Principles of wound bed preparation and their application to the treatment of chronic wounds

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Abstract

Optimal treatment of chronic wounds requires first identifying the molecular and cellular abnormalities that prevent a chronic wound from healing and then correcting them. The principles of wound bed preparation (WBP) embodied in TIME (tissue, infection, moisture and edge), provide a systemic approach to remove molecular and cellular barriers that prevent wounds from healing. This article is a concise overview of the molecular and cellular regulation of normal wound healing and practical applications of methods of WBP to promote healing.

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Introduction

Chronic wounds are a major problem that is predicted to worsen

It is estimated that 2% of Australians will be affected with venous ulcers during the course of their lives and 50 per 1000 diabetic patients currently have or have had previous foot ulcers [1]. Pressure ulcer prevalence in Australia has been reported to be 4-15% and is estimated to add close to A$350 million per year to health care costs [2].

Similarly, high rates of chronic wounds have been reported in the United States. For example, it is estimated that there were over 2 million, and perhaps up to 5 million, chronic wounds in 1998 [3]. Ulcers resulting from chronic venous stasis disease, diabetes mellitus, and pressure necrosis accounted for 70% of chronic wounds, with health care costs annually totalling approximately US$3 billion [4]. As the demographics in Australia and the United States shift toward an aging population, the incidence and health care costs of chronic ulcers are expected to increase.

Effective therapies for chronic wounds must correct the molecular and cellular abnormalities that prevent healing

To develop clinical treatments that effectively promote healing of chronic wounds, it is necessary to first understand the molecular and cellular regulation of acute, healing wounds, then identify the molecular and cellular abnormalities in chronic wounds that prevent healing and, finally, use this knowledge to develop clinical strategies that correct these abnormalities.

Molecular and cellular regulation of acute wound healing

As described in four extensive review articles, acute wounds progress through four general phases – haemostasis, inflammation, repair and remodelling (Figure 1) [5-8]. Within these phases, multiple cytokines, growth factors, proteases and extracellular matrix components play key roles in regulating cellular processes, which ultimately produce a healed wound.

Haemostasis

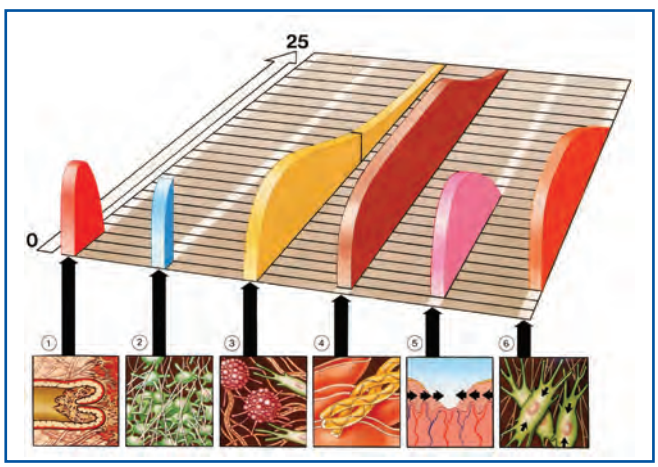
Haemostasis, the first phase of acute wound healing, involves three major processes that influence healing – haemostasis, formation of provisional wound matrix and platelet degranulation. The coagulation cascade converts soluble fibrinogen into an insoluble fibrin clot that fills the injured tissue defect and entraps red blood cells. Besides tamponading bleeding, the fibrin-rich clot serves as the provisional wound matrix, which provides the scaffold for migration of cells adjacent to the injury.

Platelets entrapped in the fibrin clot release the contents of their granules, which contain large amounts of multiple growth factors, including platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and transforming growth factor-ß (TGF-ß). Release of these pre-formed growth factors into the wound stimulates the migration and proliferation of inflammatory cells as well as fibroblasts, epidermal cells and vascular endothelial cells adjacent to the injury [5].

Inflammation

The inflammatory phase of normal healing lasts only a few days. Polymorphonuclear leukocytes (PMNs) and macrophages are initially recruited into the wound through the influence of coagulation and complement factors such as C3b and C5a and then are regulated by chemokine and cytokine activities. Chemokines (a contraction of chemo-attractive cytokines) are also involved in the recruitment and activation of neutrophils, lymphocytes, macrophages, eosinophils and basophils during the inflammatory phase. Within 24 hours, large numbers of neutrophils are drawn to the wound site, followed by activated macrophages. The inflammatory cells secrete proteases that remove damaged and denatured extracellular matrix molecules, engulf and kill bacteria and secrete reactive oxygen species that help to sterilise the wound [8].

Figure 1.



Repair

The repair phase emerges as the inflammatory phase declines and lasts several weeks, until the epidermal layer has closed the wound [6]. Cells in ischaemic regions of the injury begin secreting angiogenic factors such as vascular endothelial cell growth factor (VEGF), which stimulates vascular endothelial cells to migrate, proliferate and form new capillaries. With adequate oxygen and nutrients, fibroblasts and epidermal cells respond to growth factors such as PDGF, TGF-ß and connective tissue growth factor (CTGF) and begin synthesising new collagen, proteoglycans and other components of granulation tissue.

Some fibroblasts are stimulated by TGF-ß, CTGF and PDGF to synthesise large amounts of the specialised contractile protein, alpha smooth muscle actin, and differentiate into myofibroblasts that contract the initial scar tissue. Both the duration and location of synthesis of matrix metalloproteinases (MMPs) and other types of proteases are tightly controlled during healing. For example, during angiogenesis, vascular endothelial cells secrete MMPs that break down the basement membrane surrounding capillaries, thus enabling endothelial cells to migrate and form new capillaries. MMPs are secreted by the leading edge of migrating epidermal cells and by myofibroblasts as they migrate and contract matrix. Experiments with cultured cells, animals and human volunteers have shown that inhibiting MMPs dramatically reduce angiogensis, epidermal regeneration and matrix contraction [9]. Thus, MMPs play important roles in multiple processes of repair.

Remodelling

The remodelling phase begins after wound closure and lasts for months [7]. It is characterised by decreased fibroblast density, reduced metabolic activity of wound cells, and reduced numbers of capillaries and glycosaminoglycans and

reflected clinically by less scar erythema and oedema. Net collagen content of the scar only slightly decreases, but wound tensile strength is enhanced due to increased covalent cross-links between collagen fibres and by reorganisation of collagen fibres into a tighter network. MMPs secreted by fibroblasts in the scar play key roles in remodelling the extracellular matrix.

Molecular and cellular abnormalities of chronic wounds

All chronic wounds start as acute wounds, but their progression to complete healing is interrupted due to such conditions as repeated ischaemia, pressure necrosis, infection or metabolic disorders such as diabetes mellitus and chronic renal insufficiency. Since chemokines, cytokines, growth factors and proteases play key roles in regulating acute wound healing, it is reasonable to hypothesise that alterations in the actions of these molecules could contribute to the failure of wounds to heal normally.

A corollary to this hypothesis is that treatments that correct these molecular and cellular abnormalities would promote healing of chronic wounds. A major concept that emerged from analyses of acute and chronic wound fluids was that levels of pro-inflammatory cytokines were highly elevated in chronic wound fluids compared to acute wound fluids. For example, Trengove and colleagues reported high levels of the inflammatory cytokines TNFα, IL-1, IL-6, and IL-8 in fluids collected from venous ulcers of patients admitted to the hospital [10]. More importantly, levels of the cytokines significantly decreased in fluids collected two weeks after the chronic ulcers had begun to heal.

Harris and colleagues also found cytokine levels were generally higher in wound fluids from non-healing ulcers than healing ulcers [11]. These data suggest that chronic wounds typically have elevated levels of pro-inflammatory cytokines, and that the molecular environment changes to a less pro-inflammatory cytokine environment as chronic wounds begin to heal with conventional treatment.

A second important concept that emerged from wound fluid analyses was that protease activities were highly elevated in chronic wounds compared to acute wounds [4, 12, 13]. For example, the average level of general MMP activity in mastectomy fluids was very low (0.75µg MMP/ml) [14]. In contrast, the average level of general MMP activity in chronic wound fluids was significantly higher than in acute wound fluids from mastectomy patients by approximately 80-fold (60µg collagenase equivalents/ml in acute fluids).

More importantly, Trengove and colleagues reported the decrease in protease activity levels in chronic venous ulcers 2 weeks after the ulcers had begun to heal, a trend that was also reflected in the proinflammatory cytokine levels [14]. Yager and colleagues also found 10-fold higher levels of MMP-2 protein, 25-fold higher levels of MMP-9 protein, and 10-fold higher MMP-1 activity in fluids from pressure ulcers compared to fluids from healing, acute surgical wounds [15]. TIMP-1 levels were decreased while MMP-2 and MMP-9 levels were increased in fluids from chronic venous ulcers compared to mastectomy wound fluids [16]. Recently, Ladwig and colleagues reported that the ratio of active MMP-9/TIMP-1 was closely correlated with healing outcome of pressure ulcers treated by a variety of protocols [17].

Other classes of proteases also appear to be elevated in chronic wound fluids. Fluids from skin graft donor sites or breast surgery patients had intact α1-antitrypsin, a potent inhibitor of serine proteases, had very low levels of neutrophil elastase activity, and contained intact fibronectin [18]. In contrast, fluids from the chronic venous ulcers contained degraded α1-antitrypsin, 40-fold higher levels of neutrophil elastase activity and degraded fibronectin. Chronic leg ulcers also contained elevated MMP-2 and MMP-9, and fibronectin degradation in chronic wounds was dependent on the relative levels of elastase, α1-proteinase inhibitor, and α2-macroglobulin [19, 20].

Besides being implicated in degrading essential extracellular matrix components like fibronectin, proteases in chronic wound fluids were also reported to degrade exogenous growth factors *in vitro* such as EGF, TGF-α, or PDGF [6, 14, 21, 22]. In contrast, exogenous growth factors were stable in acute surgical wound fluids *in vitro*. Furthermore, levels of some growth factors such as EGF, TGF-α, PDGF and TGF-ß were found to be lower in chronic wound fluids than in acute wound fluids [21, 23].

A third major concept to emerge from analysis of wound fluids was that the molecular environments of chronic wounds have reduced mitogenic activity compared to the environments of acute wounds [12]. Fluids collected from acute mastectomy wounds, when added to cultures of normal human skin fibroblasts, keratinocytes or vascular endothelial cells, consistently stimulated DNA synthesis of the cultured cells. In contrast, addition of fluids collected from chronic leg ulcers typically did not stimulate DNA synthesis of the cells in culture. Also, when acute and chronic wound fluids were combined, the mitotic activity of acute wound fluids was

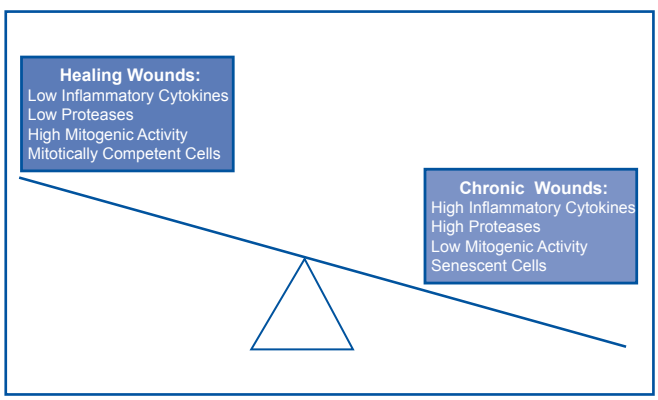
inhibited. Similar results were reported by several groups of investigators who also found that acute wound fluids promoted DNA synthesis while chronic wound fluids did not stimulate cell proliferation [11, 24, 25].

Finally, assessments of cells from chronic wounds indicate that the ability of the cells to respond to growth factors is often impaired. For example, fibroblasts cultured from chronic ulcers, venous ulcers which have failed to heal for many years, did not proliferate well in response to growth factors, whereas as fibroblasts from healing wounds or uninjured skin proliferated strongly when growth factors were added to the culture medium [26]. This premature senescence of cells in the base of some chronic wounds indicates that cellular abnormalities may contribute to the failure of some chronic wounds to respond to conventional therapies.

In general, these results suggest that many chronic wounds contain elevated levels of inflammatory cytokines and proteases (MMPs and neutrophil elastase), which cause reduced levels of active growth factors, receptors and intact extracellular matrix (ECM) proteins. These molecular alterations, combined with premature senescence of some wound cells, appear to directly contribute to the failure of wounds to heal. Thus, chronic wounds fail to heal because the molecular and cellular environment is out of balance with the conditions in acute healing wounds (Figure 2).

Figure 2. Comparison of the molecular and cellular environments of healing and chronic wounds. Healing wounds are characterised by low levels of inflammatory cytokines and proteases but high levels of mitogenic activity and mitotically competent cells. Chronic wounds are characterised by the reverse pattern, with high levels of inflammatory cytokines and proteases, low levels of mitogenic activity and senescent cells.

Modified from Schultz & Mast in Wounds, suppl F, 1998.



Wound bed preparation (WBP): removing the barriers to healing

Optimal care of chronic wounds should be based on the concept of correcting the molecular and cellular barriers that prevent healing. This concept has formalised into the principles of wound bed preparation (WBP), which is the management of a wound to promote endogenous healing or to facilitate the effectiveness of other therapeutic measures [27-31].

These four fundamental principles are summarised by the acronym TIME (Table 1):

- *Tissue:* removal of non-viable tissue or replacement of deficient tissue.
- Control of *infection* or *inflammation*.
- *Moisture imbalance*: correction of excessive moisture and prevention of dessication.
- Revision of the *edge of wound* to stimulate healing.

Wound care has progressed significantly beyond the dictum, "if it's wet, dry it and, if it's dry, wet it". WBP evolved by combining basic principles of good standard wound care and an improved understanding of the molecular and cellular abnormalities that prevent chronic wounds from healing. Applying the concepts of WBP helps to correct specific molecular and cellular differences between acute and chronic wounds, such as high proinflammatory cytokine levels, elevated matrix metalloproteinase levels, low growth factor activities, abnormal matrix and senescent wound cells. The four principles encompass many currently recommended clinical actions, but the key is to understand how the integrated actions alter the molecular and cellular profiles in chronic wounds and result in healing.

Tissue: non-viable or deficient

The first step of wound care is evaluation of the wound bed for the presence of necrotic tissue or slough/fibrinous material. Necrotic tissue consists of dead cells and their debris. In contrast, slough or fibrinous material consists of fibrin, pus and proteinaceous material. The accumulation of necrotic tissue or slough in a chronic wound is thought to promote bacterial colonisation by providing a rich growth medium, which prevents complete wound closure.

The term 'necrotic burden' was recently proposed by Falanga and colleagues [32] to describe necrotic tissue, excess exudate and high levels of bacteria present within dead tissue. Accumulation of necrotic burden in chronic wounds is generally accepted to prolong the inflammatory response, mechanically obstruct wound contraction and impede

Table 1 Principles of WBP.

CLINICAL OBSERVATIONS	PROPOSED PATHOPHYSIOLOGY	WBP CLINICAL ACTIONS	EFFECT OF WBP ACTIONS	CLINICAL OUTCOME
TISSUE NON-VIABLE OR DEFICIENT	Defective matrix and cell debris impair healing	Debridement (episodic or continuous) autolytic, sharp surgical, • enzymatic, mechanical • or biological • biological agents	Restoration of wound base and functional extra-cellular matric proteins	Viable wound base
INFECTION OR INFLAMMATION	High bacterial counts or prolonged inflammation ▲ inflammatory cytokines ▲ protease activity ▼ growth factor activity	• remove infected foci *Topical/systemic* • antimicrobials • anti-inflammatories • protease inhibition	Low bacterial counts or controlled inflammation ▼ inflammatory cytokines ▼ protease activity ▲ growth factor activity	Bacterial balance and reduced inflammation
MOISTURE IMBALANCE	Desiccation slows epothelial cell migration excessive fluid causes maceration of wound margin	Apply moisture balancing dressings Compression, negative pressure or other methods of removing fluid	Restored ephithelial cell migration, desiccation avoided Oedema, excessive fluid controlled, macreration avoided	Moisture balance
EDGE OF WOUND-NON ADVANCING OR UNDERMINED	Non migrating keratinocytes Non responsive wound cells and abnormalities in extra cellular matrix or abnormal protease activity	Re-assess cause or consider corrective therapies • debridement • skin grafts • biological agents • adjunctive therapies	Migrating keratinocytes and responsive wound cells. Restoration of appropriate protease profile	Advancing edge of wound

re-epithelialisation. Prolonged inflammation causes greater numbers of neutrophils, mast cells and macrophages to enter the wound and remain there for much longer periods of time than in acute healing wounds. The inflammatory cells attempt to phagocytise the necrotic tissue and release proteases, superoxide anions and proinflammatory cytokines, such as TNF-α and IL-1ß which perpetuate the inflammatory phase. The products of the inflammatory cells degrade growth factors, receptors and ECM proteins that are necessary for healing.

In addition, debridement of necrotic tissue, slough or foreign material helps to reduce the number of microbes, toxins and other substances that reduce host immune defenses, thereby encouraging active infection to occur[33]. Choice of debridement method (surgical or sharp, autolytic, enzymatic, mechanical and biosurgery) depends on many factors including wound size and position, the type of wound, efficiency and selectivity of debridement method, pain management, exudate levels, risk of infection and cost of the procedure[31].

In the case of non-healing chronic wounds, it may be more appropriate to perform regular or even continuous debridement rather than a single debridement at the initiation of wound care. Steed and colleagues[34] reported that healing of chronic diabetic foot ulcers was significantly increased with the frequency of debridement. The goal of WBP is to restore the wound base and functional ECM proteins that are necessary for healing to progress.

Infection or inflammation

All open wounds contain bacteria. It is the interaction of the bacteria with the host that determines the bacteria's influence on wound healing[35]. The relative number of microorganisms, their pathogenicity, in combination with host's response, dictate whether a chronic wound becomes infected or only shows signs of delayed healing.

Bacterial levels in the wound bed

Bacterial levels in the wound bed can be categorised as contamination, colonisation and critical colonisation, local infection, or spreading infection. *Contamination* is defined as

the presence of non-replicating microorganisms within a wound and does not impair healing.

Colonisation is defined as replicating microorganisms that adhere to the wound surface, but colonisation does not cause cellular damage to the host, and therefore does not impair healing. *Critical colonisation* describes the situation in which the bacterial burden in the wound is intermediate between the categories of colonisation and infection. Critically colonised wounds do not heal (or are very slow to heal), but do not exhibit the classic signs of infection such as erythema, warmth, swelling, pain and loss of function [31]. Treatment of critically colonised wounds typically includes topical antiseptic agents such as the sustained release antimicrobials, cadexomer iodine or nanocrystalline silver dressings.

In contrast to critically colonised wounds, typical clinical signs and symptoms of *locally infected wounds* are delayed healing, pain/tenderness, increased serous exudate, change in colour of the wound bed, friable, absent or abnormal granulation tissue, pus and odour [36, 37]. Similar to critically colonised wounds, topical antiseptics are used to treat locally infected wounds.

Spreading infections that extend beyond the wound margins (e.g. cellulites and ascending lymphangitis) require systemic antibiotics, often in combination with topical antiseptics.

Factors that are known to affect the bacterial burden in chronic wounds include the number and types of microorganisms present in the wound, their virulence and host factors. The number of bacteria that impede healing of open wounds is controversial, with studies showing either impaired or no impaired healing with greater than 1x105 organisms per gram of tissue [28, 38, 39].

The type and pathogenicity of the organisms, rather than the simple number of microorganisms in a wound, is a better determinate of the risk of infection. For example, the isolation of any highly virulent beta haemolytic streptococci from a chronic wound should be considered highly significant, and appropriate treatment should be initiated [40]. Most chronic wounds are usually colonised with at least three species of microorganisms [41, 42]. Some combinations of bacterial species synergistically enhance the virulence of previously non-virulent organisms, resulting in damage to the host [38, 43].

Another important component of infections is the generation of a biofilm, which may contribute to delayed healing in chronic wounds. When some bacteria proliferate, they form micro colonies that become attached to the wound bed and secrete a glycocalyx, or biofilm, that helps to protect the microorganisms from antimicrobial agents [44]. Organisms may exist as clusters of individual bacterial types or as mixed bacterial colonies. The periodic release of motile bacteria from these colonies may result in infection. These bacterial colonies can undergo several changes, expressing different genes, which may then alter the antimicrobial sensitivity of the organism. Thus, biofilms may harbour bacteria that are resistant to the effects of antimicrobial agents, such as antibiotics and antiseptics, and contribute to delayed healing.

Cadexomer iodine is an antimicrobial which absorbs wound exudate and provides sustained release of iodine in the wound bed [45]. Cadexomer iodine has also demonstrated efficacy *in vivo* against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) [46]. Because of the non-specific actions of antiseptics on cells, there has been some concern that use of topical antiseptics such as cadexomer iodine could have deleterious effects on wound healing. The evidence from nine clinical trials comparing the effects of cadexomer iodine with other forms of treatments on chronic ulcers have shown enhanced healing along with decreased pus, debris, pain and erythema [47].

Nanocrystalline silver dressings, such as Acticoat®, are comprised of a silver-coated, high density polyethylene mesh with an absorptive gauze core, which slowly releases silver into the dressing and maintain an effective antimicrobial barrier for up to 7 days. Nanocrystalline silver has been shown to be effective against a broad spectrum of bacterial strains *in vitro*, including MRSA and vancomycin-resistant Enterococcus. Silver ions have been used in the past in the form of silver sulfadiazine (AgSD) cream. Bishop and colleagues [48] found a statistically significant reduction in chronic venous ulcers treated with AgSD compared to controls. A similar finding was observed by Kucan and colleagues [49] for the treatment of pressure ulcers.

Moisture imbalance

Optimal wound healing occurs in a moist environment. Excessive fluid can cause maceration of wound margin and surrounding skin, making it more susceptible to shear forces. At the other extreme of moisture imbalance, desiccation of wounds slows epidermal regeneration by slowing migration of epithelial cells. Thus, obtaining the right moisture balance in the wound is necessary to facilitate healing. This is achieved most commonly by utilising an appropriate dressing that either absorbs excess fluid or increases hydration of the wound.

In addition to the local wound treatment dressing, it is also important to address fluid in the surrounding tissues. For example, lower extremity compression is needed to reduce peripheral oedema in chronic venous stasis disease.

Traditional wound dressings mainly serve to cover wounds and establish the proper level of hydration by either absorbing excess wound exudate or increasing moisture levels in the wound environment. Such dressings do not actively alter the wound molecular environment by releasing specific molecules into the wound or selectively absorbing molecules from the wound environment.

Traditional dressings that absorb fluids can be categorised as low, medium and high capacity. Low fluid capacity dressings include hydrocolloid dressings (e.g. Duoderm®). Moderate fluid capacity dressings include foam dressings (e.g. Allevyn®), and high capacity dressings include alginates (e.g. Kaltostat®). Gauze pads moistened with normal saline can absorb small amounts of wound fluid, but are not effective dressings for maintaining a moist environment in a clean wound because of the tendency to dry before the next dressing change. As a result, there is a high risk of traumatically removing superficial granulation tissue during dressing changes.

Thin film dressings (e.g. Opsite®) are usually permeable to gas and water vapour but are impermeable to water and microorganisms. They are useful for clean wounds with no substantial exudate. For dry wounds (or very low exudative wounds) hydrogels (e.g. Aquacel®) help to maintain a moist environment due to their high water content (70-90%).

Recently, dressing technology has shifted from establishing an optimal moisture balance in wounds to correcting specific molecular abnormalities identified in chronic wound fluids. Specifically, a dressing consisting of collagen and oxidised regenerated cellulose (Promogran®) was developed that reduced protease activities in chronic wound fluids in laboratory assays [50]. The collagen-containing dressing also reduced degradation of PDGF added to chronic wound fluid *in vitro* [51]. Randomised clinical trials demonstrated a strong trend for improved healing in diabetic foot ulcers treated with the active dressing compared to moistened gauze with a secondary dressing [52].

Edge of wound: non-advancing or undermined

Chronic wounds often develop static or slowly advancing epidermal margins. Abnormalities in cells at the edge and base of the wound, as well as alterations in the extracellular matrix, have been linked to the failure of cells to properly proliferate and migrate. For example, cultures of fibroblasts established from the edge or base of chronic venous ulcers proliferated poorly in response to serum or specific growth factors (PDGF-BB, EGF or bFGF) when compared to fibroblasts

cultured from normal dermis or acute wounds [53, 54]. Furthermore, fluids from chronic venous ulcers induced senescence in cultures of normal neonatal foreskin fibroblasts, indicating that factor(s) present in chronic wound fluids could adversely impact the capacity of normal fibroblasts to proliferate and heal wounds [55].

Histological analyses of biopsies taken from the edge of chronic venous ulcers revealed epidermal cells were in a heightened proliferative state with delayed keratinisation. In addition, the epidermal basement membrane at the wound edge was disrupted and lacked type IV basement membrane collagen, which is necessary for epithelial cell attachment and migration [56]. Thus, stimulating the edge of some wounds to advance requires correcting multiple factors in the wound, including proper debridement, inflammation and moisture balance.

Undermining at the wound edge, which occurs primarily in diabetic ulcers and pressure ulcers, also impairs healing. In diabetic ulcers with callous and overhanging tissue, a typical approach is to debride the edge of the ulcer to bleeding tissue and ensure that infection/inflammation is controlled and that moisture balance is established with the appropriate dressing.

If the diabetic ulcer does not respond to treatments based upon the principles of TIME, an advanced therapy that can be considered is topical treatment with PDGF (Regranex®). In four studies with a total of 922 patients with non-healing lower extremity diabetic ulcers of at least 8 weeks' duration, topical PDGF treatment showed a 39% increase in complete healing compared with that of the placebo gel treatment group (50% vs 36%, respectively, p=0.007) [57]. The effectiveness of PDGF was significantly increased with the frequency of debridement [34].

Another advanced therapy for diabetic ulcers that is under evaluation is the use of the metalloproteinase protease inhibitor, doxycycline. Doxycycline directly inhibits MMPs and the TNF-α converting enzyme (TACE), and indirectly inhibits elastase by preventing degradation of alpha-1 protease inhibitor by MMPs. It also selectively reduces synthesis of nitric oxide synthase, thereby reducing levels of nitric oxide. In a pilot randomised control study, daily treatment with topical doxycycline improved healing of chronic diabetic foot ulcers, perhaps by altering the molecular environment of the chronic wounds toward an acute healing wound [58].

In pressure ulcers with an extensive shelf of tissue and large undermined areas, several treatment strategies can be considered. In a clean wound, a vacuum assisted dressing can be used alone to reduce the ulcer volume until it can be closed surgically or treated with appropriate dressings. Critically colonised or infected wounds should not be treated with a vacuum assisted dressing until the infection is eliminated.

Chronic venous stasis ulcers are usually superficial, and include a hyperproliferative, non-advancing epidermal margin. Treatment typically consists of debridement of the wound edge and compression combined with an appropriate dressing to establish proper moisture balance. When the ulcer slows or stops healing even though treatments based on the principles of TIME WBP have been applied, advanced therapies such as autogenous split thickness skin graft or bioengineered skin substitutes (e.g. Apligaf®, Dermagraft®, Integra®) can be applied [59, 60].

Future directions for the treatment of chronic wounds

Optimal application of the TIME principles of WBP to chronic wounds should be based on rapid and accurate assessment of key diagnostic, cellular and molecular aspects of the wound environment that need to be corrected, and determine when the intervention was successful. Development of a diagnostic test strip that can sample the wound environment and identify specific molecular abnormalities would greatly assist in applying TIME correctly. These test strips would alert the health care provider of abnormal cytokine and protease levels so that inhibitors of inflammatory cytokines, such as IL-10, or MMP inhibitors, such as doxycycline, could be added to alter the molecular environment into an acute healing wound. Eventually, these diagnostic test strips and treatment interventions (inhibitors of proinflammatory cytokines and MMPs, and addition of growth factors) would be readily available at the bedside, in wound centres, and in physicians' offices so that immediate diagnosis and treatment can be efficiently provided.

Currently, advanced therapies such as the addition of growth factors, protease inhibitors and active dressings are used alone when healing slows or stops. Combinations of the advanced therapies may have synergistic effects. Alternatively, applying advanced therapies sequentially may improve healing as the wound progresses from one phase to the next.

In summary, the application of principles of TIME to remove the barriers to healing combined with advanced therapies provides more effective resources to heal wounds than ever before.

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