cdc.gov/grasp/diabetes/DiabetesAtlas.html

Finally, the likelihood of a wound healing is highly associated with wound-based and patient-based factors or attributes. [9,32] Examples of these attributes include the size, duration, and depth of the wound. [33,34] A published model making use of more detailed attributes (wounds ≤2cm^2, ≤2 months old, ≤ 2 in grade) has been used to successfully predict healing as well as to risk stratify wound severity. [35]

MECHANISMS/PATHOPHYSIOLOGY

a) Basic mechanisms of wound repair

A recent and extensive review of the physiological phases of the normal wound healing process details the cellular and molecular processes involved in these fundamental events.[36] The normal wound healing process is made of overlapping phases of immediate hemostasis, followed by inflammation, and the proliferative and then remodeling phases.[37] However, the process of tissue repair in chronic wounds is unlikely to fit easily this “linear” paradigm of sequential, overlapping phases.[36 37,38] Figure 2 shows a diagrammatic representation of the non-linear feature of the phases of wound healing in chronic wounds. Another important consideration is that much of what we know about in vivo aspects of wound healing in humans comes from experimental studies in animal models. Such models do not convincingly approximate chronic wounds[39], and often suffer (especially in rodent skin) from excessive contraction.[40] In fact, there are no recognized valid animal models for chronic wounds. Therefore, in the absence of true animal models, much effort has been made by developing animal models that have some features approximating delayed healing and by minimizing wound contraction, which is particularly a problem with rodent skin. [39 41 42,43]Nevertheless, suitable animal models will continue to play a key role in achieving a better understanding of pathophysiological processes (cellular and molecular) and in the testing of new therapeutic agents.[44–51] A valuable FDA guide for the use of animal testing is available: https://www.fda.gov/downloads/drugs/guidances/ucm071324.pdf.

As we stated above, reviews of the overall phases of wound healing have been recently published and address that subject very well.[36] In light of that, we will instead pay greater attention to one of those phases (inflammation) where we feel a greater focus is needed. Figure 3 illustrates the inflammatory phase of the wound healing process which, as detailed in the next section, is being increasingly recognized as playing a key role in the repair process. In addition, as in Figure 4, the focus is on investigative aspects of impaired healing that do not rely exclusively on animal studies but, instead, are derived from evidence obtained from human chronic wounds.

b) Innate Immunity in Wound Healing

Innate immunity is defined as the primary nonspecific first-line defense system protecting against pathogens and tissue damage, rather than to a specific antigen as does adaptive immunity. A continuously updated Nature.com collection on the subject is available: https://nature.com/subjects/innate-immunity. Recent reviews focused on innate immunity and the skin are available.[52–55] Most of the current knowledge on the effects of innate immunity in wound healing comes from studies in rodents. However, new studies using advanced sequencing techniques are starting to shed light on the mechanisms that may be associated with chronic wounds in humans. Below the current literature in both rodent and human wound healing is reviewed. We will be sure to distinguish mechanisms that are clearly applicable to and focused on human chronic wounds (Figure 4).

When discussing pathophysiological mechanisms leading to impaired healing and chronic wounds, a major challenge is that much of what we know has been derived from animal research studies. Therefore, a special effort is required to identify those mechanisms that have been uncovered and which are more clearly applicable or proven to take place in human chronic wounds. Figure 4 is meant to show those pathophysiological mechanisms leading to impaired healing in human chronic wounds. When injured, the initial epidermal skin barrier allows invasion by pathogens that activate the innate system and pro-reparative functions. Injured keratinocytes release danger signals, or Damage Associated Molecular Patterns (DAMPs)[56] that are recognized by cellular pattern recognition receptors (PRR) such as Toll like receptors (TLRs). PRRs also respond to pathogen-associated molecular patterns (PAMPs) present on or in invading pathogens.[57] These pathways, including increasing PRR activity, have been investigated in the context of impaired healing.[58–60] Responses are also ligand specific. DAMPs such as host noncoding double-stranded RNA (dsRNA) upregulate the keratinocyte expression of TLR3,[61] and activation of TLR3 in human keratinocytes then initiates synthesis of components needed for epidermal barrier repair such as sphingomyelin, and transglutaminase-1.[62]

As shown in Figure 4, keratinocytes are also important producers of antimicrobial peptides or proteins (AMPs). The best-studied human keratinocyte AMPs are the β-defensin (hBDs) and cathelicidin (hCAP18/LL-37) families. Keratinocytes constitutively express hBD-1, and wounding upregulates the expression of LL-37 and hBD-2, −3.[63–65]. The cathelicidin family AMP LL-37 is upregulated at edge of acute human surgical wounds, but absent from the wound edge in chronic non-healing ones; blocking its function with anti-LL-37 antibodies impairs wound epithelialization,[66] Moreover, treatment with LL-37 accelerates the healing of hard-to-heal venous ulcers.[65] Other cells may contribute to innate immunity and tissue repair, such as one subtype of myofibroblasts derived from dermal adipose precursor cells.[67] Conversely, in murine wounds, hair follicle cells can signal to myofibroblasts and reprogram them into adipocytes, suggesting new avenues for decreasing scarring.[68]

A recent study using scRNA-seq analysis showed that keratinocytes in pressure ulcers with poor healing outcomes upregulate MHC-II in response to increased IFNg levels at the wound site [69]. Furthermore, keratinocytic MHC-II upregulation impaired activation of T cells at the wound edges (Figure 4).

Langerhans cells are the antigen presenting cell in the epidermis. They express elevated levels of MHC-II and migrate to draining lymph nodes for antigen presentation. In addition, they express C-Lectins, including langerin (CD207) CD205, and CD206, which serve as PRRs and facilitate LCs motility.[70] Increased LC numbers are found at the edges of healing wounds, and lower number of LCs were found in diabetic wounds of both mice and humans.[71,72] Deletion of langerin+ cells has resulted in improved healing.[73]

Both murine and human skin contain γδ T lymphocytes,[74,75] which can promote repair. In the murine epidermis γδ T cells are also known as dendritic epidermal T cells, or DETC. Studies have shown that murine skin injury results in the activation of DETC.[76] Following activation, DETCs express keratinocyte growth factors FGF7 and FGF10, and IGF1 which can promote wound healing.[74,75] γδ T cells are also found in the dermis and express IL17 in excisional wounds, which can promote local inflammation and suppresses IGF-1.[77] In chronic wounds, γδ T cells are reduced and dysfunctional,[78] further supporting a pro-repair role of these cells in normal healing wounds,

Other resident lymphocytes, such as Tregs, have been shown to accelerate wound repair.[79,80] Tregs are usually identified by Foxp3 expression, but also express GATA-3 (a transcription factor associated with Th2 responses) via which they are able to curb fibrosis.[79] Non-cytotoxic innate lymphoid cells (ILCs) have also implicated in wound healing, and one of their subgroups (Group 2), if depleted, results in impaired re-epithelialization (Figure 4).[81,82]

Cells of myeloid origin populate the skin after injury and exhibit either inflammatory (often detrimental) or pro-reparative functions. Although neutrophils were long regarded as mainly playing a role in fighting microbial infection and in removing damaged tissues, they are actually integral to wound repair.[83–85] Persistent neutrophilic presence, however, is associated with degradation of newly formed collagen and impaired tissue repair; slow-healing wounds such as diabetic wounds are populated by large numbers of neutrophils.[49,86–88] In excisional wounds, pan-neutrophil depletion using the GR1 antibody resulted in accelerated healing in both diabetic and non-diabetic mice.[89] Interestingly, healing is impaired upon deletion of CXCR2 [90], one of the main receptors that mediates neutrophil migration.[73,91] Neutrophil extracellular traps (NETs) are made of histone-containing nuclear DNA, protease and other proteins that aid in trapping and killing microbes.[92] Diabetic patients with non-healing wounds have higher levels of neutrophils with NET markers, and non-infected diabetic mice in which NET formation was impaired healed faster.[49,88] Neutrophils exhibit phenotypic heterogeneity and functional plasticity, with a pro-reparative, pro-angiogenic subpopulation that expresses MMP9 for tissue remodeling roles which, in turn, liberates VEGF-A from the extracellular matrix.[93] Furthermore, neutrophils have been shown to express VEGF themselves.[94]

Other granulocytes that have been studied in wound healing are mast cells, whose pro-reparative functions include secretion of growth factors that result in activation of endothelial cell angiogenesis, fibroblast collagen synthesis, and restoration of epithelial barrier.[95] High numbers of mast cells are found in chronic wounds, where they impair tissue repair due to increased degranulation and increased protease activity.[96] Additionally, mast cells have been associated with fibrosis, although certain studies suggest this may not be the case.[97]

The best recognized myeloid cells in wound healing are macrophages, possibly due to their plasticity. Macrophage shift from an inflammatory, classically activated phenotype, and although this nomenclature is evolving and macrophages exhibit phenotypes along a spectrum, these are still frequently called M1and characterized by secretion of IL-1, TNFα, IL-6, IL-12, MMPs, and other cytokines, to the M2 (also called alternatively activated), anti-inflammatory, pro-reparative phenotype, characterized by production of arginase, TGFβ, CCL18, PGE2, and IL-10, and up- regulation of scavenger receptors (CD206, CD163). In *in vitro* settings, these two polarization states are characterized as being distinct and more easily controlled. However, in the wound microenvironment, macrophage polarization stages may present as a continuum, rather than a bi-polar paradigm.[98,99] In murine injury models, generation of the cytokine CCL2 leads to recruitment of CCR2+ macrophages, initially as a pro-inflammatory Ly6Chi phenotype; that population subsequently transitions to a CCR2lo/Ly6Clo phenotype that is pro-angiogenic and critical for re-vascularization.[100] Furthermore, CD301b+ macrophages have been associated with myofibroblast proliferation and wound repair, although sustained presence of these macrophages at the wound site may be linked to fibrosis and keloid formation.[67] Studies using conditional deletion of macrophages during different stages of murine healing have revealed stage-specific functionalities: deletion early in the wound trajectory diminishes granulation tissue and myofibroblast formation while depletion during the mid- stage of healing destabilizes the existing vasculature and impairs epithelialization.[101,102] Interestingly, persistence of proinflammatory macrophages at the wound site has been associated with impaired wound healing.[103]

The switch from M1-rich to the M2-rich wound milieu is crucial for the transition from the inflammatory to proliferative phase of wound healing, and this transitional process may be disrupted in chronic non-healing wounds or even exhibit heterogeneity within the same wound. Various molecules have been identified as cues for the conversion, including Th2 cytokines, and efferocytosis of neutrophils.[104] IFNβ-induced epigenetic changes are crucial for the transition of M1 to M2 macrophages. Normal healing wounds upregulate IFNβ, which promotes the expression of the histone methyltransferase Setdb2 in macrophages.[105] Setdb2 trimethylates lysine 9 on histone 3 (H3K9me3) and results in abolishment of NFκB DNA binding and transition from M1 to M2. Diabetic wounds exhibited less IFNβ levels and thus decreased levels of Setdb2, leading to increased M1 macrophages at the wound site. The metabolic status of the wound is also critical in these events and responses.[106,107]

On the flip side, studies also show that supplementation of diabetic and acute wounds with M2 polarized macrophages not only does not promote wound healing but rather delays it [108,109]. In support of this, sc-RNAseq analysis showed that healing diabetic foot ulcers (DFUs) have more M1 macrophages compared to non-healing DFUs [110], while others note that DFUs have lower numbers of neutrophils and macrophages compared to non-diabetic acute wounds [111]. These presumably contradictory results may be due to timing of the investigation relative to the transition from one subtype to the other and also lack of understanding of the specific characteristic a "healing" macrophage vs a macrophage that impairs healing [112]. In fact, a longitudinal study showed that the ratio of M1/M2 macrophages was increased in the early phases of healing DFUs compared to non-healing DFUs but during the later stages, non-healing DFUs had increased M1/M2 ratios compared to the DFUs that healed. This suggests that a sustained inflammatory response and is associated with healing failure [113].

Fibroblast deregulation has also been associated with impaired wound healing. A single cell RNAseq and spatial transcriptomic study using skin from non-diabetic, diabetic without foot ulcer and diabetic with foot ulcer donors identified a novel fibroblast cell type expressing *Il6, Tnfaip6, Mmp1,Mmp3 Mmp11, Hif1a and Chi3l1* among other genes [110]. In a mouse model of wound healing Engrailed-1 + fibroblasts were associated with increased fibrosis [114]. In agreement with this, a study examining single cell transcriptome of diabetic vs normal foot ulcers showed that fibroblasts populations were dysregulated in DFU tissues and were associated with increased expression of fibrotic and inflammatory genes [115].

The ever-expanding network of cytokines and their signaling pathways[53,55] presents a major opportunity for further investigation in failure to heal. Some of the most studied cytokines contributing to healing include CCL2 (recruiting the first wave of macrophages to the wound), IL-8 (signaling through CXCR2 in human epidermis to support re-epithelialization),[116] and Il-6. The latter is pro-inflammatory in the initial stages of healing and stimulates keratinocyte migration. IL-6 −/− animals have delayed healing, as do animals where IL-17 or IL-22 is deleted.[75,117]

The pathophysiological mechanism that converts an acute wound destined to heal into one that does not is unclear. Many factors have been associated with wound chronicity including, but not limited to the above noted aberrations in innate immune players in the wound with sustained inflammation, alterations in angiogenesis, dysregulated matrix deposition, neuropathy and impaired neuropeptide signaling, cell senescence, bacterial infection and biofilm formation and wound hypoxia. [118]

Biofilms and their formation are now well documented in chronic wounds, and likely play a critical role in impaired healing, chronic wound recurrence and, possibly, chronic wound initial occurrence. The field of biofilm is vast but several recent and representative publications now address the issue of biofilm in chronic wounds. [119–122] Biofilms effectively shield bacterial microorganisms from systemic antibiotics. Moreover, it is becoming clear that the evolution of biofilms has included the strategic evolutionary approach of them becoming polymicrobial and thus less susceptible to antibiotics that could eliminate a more uniform bacterial population. As in other sections dealing with pathophysiologic mechanisms leading to impaired healing, Figure 4 shows these various complex approaches.

Hypoxia in the wound environment results in the release of mediators that regulate angiogenesis and re-epithelialization, via activation of the transcription factor Hypoxia Inducible Factor 1 (HIF-1). [123,124] The *in vitro* and pre-clinical *in vivo* evidence demonstrating the downstream pro-reparative consequences of HIF-1 translocation to the nucleus have been extensively reviewed.[125,126] These findings have led to the proposed use of deferoxamine, a HIF-1α inducer and stabilizer as a therapeutic for chronic wounds. [123,124] Indeed, a current clinical trial is underway to examine if topically applied deferoxamine can improve healing in diabetic foot ulcers [127], and a patch engineered for its transdermal delivery deferoxamine is being trialed for the treatment of sickle cell ulcers.[128,129] The outcome of these trials may provide novel approaches to both preventing and treating chronic ulcers.

d) Advances in Mechanistic Approaches to Impaired Healing

Non-coding RNA—Since their discovery the role of noncoding RNAs (ncRNA) has been expanding. Most of the work has focused on a subset of ncRNAs, the microRNA (miRNA or miR), which are about 22–23 nucleotides long and perform regulatory functions.[130] Their expression is tissue and cell-type specific, and early investigations of the cutaneous role of miRNAs focused on their expression patterns and roles in maintaining stemness in epithelial and hair stem cell populations.[131,132] The expression pattern of a multitude of miRNAs changes during different phases of wound repair.[133–136] One of the most studied is miR-21 that is upregulated in both keratinocytes and fibroblasts at the wound margin in murine wounds and whose inhibition impairs healing in that animal model.[137] Its expression promotes an anti-inflammatory phenotype in human macrophages *in vitro* and modulates conversion of rat mesenchymal stem cells to fibroblasts.[138] However, it is over-expressed in venous ulcers,[139] possibly inhibiting epithelialization. Dermal remodeling is modulated by miR-29, which targets several transcripts responsible for generating the extracellular matrix and can decrease wound contraction and collagen deposition.[140,141] On the other hand, suppression of miR-29 downstream actions by competitive binding to the long non-coding (lnc) RNA H19n is associated with increased extracellular matrix synthesis and fibroblast proliferation; however, over-expression of lncRNA H19 may improve healing in diabetic mice.[142] There is evidence that miR-127–3p regulates the transition from the proliferative phase to the remodeling phase of murine wounds, possibly by inhibiting the proliferation of myofibroblasts.[143] The role of this miRNA in human wound healing has just recently been explored, and may function as an epigenetic activator regulating the transition from repair to remodeling during skin wound healing.[143,144] Another miR under investigation for potential therapeutic modulation of healing is miR-210, which is induced by tissue hypoxia and targets keratinocyte proliferation.[145] Wound inflammation and its resolution may be controlled by expression of specific miRNAs such as miR-132, which is diminished in human diabetic wounds.[146,147]

The subset of long ncRNA (lncRNA), with 200 or more nucleotides, targets genetic networks and has been found to have epithelial functions.[148] Notably, two lncRNAs have been found to regulate the balance between proliferation and differentiation in human organotypically cultured epidermis, with the Anti-differentiation Noncoding RNA (ANCR) maintaining progenitor status of basal keratinocytes in human epidermis; its counterpart, Terminal Differentiation-Induced Noncoding RNA (TINCR) promotes differentiation in the upper epidermal compartment.[149,150] Recent studies have suggested therapeutic approaches to accelerated healing by targeting specific lncRNAS, either by inhibition of a novel lncRNA, Wound and Keratinocyte Migration Associated lncRNA 2 (WAKMAR2) present in non-healing wounds, or induction of expression of the pro-proliferative lnc-RNA Gas5 by the use of statins.[151,152] One recently discovered mechanism by which lncRNAs can regulate cell function is by competitively bonding, or “sponging”, multiple other miRNAs to limit their function.[142,153,154]

Microbiome—With the advent of new molecular tools, the role of the wound microbiome is being increasingly investigated as a contributor to wound chronicity.[155,156] The prolonged inflammatory state of the chronic wound is associated with persistent infection or biofilm formation.[120] A causal role has been demonstrated in many studies using animal models. Application of a bacterial inoculum or transfer of preformed biofilm results in delayed healing.[157] The transfer of the skin microbiome of a mouse having a mutation that impairs the innate immune response and healing to a wild type mouse results in WT acquisition of the impaired healing phenotype. [158] *Staphylococcus aureus* and *Pseudomonas aeruginosa* are among the most commonly encountered by either molecular or culture-based techniques.[156,159] However, the classic skin commensal bacterium, *Staphylococcus epidermidis*, has recently been shown to induce a type17 immune program and accelerated repair through mechanisms linked to antimicrobial peptide production and a subset of skin resident T cells that recognize specific commensals (‘commensal-specific T cells’).[160] This commensal organism also upregulates the expression of the antimicrobial molecule, Perforin-2 in keratinocytes.[156] Thus, the link between specific microbes within the wound microbiome and wound chronicity remains tenuous.

A better prognosticator of wound chronicity may be the determination of overall bacterial diversity of the wound microbiome.[161] A negative linear relationship has been documented between decreasing wound diversity and wound healing rate in two cohorts (each of about 80 patients) with chronic wounds.[162] Similar associations have been noted in the wounds of patients with dystrophic epidermolysis bullosa.[163] Additional mechanistic progress may be achieved in future studies through deeper sequencing afforded by shotgun metagenomics.[164]

In addition, host neuroimmune mechanisms that mediate the inflammatory response to pathogens and impact wound repair are being discovered. Nociceptors in the skin that express the transient receptor potential cation channel subfamily V member 1 (TRPV1) are activated by a cutaneous challenge of *Candida albicans* and respond with expression of the neurotransmitter CGRP. The downstream consequences are activation of dermal dendritic cells to generate IL-23, which then induces the production of the protective, antimicrobial and pro-reparative cytokine IL-17A by dermal $\gamma\delta$ T cells.[165] On the other hand, skin infection with *Streptococcus pyogenes* also activates TRPV1+ nociceptors to release CGRP, but the result is inhibition of neutrophil recruitment and function, which could be reversed by administration of a CGRP antagonist.[166,167] These divergent nociceptor responses may be due to splice variants, or may be pathogen specific.[162] The potential for TRPV1- targeted therapies to improve healing may require personalized medicine approach tailored to the channel splice variant expressed or specific wound pathogen.[168]

While most studies of the wound microbiome have focused on bacteria, emerging work implicates the wound 'mycobiome' and 'virome' as potential contributors to wound chronicity. Fungal components are present in the wound microbiome and their abundance is associated with longer healing times.[169] Fungal activation of a fungal-specific T cell Th17 response can exacerbate local inflammation and regulate healing.[170] Although viral components are recognized as members of the wound microbiome, and viral modulation of inflammatory responses are documented, at this time there are few studies of the impact of the viral component on wound outcomes or chronicity.[59]

The common presence of antibiotic-resistant bacteria in wounds[155] has prompted the search for non-antibiotic approaches. Indeed, a recent study has demonstrated that application of topical antibiotics to a wound significantly impairs healing in mice, and possibly in humans as well.[171] Multiple agents are being investigated including therapeutic bacteriophages, antimicrobial peptides, repurposed drugs, cold plasma treatment, photodynamic therapy, probiotics and bioelectric dressings.[172,173] Trials of both topically administered probiotics in preclinical wound models, and orally administered probiotics in patients have been reported.[174] In one randomized controlled study, supplementing patients with orally ingested probiotics improved healing in the treated cohort of diabetic foot ulcer patients.[175] The gut-skin axis and gut dysbiosis are being implicated in the pathogenesis of skin diseases such as atopic dermatitis and psoriasis and ongoing investigation may uncover the impact of this signaling axis on wound healing.[176]

DIAGNOSIS, SCREENING AND PREVENTION

Chronic wounds are quite heterogeneous in their etiology, pathogenesis, approach to therapy, and prevention. Therefore, clinical evaluation is an essential step to reach a final diagnosis and to establish a specific therapeutic strategy. Table 1 describes the salient features of the main types of chronic wounds: arterial, venous, diabetic and pressure ulcers. Table 1 also lists what are not regarded as atypical wounds, which encompass a group of disorders that are less common but have also proven difficult to heal. These atypical wounds and their diagnosis and management are described in recent publications. [1] Regardless of the type of wound and underlying cause or etiology, a comprehensive assessment of the patient's condition, including the medical and pharmacologic history, is essential. Table 1 summarizes the diagnostic features to be analyzed from the history, physical exam, and imaging or laboratory testing. In addition to immediate surgical considerations that arise during the diagnostic phase, such as surgical debridement and planning for grafting or vascular and orthopedic procedures, the fundamental clinical criteria involve three major components of the wound: the wound bed, wound edges, and surrounding skin. Figure 5 outlines the diagnostic and interventional approaches.

Clinical features of chronic wounds

The major category of typical chronic wounds are vascular ulcers (including those due to venous and/or arterial insufficiency, diabetic foot ulcers (neuropathic, vascular, or of mixed etiology from neuropathy and vascular obstruction), and pressure or decubitus ulcers. The etiology of these chronic wounds is easily diagnosed by clinical inspection due to such characteristics as body location, adequate arterial pulses, presence of neuropathy, wound bed, unusual features in the surrounding skin, among other typical features (Table 1).

The features of venous leg ulcers (VLU or stasis ulcers) reflect the main pathophysiology involving incompetent perforator (or communicating) veins between the deep and venous system and failure of the venous ambulatory pressure to decrease upon walking or exercise (venous hypertension). [177,178] The CEAP classification for VLU has been found useful in different guidelines, and refers to the use of Clinical signs, Etiological cause, Anatomical distribution, and Pathophysiological dysfunction.[179,180] With this scoring system each parameter is divided into several stages, which enables the clinician to score the complexity of the patient's condition. The VLU are typically located above the medial malleolus, more commonly on the medial side of the leg, have irregular edges, and tends to be superficial, i.e., not involving muscle or bone. Surrounding varicosities and edema are common. The wound bed is typically yellow or pale red due to fibrinous material deposition, and there can be considerable exudate even in the absence of infection. The ulcer is not very painful, notably except for when the ulceration is close to or over the malleoli and thus near the nerve-rich periosteum. The surrounding skin is often characterized by indurated and hyperpigmented skin, which is labeled lipodermatosclerosis and represents a deeply fibrotic process.[48,178,181–183] Arterial ulcers tend to occur more commonly on the lateral side of the lower leg, are often distally located (such as the dorsum of the foot of toes), and display poor proximal (femoral or popliteal) or distal (dorsalis pedis or posterior tibialis) pulses by palpation or Doppler examination. Arterial ulcers are smaller in size than VLU, are deeper and often down to muscle and even tendons or bone, more painful, and regular in configuration (punched-out appearance). The wound bed often displays black necrotic tissue. Their surrounding skin may show atrophy, with pale skin color and loss of hair. Diabetic foot ulcers a categorized as being neuropathic, ischemic, or neuro-ischemic, according to their preponderant etiological and pathogenic mechanisms. Neuropathic diabetic ulcers usually occur in areas of high pressure on the plantar surface of the foot and, typically, over the metatarsal heads. They may be preceded by an inflammatory process leading to distortion of the underlying bones (Charcot foot). Callus formation is common and contributes to the pressure on the insensate foot. Diabetic ulcers in which ischemia plays a substantial role will show absence of or poor pedal pulses, typical ischemic atrophic changes in the skin, and a necrotic wound bed at an early stage. It is critical to realize that small diabetic ulcers may involve a much larger portion of the foot and may become easily infected, with tunneling and the need to amputation. Thus, diabetic ulcers are always in danger of causing devastating clinical effects. Pressure ulcers are located over bony prominences, such as the heel, hip, and sacral areas. These ulcerations may exhibit skin redness (Stage I) that becomes non-blanchable during digital palpation. They may rapidly advance to deep ulcers involving the dermis (Stage II) and subcutaneous tissues (Stage III), and even tendons and bone (Stage IV). An extensive review of the subject was recently published. [184]

Diagnostic work-up

The clinical guidelines for vascular wounds assessment are well established.[185] In venous leg ulcers there is no valid test other than clinical evaluation and the need to always exclude arterial insufficiency. However, the detection of incompetent perforators on Doppler Duplex Scanning is helpful in the diagnosis. The vascular diagnostic work up will always start with pedal pulse palpation, which might be difficult in obese patients, those with leg edema, and in patients with concomitant diabetes having extensive vascular calcification.[186 184] Diagnostic tests for ulcers due to arterial insufficiency include continuous wave Doppler (CWD), ankle brachial pressure index (ABI), and transcutaneous oximetry ($TcPO_2$). In patients with diabetes the Doppler probe must be used according to systolic toe pressure (TP) and toe brachial index (TBI).[187] The results and, particularly, the interpretation of those tests will guide the medical and surgical management and the possible complications. The ABI includes the measurement of the Doppler signal, by dividing the ankle systolic blood pressure over the brachial systolic blood pressure; a value of 1.0 or 1.1 is considered normal, while a ratio of 0.8 or less is abnormal. However, because of calcification in blood vessels, the ABI is unreliable in the assessment of diabetic ulcers. Palpation of the pedal pulses is a subjective test for the detection and intensity of the pulse of the dorsalis pedis and posterior tibialis arteries; the detection depends on the clinician's skills and experience. The use of a Doppler probe for CWD test helps by recognizing the arterial signal with either tri-phasic or bi-phasic sounds in normal patients, and monophasic or absent sounds in peripheral arterial disease. Measurements of $TcPO_2$ are time consuming, and they are most valid for diabetic foot assessment, where a value of less than 40mm Hg is related to poor oxygen perfusion and may even be used to determine the level of eventual amputation.[188] Pressure (decubitus) ulcer assessment is mainly used to understand the true wound size and depth, and has evolved from pure clinical evaluation to imaging techniques in more difficult cases.[189] The main problem with pressure ulcers assessment is to completely assess the depth and size, as these wounds typically have undermined edges; there are several 2D and 3D assessment options ranging from high frequency ultrasound to laser scanner imaging or optical coherence tomography.[190]

The role of the bacterial swab in chronic wound management is now clear; it should be considered only if clinical signs of infection are present.[191] Symptoms and signs of wound infection are more critical in diabetic foot and pressure ulcers compared to VLU, and they present with the following clues: increased exudate and foul odor from the wound, increased wound pain, localized swelling, increased heat of the surrounding skin, friable and hyper-granulating wound bed, and increased erythema and cellulitis. Increasing evidence suggests that biofilm formation and persistence plays an important role in chronic wound formation, chronicity, and possibly recurrence.

Wound biopsy

When wounds fail to heal in spite of appropriate therapeutic management, it is important to perform a wound biopsy, which is essential in confirming the clinical wound diagnosis, to understand the failure to heal and respond to treatment, and for ruling out neoplasia. The biopsy should be performed at the wound edge, and should include a portion of the surrounding skin.[192] It has been shown that, peculiarly, the biopsy site will heal faster than the actual wound bed tissue.[193] The wound biopsy is also helpful in the diagnostic work up in atypical wounds, which include inflammatory wounds, such as vasculitis and those wounds due occlusion of microvascular channels from cryoglobulins and cryofibrinogens.[194] The latter ulcers due to cryoproteins are surrounded by a small blood vessels of linear hyperpigmentation often termed “micro livedo”.[194] In pyoderma gangrenosum, a diagnosis of exclusion, the biopsy is going to exclude other vasculopathies, including calciphylaxis.[195]

Biomarkers

TNF-alpha assessment in pyoderma gangrenosum and in non-healing VLU has shown a good correlation with the prognosis of specific groups of patients.[196,197] TNF alpha was found to be elevated in non-healing VLU, and, when elevated, its therapeutic use has proved helpful in chronic wounds.[196] Osteopontin is another biomarker used to detect the presence of calciphylaxis.[198] The level of specific metalloproteinases have shown promising results in non-healing chronic wounds.[199] The elevated level of matrix metalloproteinases in wound fluid from non-healing wounds has been correlated with reduced healing rates.[200]

Prevention

Various guidelines and recommendations have been proposed for the primary and secondary prevention of chronic wounds.[201] An increasingly recognized important component of prevention and ulcer recurrence is that, in the absence of proper and continued guidance and teaching, patients with chronic wounds are generally not able to follow effective self-management strategies. Prevention of VLU is mainly using compression leg bandages and stockings. The continued use of compression stockings will help reduce the rate of recurrence. Modern textile materials have helped in increasing compliance (adherence) from patients and practitioners. Control of leg edema is best achieved using stockings and leg elevation. A United Kingdom initiative called Lindsay Leg Club has helped in reinforcing compliance/adherence. Prevention of diabetic foot ulcers is very complex and demanding and is best achieved using a multidisciplinary foot care unit. The risk of developing foot ulcers has been stratified into low, moderate, and high risk according to the extent of neuropathy and limb ischemia.[202] Assessment of foot biomechanics and sequential use of orthoses, together with constant follow up, have shown to reduce the risk of foot ulceration in patients with diabetes.[203] Prevention of pressure (decubitus) pressure ulcers is challenging but essential, and is initially achieved by using a risk assessment scale The Braden scale is widely used,[184,204] but several assessment scales have been implemented according to different categories of patients at risk. The approach in patients with pressure ulcers is to minimize pressure, shear, and moisture by repositioning the patient with scheduled protocols, and by avoiding contact of the chronic wound with urine, feces, and other fluids. In patients simply not able to receive proper repositioning the use of multi-layered silicone dressings in areas at risk has shown to decrease the incidence of pressure ulcers.[205] There is a need to more actively screen certain populations at risk for chronic wound development. Finally, it should be noted that the chronic wounds described in this discussion are more applicable to first world “developed” countries, and less so in less developed countries, where lifespan is decreased, tropical and infectious diseases are more common, and medical and laboratory assessment less available. It should be noted here that a more proactive approach is needed in identifying populations at risk for different types of ulcerations. Such an approach would help in determining how we can best prevent ulcerations from occurring in vulnerable groups.

MANAGEMENT

Scale of the clinical problem

The extent and cost of treating wound problems in modern day practice is not completely known but recent publications would suggest that chronic wounds may affect up to 5% of the adult population and consume up to 10% of health care expenditure. It has also been shown that the number of patients with wounds increased by 70% with an increase in cost of care of 45% over a 5-year period.[206–208] In the past, clinicians may have been slow to recognize the nature and extent of the challenge of this problem, and there was a general lack of appreciation of the need to develop a system of optimal clinical delivery and care that takes advantage of emerging treatments and technologies. This situation is compounded by the fact that "precision medicine" is still in its infancy when it comes to chronic wounds; better molecular diagnostic and therapeutic tools are needed to focus on individual patients.

Despite the above considerations, the management of chronic wounds has improved dramatically in the last 2–3 decades. The term chronic wound is often used but there are several definitions of chronic wounds that can result in an inability to compare results of an intervention from different studies.[4,209,210] Tables 2 illustrates therapeutic options to be considered, including dressings, devices, drugs, and surgical and biological approaches.[120,211–214] For the 3 main types of chronics wounds, Table 3 illustrates which therapies might be considered to treat specific wound types if a comprehensive treatment were to be provided. Figure 5 is an algorithm approach for how to address different interventions for treatment. It is important to recognize that basic clinical approaches also rely on proper surgical management. In addition to vascular reconstruction, grafting, and limb salvage, debridement is critical for removing necrotic tissue. Indeed, what had been termed "maintenance debridement" may be required to keep the process of healing going in the right direction.

Evidence-based approach for treatment selection

The Cochrane Wounds Database details many useful reviews and provides valuable insights: https://wounds.cochrane.org/news/reviews. The available evidence shows the paucity of currently available data on which to draw conclusions, including studies focused on dressings, devices, drugs, surgical and biological approaches. The inability to draw definite conclusions is multifactorial, and it may have to do with poor statistical power analysis when designing clinical research trials, among other clinical and treatment factors. At the present time several recommendations are still based on expert opinion and clinical guidelines.

The stimulus for establishing chronic wounds management guidelines came largely from an emphasis on wound bed preparation (WBP), which has continued up to date.[120,211–214] There are basic approaches to the treatment of chronic wounds that are rooted in both experience and evidence: compression for venous ulcers, off-loading for diabetic neuropathic ulcers and pressure ulcers, debridement of necrotic tissue, stenting and revascularization to restore blood flow and oxygen, hyperbaric oxygen for chronic wounds unresponsive to treatment. We now have better wound dressings than we had even 10–20 years ago, and which are based on the recognition that moist wound healing can help chronic wounds as well, or at least the formation of granulation tissue. We like to use film wound dressings for less exudative wounds, foams to absorb excessive exudate, hydrocolloids to initiate debridement, alginates to provide moisture to very dry wound beds. Composite of these dressings and variations have been developed, as for example the delivery of antiseptics, antimicrobials from the dressings.[215] For standard and basic therapies, certain considerations are in order.

Diabetic foot ulceration

Diabetic foot ulceration is a complex, common, and challenging problem.[210,216–218] It consumes around 25% of total cost of managing all complications of diabetes including diabetic eye, heart, and renal disease. The major underlying reasons for the development of diabetic foot ulcers are peripheral nerve damage, alterations in vascular flow and/or blood vessel physiology may be present and be associated with increased risk of infection.[219]

Venous Disease—For patients with venous ulceration the cornerstone of standard treatment remains lower leg compression. Much research has centered around the level of sub bandage pressure that needs to be applied and how to achieve it with bandages or stockings. A systemic agents that acts in part through vasodilation or effect on white blood cells was finally shown to enhance the healing of venous ulcers when given at double the dose that was previously tested in controlled randomized trials.[220] This finding was later confirmed by a meta-analysis.[221]

Pressure injury

Pressure ulcers or injuries remain a common and expensive problem.[222] Much effort has been spent on developing and evaluating pressure relieving properties in mattress and cushion material. However, many factors in individual patients including incontinence, nutrition, mobility pain level, co-morbidities, environment and skill level of staff caring for a patient the impact of intervention on both the development and resolution of pressure injury. There are also special subgroups of patients at increased risk of developing pressure-induced ulcers including spinal cord injury, congenital or progressive neurological diseases, and individuals who are elderly and mentally infirm. It is likely that varied factors are operating in individual patients who develop these wounds. At this time, it is unknown how biological differences can explain different outcomes in subgroups or individual patients.[223]

Biologically-based treatments

Despite the difficulties in identifying and studying specific treatments, over the last 2–3 decades enormous progress has been made in advanced therapeutic approaches that could potentially benefit chronic wounds. One of the first such targets was the use of topical growth factors that are important in the healing cascade. [37,224] The first topically applied recombinant growth factor approved by a regulatory agency (FDA) for the treatment of diabetic neuropathic foot ulcers was PDGF.[37,225–229] The topical PDGF approval also helped to highlight the importance of wound debridement. [225,230] A review of the present situation with the topical application of growth factors, including PDGF, suggests that there are limitations to this type of therapy. [231]

Following and in fact around the same time as interest was focused on growth factors, there was a great deal of activity in tissue engineering to treat chronic wounds. Here the ability to grow epidermal and dermal cells in the laboratory were felt to be another way to enhance healing.[232–235] The use of this “artificial skin” or bioengineered skin (cellular or cellular) has led to several publications and, in some cases, regulatory approval for the treatment of venous and diabetic foot ulcers, as well as mechanisms of action.[186,232,233,236–239] Important and interesting take-away points from some of these clinical research studies and laboratory research are the following: bioengineered skin constructs (both cellular and acellular) can accelerate the healing/closure of venous and diabetic foot ulcers; the percentage of improved healing over control treatment is no more than about 20%, and that the constructs are short-lived within the wound, thus not acting as a skin replacement but rather as a stimulus for wound repair. A peculiar and interesting consideration is that many of these bioengineered constructs have been approved and marketed with a name ending in “graft”. This decision has actually led to disappointment by clinicians, especially surgeons, who view “grafts” a more permanent solution to wound resurfacing. Moreover, repeated treatments with these advanced therapies may be required, thus diminishing their cost effectiveness. Another important consideration is that such advanced therapies are for the most part not available to less privileged patients and those in third world countries.

Despite these setbacks in advanced approaches progress being made in the more mechanistic aspects of wound healing Is likely to lead to improved and more cost-effective treatment. It could still be stated that expensive ore focus on a technology that might have theoretical benefits in assisting healing rather than identifying the aberrations that are present in an individual patient and which are preventing healing.[240]

In addition to biological factors influencing healing, it is increasingly recognized that social and psychological factors can influence outcome.[241] An important example of the challenge of research is the wounds seen in patients suffering from terminal disease from other comorbidities. It is clearly inappropriate to expect full wound closure in that setting. However, it is both proper and desirable to evaluate interventions that influence pain, smell, discharge, and patients Health Related Quality of Life by measures other than complete wound closure or speed of healing.[242]

QUALITY OF LIFE

Ways in which quality of life is altered by chronic wounds

Chronic wounds have a negative effect on health-related quality of life on account of the physical injury, the treatment required, the chronicity of the condition and the likelihood of recurrence. Over two decades ago, research conducted by Franks and Moffat[243] indicated that leg ulcer patients experienced significantly poorer quality of life than matched controls in the areas of emotional, social and physical health. Today it is well established that chronic wounds are painful and affect physical role and function,[244] can cause mental health concerns[245] and may limit social and workforce participation.[246] Chronic wounds can present a financial burden to those who must self-fund their treatment.[247] Wound treatment and prevention have improved in recent decades. However, mitigation of the negative effect of chronic wounds on quality of life remains an unresolved challenge. Box 1 illustrates the many facets of quality of life, and how patients are affected. There are three main domains that are altered in patients with chronic wounds: physical, emotional, and social. For each domain, Box 1 lists the main facets/components of one's existence that are altered, often in a fundamental way. It is important to recognize that these domains overlap, and the consequences of each are interrelated. In many ways, Box 1 exposes the need for what, ideally, should be a multidisciplinary approach to patients with chronic wounds; it is extremely challenging to address all the listed needs.

Measuring quality of life

The introduction of patient reported outcomes and patient centered care has required healthcare providers to identify and respond to what is most important to the patient. It is now common for research to measure quality of life, for example using generic instruments such as the SF 36[248] and the EQ-5D.[249] Disease specific measures include the Cardiff Wound Impact Schedule[250] and the Diabetic Foot Scale[251] which are clinically useful and responsive as they include items that are specific to the condition of interest. Quality of life is often a secondary outcome measure and research that investigates quality of life as the primary purpose of the study should be conducted if quality of life improvement is to be realized by people who have chronic wounds.

Management of the wound and care of the patient

Quality of life must be assessed early in the care episode so that needs are identified and interventions can be targeted. Interdisciplinary team work is necessary[252] and a genuine partnership with the person who has the wound is essential. Traditional models of treatment and care (provided in the doctor's clinic and in the home by nurses) have evolved to better meet the holistic need of patients. Advancements include specialist wound clinics that bring together multidisciplinary expertise,[252] community based interventions such as Leg Clubs which offer opportunity for social support[253] and self-management approaches to optimize the persons involvement in their care.[254] Engagement with informal care-givers who support those affected by chronic wounds has also been recognized and their participation in wound care may enable improved quality of life.[255]

Co-morbidities and chronic wounds

Chronic wounds are a symptom, manifestation or consequence of the health conditions that cause the greatest disease burden in society (for example vascular disease and diabetes) therefore chronic wounds should be considered a priority at the health policy level.[256] People who experience chronic wounds often present with multiple co-morbidities[257] and therefore many inter-related factors that may affect their quality of life. Our understanding of quality of life is, in the most part, informed by research conducted with the patients who are the easiest to access but not necessarily the most vulnerable. Future research should target people who experience less prevalent wounds, who live in developing countries, who are from diverse backgrounds, who experience cognitive impairment and who are at end of life.

OUTLOOK

Considerable progress has been made in our understanding of the healing process of the skin and the impaired healing that characterizes chronic wounds, and we have discussed the epidemiology and the adverse effects on quality of life. Still, many opportunities exist for improving the outlook in this challenging field.

Stem cells

Although all tissues, including skin, have resident stem cells, bone marrow mesenchymal stem cells (BM MSC) have been the most widely investigated for their potential to accelerate and/or improve aspects of wound healing. *In vitro* and *in vivo* studies demonstrate that MSC exhibit multiple pro-reparative functions, including the ability to migrate to sites of injury, stimulation of proliferation of wound resident cells, and wound angiogenesis; these effects take place via secretion of growth factors and cytokines, suppression of inflammation, and generation of anti-microbial peptides.[258–260] Additionally, MSC are easily harvested and cultured, and autologous or allogenic MSC can be administered without much risk of immune rejection. Adipose tissue is another easily accessible source for stem cells having some multipotent properties like bone marrow-derived MSC. [261,262] Tissue resident stem cells, such as dermal or epidermal stem cells, have also been proposed as therapeutic mediators of repair.[263,264] but require autologous sources to prevent immune rejection, a factor that limits their availability. Other sources of stem cells that have been less fully investigated for use in impaired healing are derived from umbilical cord or Wharton's jelly.[265] An emerging concept had been termed "priming" of advanced therapeutic agents.[266] Several lines of investigations support this concept. The pro-reparative efficacy of stem cells may be amplified by conditioning by hypoxia, TGF-β1, or other drugs, or by lentiviral transduction of MSC for VEGF expression.[266–269] This priming approach resembles the one proposed for bioengineered skin before its clinical use in chronic wounds.[266] Additional strategies to improve therapeutic efficacy include co-administration with other cell types, such as fibroblasts, or modulation of the co-administered extracellular matrix 'niche'.[268,270,271] A promising study was focused on well characterized cultured autologous bone marrow-derived MSC delivered to hard-to-heal wounds in a fibrin spray.[272] Subsequent trials in chronic wounds treated with autologous BM MSC have reported improvement in healing.[273,274] Although promising, MSC therapy has limitations that include the very few clinical trials from which to draw conclusive evidence, and the lack of uniform protocols for clinical administration. Ongoing work will address these issues, as in a preliminary recently published controlled and prospective randomized clinical trial.[275]

Exosomes

One approach to standardization of cellular therapy may be through the use of MSC-generated exosomes that have been shown to have similar pro-reparative functions as their cellular parents, primarily in animal models of wound repair and *in vitro*. Their cargo of miRNAs, Wnt ligands, growth factors, cytokines and signaling lipids provide the mechanism for paracrine stimulation of wound resident cells.[276–279] Exosomes cargo has also been demonstrated to orchestrate cell migration, including that of murine dermal fibroblasts.[280] Numerous preclinical studies, using many variations of cellular sources of exosomes, have shown improvement in healing.[281] The lack of standardization precludes identification of the optimal exosome approach, and will undoubtedly be remedied. The limitations for therapeutic use may also lie with the large numbers of cells required to generate quantities sufficient to effect pro-reparative functions, and the variable cargo contents dependent on parental cell strain.[277]

Unifying concept of wound bed preparation

This concept of wound bed preparation (WBP) was first proposed to provide diagnostic/ prognostic considerations, a framework for unifying mechanisms of tissue repair, and for maximizing available and future treatments. The concept of WBP also recognized the need to address biochemical and molecular factors. [211] This all-encompassing concept was later refined to better address biochemical and therapeutic considerations.[212–214] Figure 6 shows a proposed scoring system for WBP (VF, 2021), addressing certain parameters to address and improve.

Final comments

Despite established progress and emerging technologies, certain obstacles remain quite challenging; these obstacles are also fertile ground for research. The finite speed at which keratinocytes are capable of migrating from the edge and resurface the wound is a perplexing phenomenon.[282,283] These studies suggest the existence of what has been called "keratinocyte speed limit".[37,284] Indeed, a rate of healing (advancement of keratinocytes from the edge towards the wound's edge) of less than 0.75 mm/week is associated with a poor diagnosis and failure to heal. Conversely, we have not been able to demonstrate a rate greater than 1.4–1.5 mm/week even in healing wounds.[284] Another major challenge is that chronic wounds have anatomical and physiological abnormalities that cannot be adequately corrected with present knowledge and approaches. Regrettably, at least until a so-called quantum jump in our capabilities is achieved, we might need to attenuate our ideal focus on tissue regeneration. Certain realities must be recognized. As we evolved into more complex organisms, we lost the ability to heal by a regenerative process. Instead, we developed mechanisms to stop bleeding after injury and achieve closure of acute wounds rapidly, even if that meant reliance on scarring. That evolutionary approach served us well as long as our lifespan was limited. However, with increasing aging, we developed disease processes (diabetes, circulatory problems, pressure injury, etc.) that evolution simply did not prepare us for. Yet another possible reality, supported by early experimental evidence, is that chronic wounds may not be as dormant as we think; additional stimulation may actually increase their energy requirements. [285] Certain approaches deserve additional consideration. We may also have to explore ways to render cellular components and extracellular matrix more resistant to the effects of pressure and ischemia. Therefore, although much of our focus has been to find new ways to stimulate and accelerate the healing process, opposite and unusual paradigm shifts may be required from the laboratory and clinical standpoints.

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Box 1.

Quality of life domains affected in patients with chronic wounds.

Physical domain

- Physical deformity
- Wound pain, odor, exudate
- Reduced mobility
- Suboptimal sleep
- Impaired activities of daily living
- Feeling unwell from side effects of treatment modalities
- Worsening of comorbidities

Emotional domain

- Stress, anxiety, depression
- Frustration, worrying, helplessness
- Dissatisfaction with health care givers
- Fear of trauma to the wound, recurrence
- Stigma and worsening self-esteem
- Lack of life enjoyment

Social domain

- Withdrawal from family and friends
- Loss of independence
- Altered sexual
- Difficulty with domestic duties, hobbies, recreation
- Lack of acceptable clothing and footwear
- Difficulty with work, studying, shopping
- Time required for wound treatment, medical appointments

Figure 1:

Representative clinical photos of typical chronic wounds. A) Diabetic ulceration due to both neuropathy and arterial insufficiency. The forefoot has been previously amputated; B) Venous ulcer with surrounding lipodermatosclerosis on the medial aspect of the ankles; C) Deep decubitus (pressure) ulcer in the sacral area; D) Neuropathic diabetic ulcer on the sole in a diabetic patient with Charcot foot; E) Extensive ulceration of the lower leg due to combined venous and lymphatic disease. The deep red granulation tissue is not normal and may signify bacterial colonization. The edges of the wound (surrounded by indurated and fibrotic skin) and the island of skin in the wound be are unable to migrate onto the wound bed.

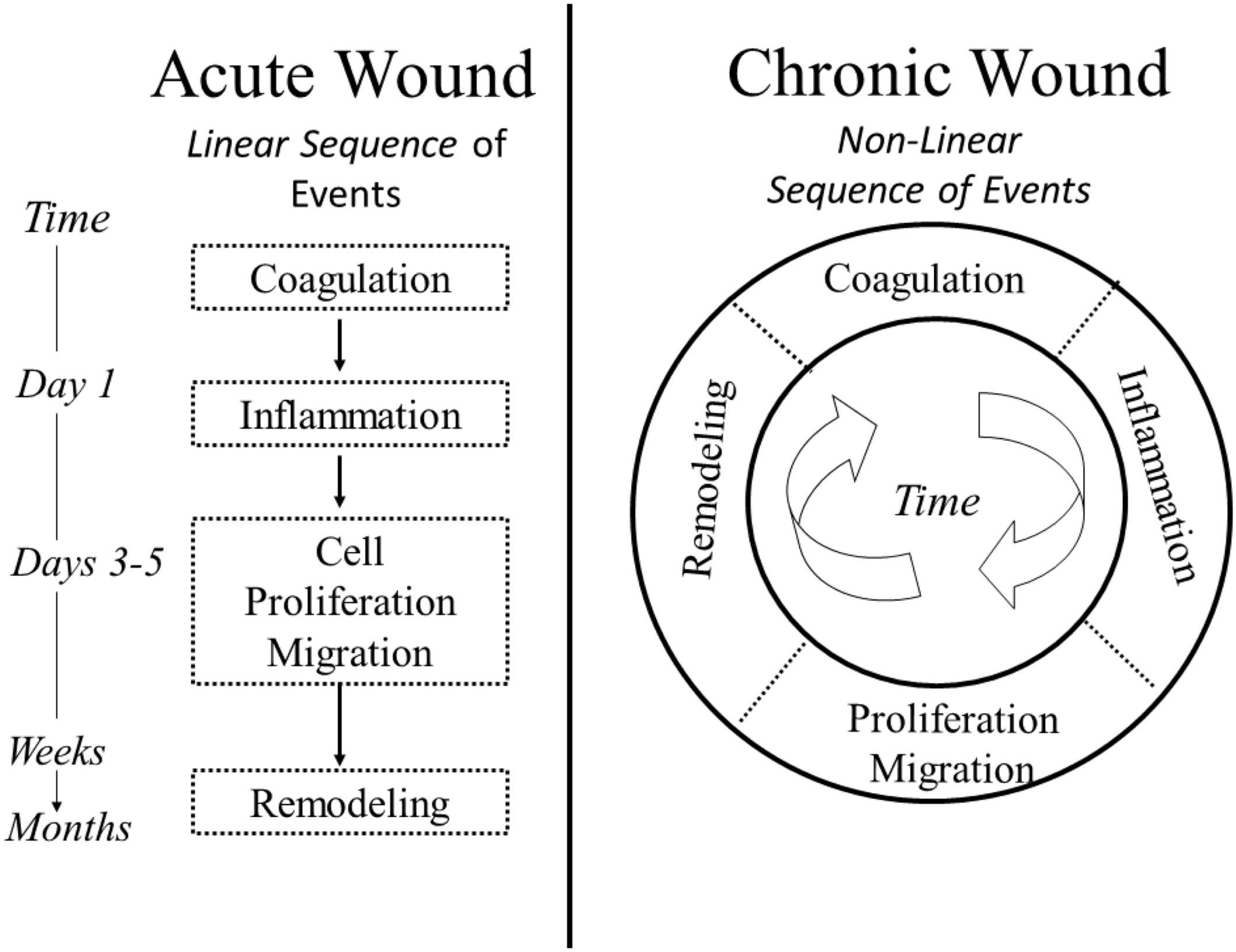

Figure 2.
Diagrammatic representation of the wound healing process in acute wound healing and in chronic wounds. Unlike the linear relationship of the recognized phases of normal wound healing (A: left panel), chronic wounds (B: right panel) are characterized by a process whereby the different phases take place at random and with no defined timeframe. Some parts of the wound are in different phases of healing.

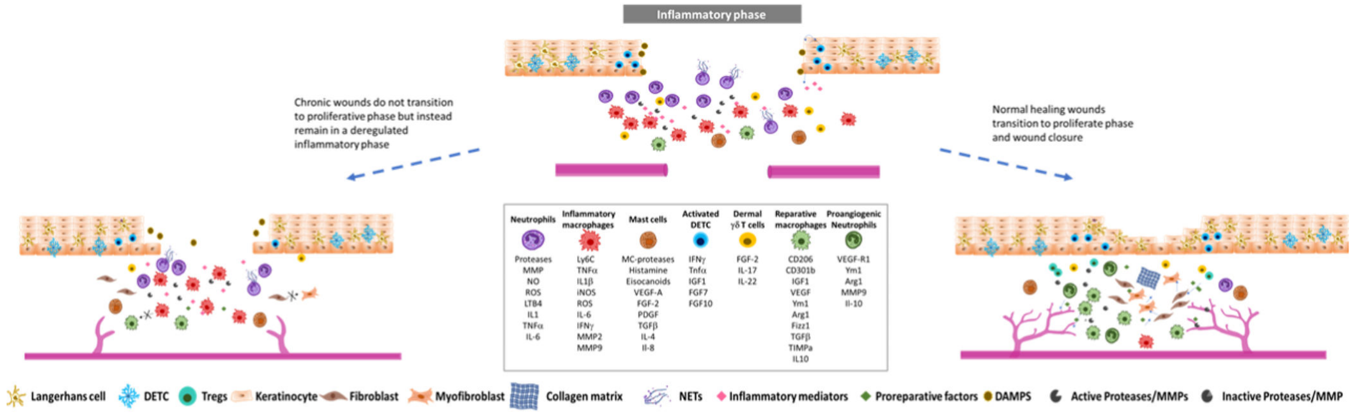

Figure 3:

Schematic representation of the inflammatory component of wound healing process in normal healing and chronic wounds. After the inflammatory phase, normal healing wounds can transition to the proliferative phase of wound healing which is hallmarked by the shift of the responses of immune cells to anti-inflammatory and proliferative to allow tissue repair (right panel). Chronic wounds are instead characterized by a stagnant and deregulated phase which fails to quench local inflammatory responses and does not progress to tissue repair (left panel).

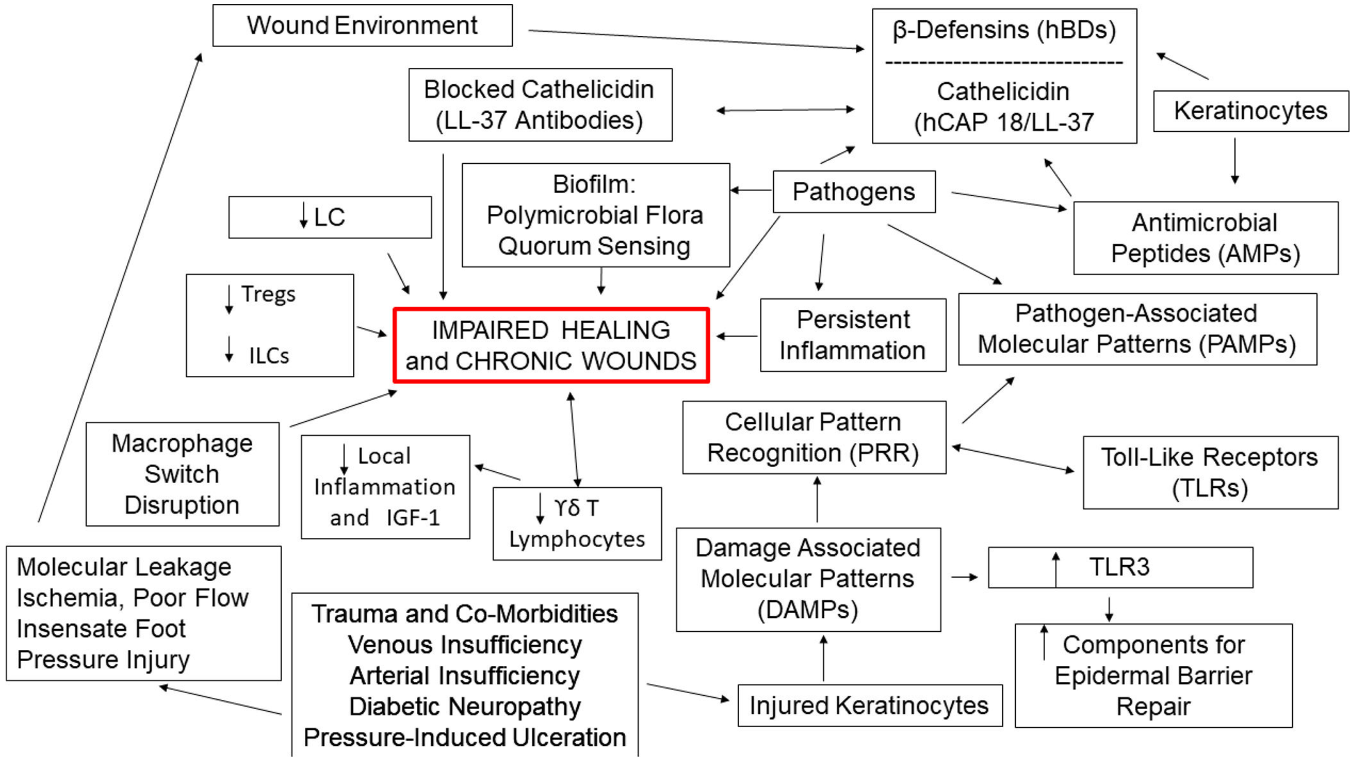

Figure 4:

Representation of key component that characterize the path to impaired healing in chronic wounds.

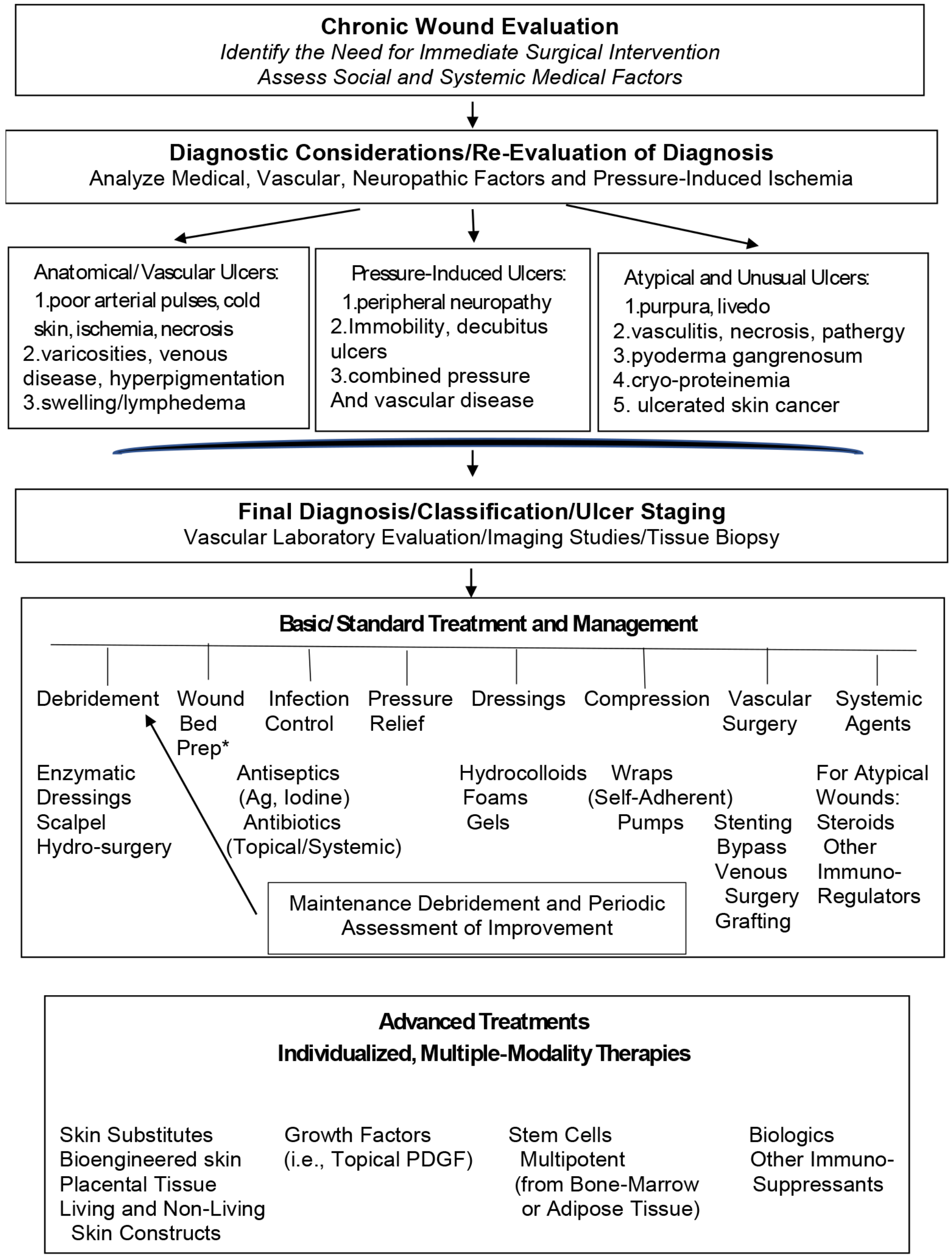

Figure 5:

General algorithm for the evaluation, diagnosis, and treatment strategies for chronic wounds.

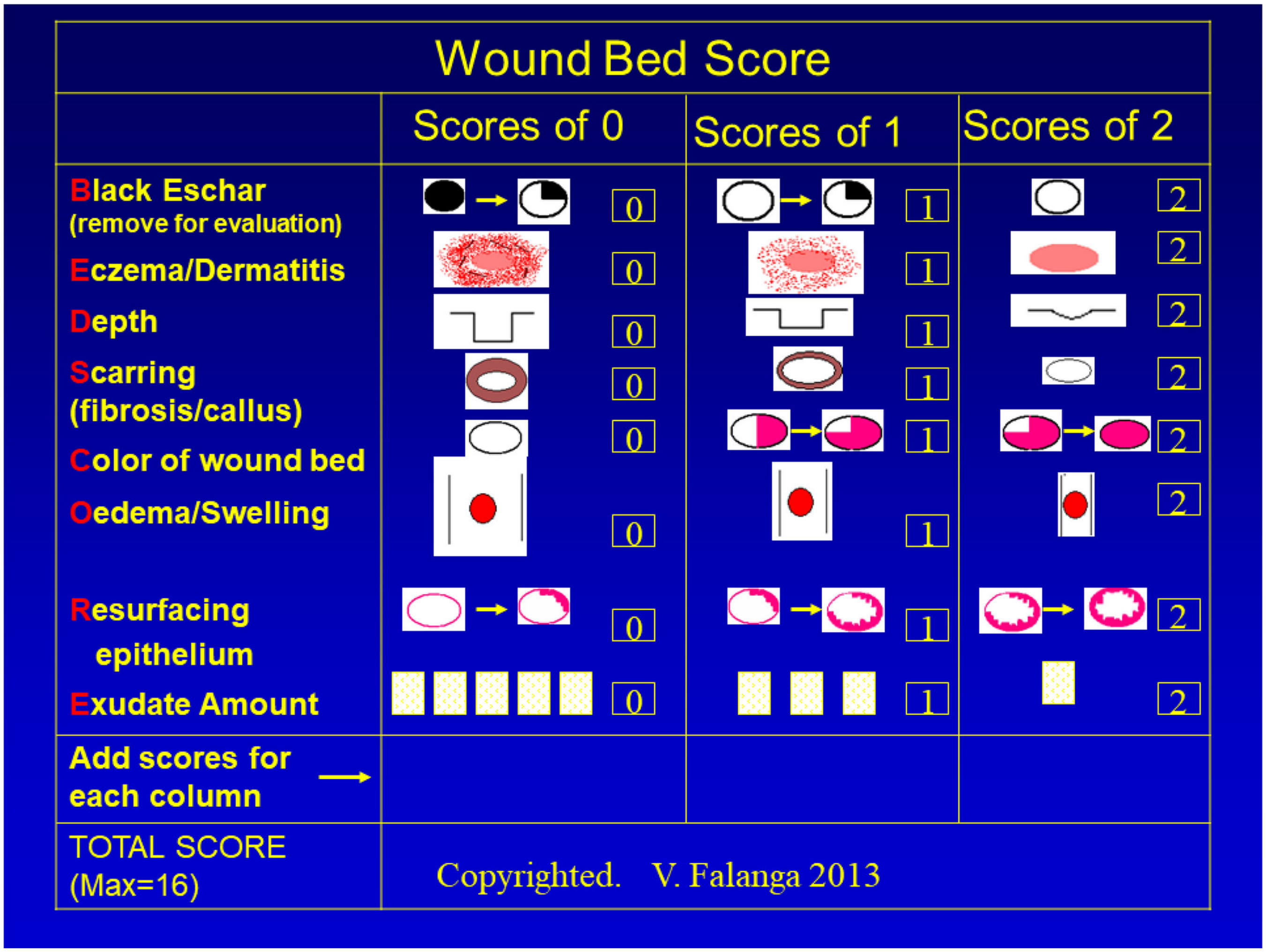

Figure 6:

Representation of some critical clinical components of wound bed preparation, with a scoring system (wound bed score or WBS) that may be useful for assessment, follow up, and for decision making in the use of therapeutic agents.

Table 1.

Chronic Wounds and Common Diagnostic Features

Type of Chronic Wound				
	Typical Body Location	**Cause**	**Physical Exam Findings**	**Diagnostic Testing**
Pressure Ulcers	Sacral areas and Hips	Inability to move, pressure	Paralysis, aftermath of spinal injury	Rule out osteomyelitis
Arterial Ulcers	Lower extremities, especially ankle and toes	Athero-sclerosis	Pale skin and poor or absent peripheral arterial pulses. Hair loss on lower extremities	Doppler ankle/brachial index measurements. Vascular studies, angiography.
Venous Ulceration	Medial aspect of lower legs	Venous hypertension	Lower leg varicosities, hard indurated skin (lipodermatosclerosis) hyperpigmentation, edema	Doppler Duplex Scanning. If suspected, biopsy to rule out malignancy. Doppler ankle/brachial index measurement
Diabetic Ulcers	Toes, soles	Neuropathy ± arterial obstruction	Insensate foot, lower extremity, and foot arterial pulses	Neuropathy testing. Vascular studies if pulses are not adequate
Atypical chronic wounds	Any location, but more common on lower extremities		Livedo net-like skin pattern, purpura, signs of previous radiation. Undermined wound edges	Serum cryoglobulin and plasma cryofibrinogen. Biopsy to rule out skin cancer, occluded dermal blood vessels or vasculitis

Table 2.

Standard Treatments of Chronic Wounds

Types of Chronic Wounds		
Venous Leg Ulceration	**Diabetic Foot Disease**	**Pressure Injury**
Compression	Off-loading device	Pressure relieving mattress
Vascular reconstruction	Padding and protection	Incontinence management
Endovascular stenting	Vascular/endovascular intervention	Nutritional support
Superficial vein ablation	Oral/IV antibiotics	Mobility maximized
Steroids topical/systemic	Debridement	Adaptions to home
Cytotoxic/immunosuppressive drugs	Amputation	Adapted wheelchairs
Skin grafting/flap surgery	Metabolic control	Surgery

Table 3.

Spectrum of Standard Therapeutic Options for Chronic Wounds

Therapeutic Approaches				
Wound Dressings	**External Devices**	**Drugs/Pharmaceuticals**	**Surgery**	**Biological Approaches**
Low adherent	Negative pressure	Antibiotics	Debridement	Protease modulating agents
Alginates	Electrical stimulation	Anti -inflammatory agents	Revascularization	Platelet rich plasma
Hydrogels	Off-loading foot devices	Vasodilators	Bone resection	Recombinant growth factors
Hydrocolloids	Specialized mattresses	Corticosteroids	Skin graft	Tissue engineering constructs (acellular or cellular; living or non-living cells)
Foams	Biofilm disruptors	Immunosuppressants	Flap surgery	Extracellular matrix replacement
Antimicrobial	Phototherapy	Topical stimulatory agents	Amputation	Stem cells