

Wound healing and its impairment in the diabetic foot

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Optimum healing of a cutaneous wound requires a well-orchestrated integration of the complex biological and molecular events of cell migration and proliferation, and of extracellular matrix deposition and remodelling. Cellular responses to inflammatory mediators, growth factors, and cytokines, and to mechanical forces, must be appropriate and precise. However, this orderly progression of the healing process is impaired in chronic wounds, including those due to diabetes. Several pathogenic abnormalities, ranging from disease-specific intrinsic flaws in blood supply, angiogenesis, and matrix turnover to extrinsic factors due to infection and continued trauma, contribute to failure to heal. Yet, despite these obstacles, there is increasing cause for optimism in the treatment of diabetic and other chronic wounds. Enhanced understanding and correction of pathogenic factors, combined with stricter adherence to standards of care and with technological breakthroughs in biological agents, is giving new hope to the problem of impaired healing.

The healing of a wound requires a well orchestrated integration of the complex biological and molecular events of cell migration, cell proliferation, and extracellular matrix (ECM) deposition. Cellular responses to inflammatory mediators, to growth factors and cytokines, and to mechanical forces must be appropriate and precise. These fundamental processes are similar to those guiding embryogenesis, tissue and organ regeneration, and even neoplasia.^{1–4} However, definite differences exist between adult wounds and these other systems. In cutaneous injuries that heal readily and do not have an underlying pathophysiological defect (acute wounds), the main evolutionary force may have been to achieve repair quickly and with the least amount of energy. Hence, such wounds heal with a scar and no regeneration. In wounds with pre-existing pathophysiological abnormalities (chronic wounds, such as diabetic ulcers), evolutionary adaptations have probably not occurred; impaired healing is the result. However, there is much cause for optimism for the treatment of chronic wounds, because of tremendous strides in our scientific understanding of the repair process and how that knowledge can be used to develop new approaches to treatment.

Basic aspects of normal wound healing

The type, size, and depth of cutaneous injury have important implications for events at the cellular and molecular level. Scalpel injury (ie, after surgical procedures) causes less overall and diffuse tissue damage than burns or radiation, can be primarily closed (by suture), and generally results in less scarring. Small and superficial cutaneous defects can resurface mainly by epidermal migration, and do not have to rely on actual keratinocyte proliferation and its more substantial lag

time after injury. Wounds that are restricted to the superficial layer of the dermis (partial-thickness wounds) still have a reservoir of keratinocytes in the hair follicles and other skin appendages left in the wound bed, and thus can heal both from the edges and from within the wound. Conversely, full-thickness wounds can only heal from the edges, and contraction plays an important mechanism for wound closure in these deeper wounds.^{5,6}

Events and phases of wound healing

The fundamental biological and molecular events after cutaneous injury, with information mainly derived from experimental wounds in animals, cannot be separated and categorised in a clear-cut way. However, it has been useful to divide the repair process into four overlapping phases of coagulation, inflammation, migration-proliferation (including matrix deposition), and remodelling. These phases are shown in figure 1, which also highlights the main events during each phase and the key types of cells implicated. Whereas acute wounds go through the linear progression of overlapping biological and molecular events illustrated in figure 1, chronic non-healing wounds do not. Some areas of chronic wounds are in different phases at the same time and, presumably, progression to the next phase does not occur in synchrony. These overall differences between acute and chronic wounds are not restricted to lack of progression alone. Certain events occur abnormally in the healing-impaired wound, highlighting the need to be cautious in extrapolating lessons learned from acute wounds to the situation in chronic wounds. Studies in animals have shown that isolated abnormalities can markedly modulate the healing process.⁷ Impaired healing is found in mice with combined deficiency of molecules that have a critical role in inflammation (E-selectins and P-selectins), and in mice without plasminogen, urokinase plasminogen activator, and tissue plasminogen activator (double knockout), fibroblast growth factor-2 (basic fibroblast growth factor), or inducible nitric oxide.^{1,2,7} Conversely, decreased healing occurs in transgenic mice overexpressing some tissue metalloproteinases (eg, matrix metalloproteinase [MMP]1) and antisense to CD44, the receptor for hyaluronic acid.⁷ Unexpectedly, some mutations lead to accelerated healing,

Search strategy and selection criteria

I searched PubMed by matching "wound healing" and "wounds" with the search terms "keratinocytes", "diabetes", "hemidesmosomes", "integrins", "MMPs", "contraction", "neuropathic ulcers", "gene therapy", "stem cell therapy", "growth factors", "tissue engineering". I mainly selected publications from the past 6 years. Relevant articles and book chapters were also included. No restriction was applied on language of publication.

as reported with Smad-3 or skn-1a knockout mice.⁸ These findings offer the promise of improving healing in human beings, by manipulating growth factors, ECM, and signalling pathways.

Phases 1 and 2: coagulation and inflammation

The different phases of wound healing not only overlap, but also have ramifications beyond their more obvious immediate purposes. Soon after injury, a fibrin plug forms and inflammatory cells are quickly recruited to the wound. Coagulation is needed for haemostasis and wound protection. The fibrin plug consists of platelets embedded in a meshwork of mainly polymerised fibrinogen (fibrin), fibronectin, vitronectin, and thrombospondin; it is an immediate way to ward off bacteria and provide temporary wound coverage,^{3,7} but also has other roles. During their incorporation within the plug, platelets aggregate and release a wide range of growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor (TGF) β 1.^{1,3} These and other growth factors, the activation of which also depends on pH and other parameters within the injured tissue, have an early role in cell recruitment and a later one in ECM formation.⁷ As another example of multiple effects, thrombin polymerisation of fibrinogen to fibrin yields fragments, such as fibrinopeptides A and B, which can recruit inflammatory cells to the wound.³ Then, through the endothelial expression of selectins, leucocytes are slowed down in the bloodstream enough that stronger forces generated by binding to integrins will help their movement through endothelial gaps and into the extracellular space (diapedesis).² Again, these inflammatory cells recruited to the wound have several purposes. Neutrophils and macrophages, whose function is impaired in diabetes, aid in wound debridement. However, both cell types produce several key growth factors and mediators that keep fuelling the repair process: for example, connective tissue growth factor was first identified in neutrophils.³

Immediately after injury, the wound is hypoxic because of damage to the blood vessels. This seemingly deleterious situation has some beneficial effects, and might help prepare for the next phase of healing. Hypoxia increases keratinocyte migration, early angiogenesis, proliferation and clonal expansion of fibroblasts, and the transcription and synthesis of crucial growth factors and cytokines, including PDGF, vascular endothelial growth factor, and TGF β 1.⁹ Later, within the next 2–3 days, inflammatory and dermal cells recruited to the injury site produce a powerful armamentarium of growth factors and cytokines.⁷ Circulating monocytes take up residence at the injury site as tissue macrophages, and so do fibroblasts and endothelial cells as they form the early granulation tissue that begins the process of contraction.

Phases 3 and 4: migration-proliferation and remodelling

As the inflammatory phase of wound healing is toned down (figure 1), wound contraction begins, but stable

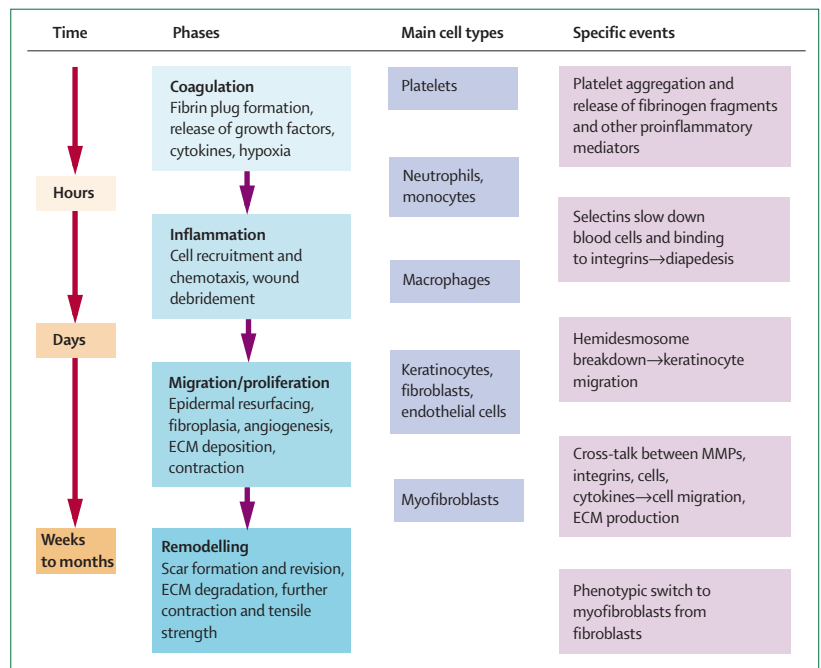


Figure 1: Phases of wound healing, major types of cells involved in each phase, and selected specific events

wound closure needs to be addressed. Formation of ECM proteins, angiogenesis, contraction, and keratinocyte migration are essential components of these phases. Matrix proteins, including collagens, fibronectin, and vitronectin, provide substrates for cell movement, vehicles for changing cell behaviour, and structures that return function and integrity to the tissue.¹⁰ Angiogenesis makes possible the re-supply of oxygen and other nutrients. Contraction, aided by the formation of ECM, granulation tissue, and the emergence of myofibroblasts, is a rapid and efficient way of achieving wound closure. The balance between contraction and keratinocyte-dependent closure has much to do with the depth and location of the wound and the presence of complications due to infection, and seems to be impaired in diabetic wounds. Another critical balance is the deposition, persistence, and dynamic remodelling of ECM proteins. Excessive deposition of some matrix proteins, such as collagens and fibronectin, has been reported in diabetic wounds.⁹

The role of integrins

As shown in figure 1, cell movement is critical for fibroplasia, angiogenesis, and keratinocyte-dependent wound closure. Keratinocytes need to migrate through or below the fibrin meshwork, and fibroblasts and endothelial cells are recruited to the nascent granulation tissue. At this time, MMPs and other enzymes (tissue plasminogen activator and urokinase plasminogen activator) are needed to free cells and structures from their more stable surroundings.^{7,11} Integrins are also essential, because they represent the language by which cells communicate with the matrix and with each other.

Integrins are α and β transmembrane cell-surface receptors that bind the ECM to cytoskeletal structures. There are at least 24 $\alpha\beta$ heterodimers, which are formed from a pool of 18 different α and eight β subunits. For cells to move, they have to be freed from their stable configuration and location in the tissue.¹¹ Dermal fibroblasts, which up to 3–4 days after wounding are in a resting state with dermal collagen, undergo a switch from $\alpha 2$ to $\alpha 3$ and $\alpha 5$ integrin subunits, the latter being more efficient in negotiating the migration of fibroblasts through the early fibrin-rich matrix. Basic fibroblast growth factor is crucial to angiogenesis and can induce vascular endothelial growth factor, the flt-1 receptors of which are upregulated on endothelial cells and are decreased in wounds with impaired healing. Again, several interactions are implicated. For example, endothelial cells are incapable of responding to angiogenic stimuli without the expression of $\alpha v\beta 5$ integrin. During migration, endothelial cells exhibit high-affinity forms of $\alpha v\beta 5$.^{7,9,11}

Keratinocyte migration

The disassembly of hemidesmosomes, which provide anchorage of the basal keratinocytes to the underlying basement membrane, is a good example of a highly organised structure being torn down for the purpose of cell migration. This disassembly and keratinocyte migration require cross-talk between growth factors, MMPs, integrins, and structural proteins. Among critical factors affecting hemidesmosome disassembly are the unravelling of laminin-5 binding to $\alpha 6\beta 4$ integrin, receptor clustering, interactions of integrins with ECM components, formation of lamellipodia needed for cell movement,^{12,13} the molecular switches GTPases (Rho, Rac, Cdc42),^{13,14} and the state of phosphorylation of the integrin subunits. For example, a shift from the stable and resting assembly of laminin-5 with $\alpha 6\beta 4$ is caused by phosphorylation of this integrin heterodimer, causing binding to the unlocked $\alpha 3\beta 1$ integrin and facilitation of lamellipodia formation and keratinocyte movement.¹³ In addition to lamellipodia extension, basal keratinocytes leapfrog over the basal cells near the wound. Interestingly, in the embryo and probably in the adult cornea, a purse-string mechanism for wound closure is operative, so that an actin cable forms within minutes, followed by keratinocytes being pulled together.¹ Kinases, such as mitogen-activated protein kinase, are activated in basal and suprabasal keratinocytes by further action of integrins or the release of interleukin 1α . Calcium concentrations and entry into the cells also have a central role in migration, proliferation, and differentiation.¹⁵

MMPs and other enzymes are important components of the wound that facilitate cell movement and the eventual remodelling of ECM. To negotiate the fibrin clot, keratinocytes need to upregulate tissue plasminogen activator and urokinase plasminogen activator. The interactions between $\alpha 3\beta 1$, keratinocytes, and collagen

ultimately lead to induction of MMP1 (collagenase 1 or interstitial collagenase). Acting like molecular scissors, the MMPs help regulate matrix degradation and cellular movement. Type 4 and type 7 collagen, essential components of the basement membrane and anchoring fibrils, are cut by MMP9. Other non-collagenous matrix components are degraded by MMP10 (stromelysin-2).^{3,11,16}

Keratinocyte proliferation

Keratinocytes start migrating to fill the wound defect within a few hours after injury. A keratinocyte proliferative burst then occurs, which is especially important for larger wounds where migration of cells alone is insufficient to close the defect.² Keratinocyte proliferation also involves regulation of *TP53*, *CKAP4*, and *TP73* tumour suppression genes, epidermal growth factor, and TGF α , among other signals. Fibroblast and keratinocyte proliferation rely in part on the TGF β -related activins.¹⁷

Wound contraction and ECM remodelling

Although keratinocyte migration is very important in wound closure, events leading to angiogenesis and wound contraction play a major part in both acute and chronic wounds. Failure of timely and rapid contraction seems to be a major problem in diabetic ulcers, for example. Within a week from injury, several events have occurred, including matrix deposition, aided by such growth factors as PDGF, TGF β , fibroblast growth factors, and vascular endothelial growth factor, phenotypic changes in fibroblasts to myofibroblasts (TGF β -induced), and early remodelling.³ Thus, overall, ECM is formed, providing initial support and a conduit for cell migration, and begins to be degraded, as a result of serine proteases and MMPs. Sequential deposition of collagens, first type 3 and then type 1, and their hydroxylation peak at about 3 weeks. The wound continues to contract, with maximum tensile strength being 60% of the previously unwounded skin.^{3,6}

Impaired healing: the diabetic ulcer

The linear progression paradigm for normal wound healing shown in figure 1 has been highly valuable in understanding the basic biology of tissue repair. However, one should not oversimplify. Even during the normal process of wound healing complications can occur, including infection, thrombosis, and ischaemia. Also, lessons learned from experimental models, on which figure 1 and the previous discussion are based, cannot be completely extrapolated to the situation encountered in diabetic wounds. There, intrinsic pathobiological abnormalities and extrinsic factors contribute to an even more complex wound microenvironment. Unfortunately, a valid model of chronic wounds in animals has not yet been developed. Clinical and experimental evidence suggests that diabetic ulcers and other types of chronic wounds do not follow an orderly and reliable progression of wound healing. Parts of the chronic wound may be stuck in different phases, having lost the ideal synchrony

of events that leads to rapid healing.^{18,19} In the case of diabetic ulcers, healing impairment is caused by several intrinsic factors (neuropathy, vascular problems, other complicating systemic effects due to diabetes) and extrinsic factors (wound infection, callus formation, and excessive pressure to the site). Traditionally, this set of predisposing abnormalities in diabetes has been referred to as the pathogenic triad of neuropathy, ischaemia, and trauma. However, this too is an oversimplification. One pathogenic abnormality can lead to another, and vicious cycles of pathogenicity develop in the diabetic foot. Moreover, the triad does not specifically mention infection, which plays a major role in healing impairment, hospitalisation, and high incidence of limb loss.^{20,21} Diabetic ulcers are also quite heterogeneous, depending on the underlying predominant abnormality. In a sense, no diabetic ulcer is completely pure from a pathogenic standpoint. An ulcer can be mainly attributed to vascular occlusion or neuropathy. However, neuroischaemic ulcers are common, if not the rule.²² Moreover, infection, the location of the ulcer, and foot deformity and callus have to be factored in. For this reason, developing an adequate and universally agreed classification of diabetic ulcers from a pathogenic standpoint has been a challenge. An extremely useful series of publications and updates have come from consensus statements by an international working group on diabetes, which highlights these important considerations and the need to properly define abnormalities and classifications.^{23–25}

Vasculopathy and endothelial cell abnormalities

Patients with diabetes, particularly those with type 1, have more macrovascular disease than non-diabetic people, with more distal distribution from the superficial femoral artery to the pedal arch and involvement of the metatarsal artery.²⁶ Microcirculatory deficiencies occur early in diabetes. These abnormalities include a reduction of capillary size, thickening of the basement membrane, and arteriolar hyalinosis. The thickening of the basement membrane interferes with physiological exchanges, and leads to altered migration of leucocytes (contributing to infection), decreased maximal hyperaemia, and abnormal autoregulatory capacity.^{27,28} Impaired endothelial function might involve a reduction of nitric oxide synthetase. Importantly, the lumen of microvessels is not decreased in diabetes.²⁹ The long-standing myth of small-vessel disease accounted for the unfortunate and incorrect notion that revascularisation would not help diabetic patients. Nevertheless, although true luminal occlusion of small blood vessels does not occur, bloodflow is maldistributed. Abnormal bloodflow might also explain the development of Charcot foot, which results in dramatic changes in bone alignment and great susceptibility to pressure forces in the insensate foot.

Clear links exist between vasculopathy and neuropathy in the diabetic foot. Shunts in the microcirculation, together with the presence of sympathetic nerve

denervation and autonomic neuropathy, lead to the maldistribution of bloodflow. Poly (ADP-ribose) polymerase (PARP), a nuclear enzyme responsive to oxidative DNA damage, can also lead to cell necrosis and changes in microcirculatory reactivity. In diabetic neuropathy, the neurovascular response, dependent on the C-nociceptive nerve fibres and adjacent C fibres, is impaired, leading to defects in the secretion of substance P, calcitonin gene-related peptide, and histamine. Hence, vasodilatation is impaired, particularly in situations of stress from trauma and pressure.^{30,31}

The assessment of bloodflow in the diabetic foot is complicated by the presence of medial calcification, which renders simple measurement of ankle-brachial pressure index unreliable.³² Thus, non-invasive assessment for vascular disease requires other tests, including absolute ankle pressure, toe pressure measurements, and colour duplex ultrasonography.²² Measurements of transcutaneous oxygen, especially around the wound, might also be helpful from a prognostic standpoint.³³

Neuropathy

Some neuropathological problems have already been mentioned, as they are tied to microcirculatory defects. Motor, sensory, and autonomic fibres are all affected. The consequences are predictable. Because of sensory deficits, the diabetic patient does not have protective symptoms guarding against pressure and heat. Thus, trauma can initiate the development of an ulcer. Absence of pain, probably combined with abnormal vasodilatory autoregulation, contributes to the pathogenesis of Charcot foot, which further impairs the ability to sustain pressure. Similarly, the addition of motor fibre abnormalities leads to undue physical stress on the insensate foot, the development of further anatomical deformities (arched foot, clawing of toes), and might play a part in the development of infection, since bacterial growth is enhanced in tissues with high compressive forces.^{20,22,34–36}

Although intuitively correct, the link between glucose control and the development or stabilisation of neuropathic abnormalities is not absolutely proven. This type of evidence would necessitate randomised prospective trials that would clearly not be ethical. However, long-term trials aimed at determining the relation and correlations in a large cohort of patients might be possible. Measurements with the Semmes-Weinstein 10 g nylon monofilament can assess protective sensation. Vibratory sensation can be measured with a biothesiometer.^{34,35}

Infection

Infection is not a stated component of the pathogenic triad for development of diabetic foot ulcers, but is an extremely important cause of morbidity and hospitalisation, amputation, and impaired healing. Whether it has a role in the initial development of the ulcer, especially when combined with trauma, is unclear. There are several reasons for the increased incidence of infection in the

diabetic foot compared with other types of chronic wounds. The role of stress and compressive forces favouring overgrowth of bacteria has been mentioned,³⁶ as has decreased function of macrophages and neutrophils.^{37,38} However, it is the combination of factors, including vascular abnormalities, that has the essential role in this major complication. Infection can spread rapidly in diabetic ulcers. Limb-threatening cellulitis, abscesses, and osteomyelitis need immediate attention. High bacterial burden without the classic signs of infection is also detrimental to healing.³⁹ The presence of bacterial biofilms in diabetic wounds is still speculative. As discussed earlier, temporary hypoxia after injury may be beneficial in stimulating cell movement, angiogenesis, and production of growth factors. However, prolonged hypoxia is detrimental, in part by exaggerating these early physiological events and by causing reperfusion injury and the formation of oxygen radicals. Together with hyperglycaemia and other metabolic effects of diabetes, hypoxia adversely affects neutrophil and macrophage function.⁴⁰

Debridement: multiple beneficial effects

Proper debridement involves removal of the necrotic wound bed and callus, as the latter can contribute to increased pressure on the insensate foot.^{41,42} In a retrospective analysis, debridement increased the therapeutic effect of topically applied PDGF.⁴² However, we have now begun to realise that debridement, by removing diseased tissue, actually corrects several cellular and molecular abnormalities. One hypothesis is that debridement resets the stage for proceeding towards the normal wound healing sequence.^{9,43} Several observations and mechanistic reports lend support to this still unproven view. Diabetic ulcers appear to be stuck in the proliferative phase, with an excess of matrix proteins, including fibronectin.¹⁹ Thus, remodelling or turnover of matrix might be inadequate, which ultimately affects cell migration and probably the stability of the healed wound. These abnormalities could have consequences for growth factors, which can become trapped and unavailable for the healing process.⁴⁴ Directly or indirectly, hyperglycaemia alters the balance of MMP concentrations and proteolytic activity. Diabetes is associated with decreased concentrations of urokinase plasminogen activator and increased tissue plasminogen activator inhibitor, a situation that might result in decreased fibrinolysis and impaired matrix deposition.⁴⁵

Wound cell abnormalities

Very importantly, some of the resident cells in diabetic ulcers become phenotypically altered. Fibroblasts isolated from diabetic foot ulcers are probably senescent and show a decreased proliferative response to growth factors.¹⁸ Similar studies in other types of chronic wounds are in agreement with these findings, having shown decreased fibroblast response to TGFβ1,⁴⁶ platelet-derived growth factor,⁴⁷ and other cytokines.⁴⁸ Evidence suggests that phenotypic changes in wound cells are not due only to

replicative senescence, but are perhaps caused by more complex interactions between the resident cells and the chronic wound.⁴⁹ Chronic wound fibroblasts do show decreased expression of type 2 TGFβ receptors, with impaired phosphorylation of transduction signals, including Smad2, Smad3, and mitogen-activated protein kinase.⁵⁰ Although much more work is needed to clearly define the phenotypical abnormalities in diabetic wound cells, these findings have clear-cut implications for therapeutic intervention. For example, growth factors delivered in a simplistic topical approach might not find a regularly suitable and responsive target cell population.

Other cellular abnormalities exist in diabetes. Macrophages in diabetes show a decrease in release of cytokines, including tumour necrosis factor α, interleukin 1β, and vascular endothelial growth factor.³⁸ Excessive activation of some MMPs, such as MMP9, can impair cell migration and lead to breakdown of some necessary matrix proteins and growth factors.⁵¹ Although there is no direct evidence that the proliferative activity of keratinocytes is affected in diabetes, migration may well be impaired and studies with cells from diabetic wounds are needed.⁵²

Therefore, going back to the original premise mentioned earlier, it is possible that proper debridement of diabetic ulcers corrects many more subtle abnormalities, at least partly, by removal of altered resident cells and matrix material.

Correcting impaired healing

Is the diabetic foot ulcer truly a chronic wound, and is impaired healing simply the result of failure to provide timely treatment for the ulcer or due to poor patient compliance? This point of view might apply to purely neuropathic ulcers, in which offloading alone can lead to rapid healing. However, as stated earlier, diabetic ulcers are heterogeneous. The treatment and the outcome depend very much on the presence or extent of arterial insufficiency, the degree of neuropathy, the ulcer location, presence of Charcot deformity, and the persistent propensity to infection.

Figure 2 provides a guideline for the approach to diabetic ulcers. Thorough assessment of the patient and the wound is crucial, as is the immediate need to control glucose concentrations, treat infection, and correct perfusion abnormalities. Offloading is crucial, and has been the subject of much discussion. Areas of controversy exist. For example, a recent systematic review⁵³ favours the use of hyperbaric oxygen in the treatment of diabetic foot ulcers. However, even that review admits to methodological problems and the need for further studies. Similarly, although moist wound healing is widely practised in the management of other types of chronic wounds, the answer in diabetic ulcers is more difficult; a more delicate balance may be needed to avoid maceration of tissues while promoting conditions that prevent eschar formation and facilitate cell migration within the wound.⁵⁴ Control of oedema and removal of exudate are important. There is

now some evidence that negative wound pressure may lead to faster healing.⁵⁵

The diabetic wound needs to be assessed both from the pathogenic standpoint and for its extent. The Wagner classification can be used to assess extent.⁵⁶ Surgical intervention, including vascular reconstruction and debridement may be required immediately. Ways to achieve the optimum wound bed have been discussed.⁴³ Figure 2 addresses optimisation of the wound bed as “good clinical practice” (a term adopted by regulatory agencies overseeing clinical trials that covers both evidence-based approaches and widely accepted ones that have yet to be proven conclusively). In previous work I have referred to some of these steps as “wound bed preparation”.⁴³ The remainder of the paradigm shown in figure 2 gives suggestions for when to use more advanced therapies. Even after complete wound closure, constant vigilance is required, in terms of glucose control, daily attention to any breaks in the skin, and offloading. Preventing wound recurrence is of critical importance.

Technological advances

Recent technological advances have led to very promising breakthroughs in the treatment of diabetic and other types of chronic wounds.⁵⁷ The realisation of the crucial role of growth factors in normal wound healing has already led to the development and regulatory approval of topically applied growth factors, particularly PDGF-BB.^{41,58} Four placebo-controlled trials of PDGF-BB in neuropathic ulcers have been done, with the best result being a 15% increased incidence of wound closure at 20 weeks (50% healing in the growth factor-treated group).⁵⁹ There is a need to improve these results with growth factors. Greater efficiency of delivery of growth factors, by gene therapy or by cell therapy, is now possible and being tested.^{60,61} Better understanding of the phenotypic changes in resident cells, which may be unresponsive to growth factors and which were discussed earlier, may further improve the therapeutic outcome of growth factor therapy. In addition to the use of growth factors, there has also been considerable interest in the application of ECM proteins to accelerate healing of diabetic foot ulcers, including collagen and hyaluronic acid. In the future, we will probably see combination therapies of ECM with growth factors, provided we can overcome the regulatory hurdles.

Growth-factor therapy requires knowledge about the dose of peptide to be used and, from a regulatory standpoint, is a challenge if multiple cytokines and growth factors are to be tested in clinical trials.^{9,57} Partly for these reasons, cell therapy with bioengineered skin has had recent success in both testing and results. Two main types of living bioengineered skin have been tested and proven to be effective in diabetic neuropathic foot ulcers. In a randomised 12-week trial of 208 patients with neuropathic ulcers, a bilayered construct comprising living fibroblasts and keratinocytes from neonatal foreskin led to complete wound closure in 56% of patients, compared with 38% in

controls.⁶² Notably, patients in the active group had decreased incidence of osteomyelitis and amputation, possibly because of faster healing. However, the study was not initially powered to study these complications. In another 12-week randomised study with living foreskin fibroblasts in a vicryl mesh, incidence of complete wound closure of neuropathic foot ulcers was 30% in the active group and 18% in the control group.⁶³ Widely differing incidences of wound closure have been reported in the control groups of these trials with PDGF and bioengineered skin. The reasons for this discrepancy are unclear. The results might reflect different extents of disease and variable adherence to offloading in different protocols and groups of investigators. The extent of debridement might also vary between different study sites. Another criticism of these trials is that optimum offloading, by total contact casting or other variations, can presumably achieve similar or even better results.⁶⁴ However, types of offloading methods have not been

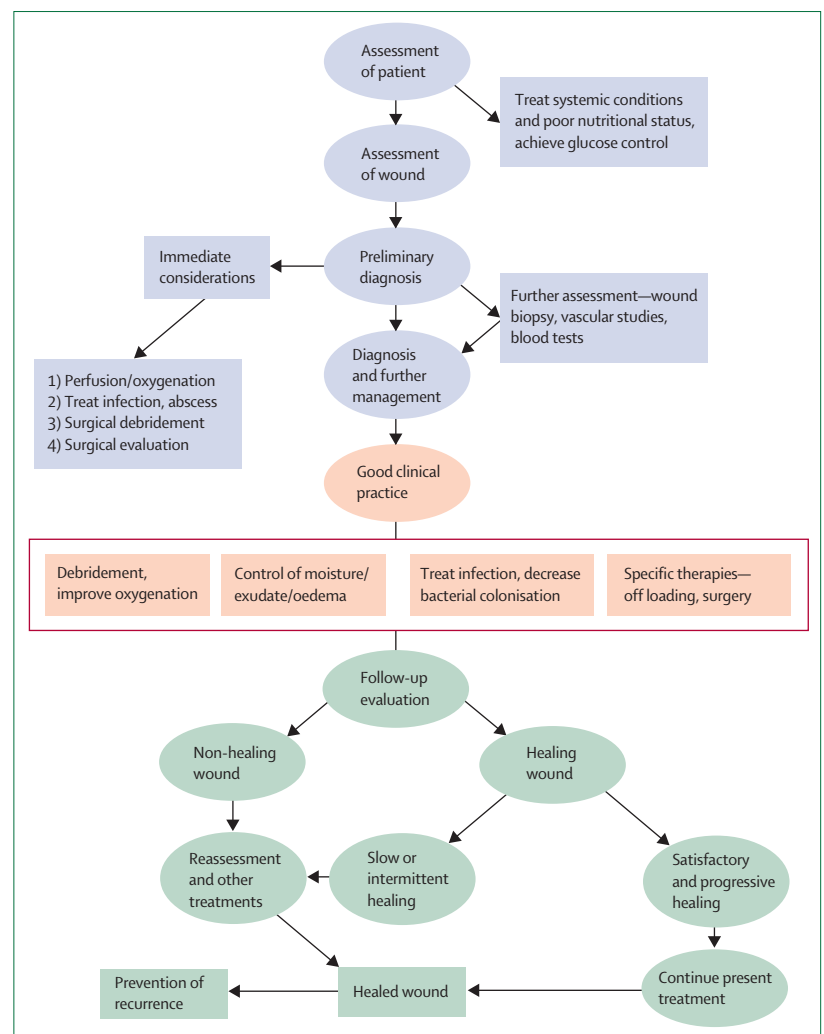


Figure 2: A management strategy for treatment of diabetic foot wounds, taking into account systemic and wound-related pathophysiological abnormalities

compared directly with advanced biological treatments (or even a combined approach). Still, from a therapeutic and a purely scientific standpoint, these results are important since they have shown a beneficial effect of biological agents in the treatment of chronic wounds. The mechanisms of action of bioengineered skin might involve new matrix deposition, increased availability of growth factors, and perhaps recruitment of stem and progenitor cells to the wound site.⁶⁵ However, strong evidence exists that true engraftment or prolonged persistence of cells from these allogeneic constructs does not occur.^{66,67}

Ultimately, for chronic wounds to heal in a timely fashion and even be resistant to the pathophysiological forces driving their recurrence, structures may need to be reconstituted and the wound site repopulated with healthier cells. There is great interest in delivery of stem or progenitor cells, either applied topically or recruited from the circulation.⁶⁸ Some preliminary work suggests that topically applied autologous bone-marrow cultured cells can heal human chronic wounds that are recalcitrant to other treatments, including growth factors and bioengineered skin.⁶⁹ Recruitment of CD34+ cells from the circulation have shown promise in ischaemic limbs.⁷⁰ Also, CD34+ cells from diabetic animals produce fewer endothelial cells than in control animals.⁷¹ However, delivery of stem cells to the wound may need to be followed by other interventions, such as split-thickness skin grafting or application of growth factors or bioengineered skin (Falanga V, unpublished). One hypothesis is that stem cells need to be “directed” for favourable differentiation that benefits the wound. Clinical decisions about when to use advanced or more experimental therapies can be based on healing rates. Studies in venous and diabetic ulcers suggest that advancement of more than 0.7 mm per week is 80% sensitive and specific for eventual wound closure.⁷²

As stated earlier, advances in the treatment of chronic wounds, particularly diabetic wounds, are promising. However, the intrinsic pathophysiological abnormalities that lead to ulceration in the first place cannot be ignored. At the moment, no known therapy will be effective without concomitant correction of ischaemia, treatment of infection, and adequate offloading.^{20,35} To address these issues, a multidisciplinary team is often needed. Still, the stage is set for incorporating in the approach to impaired wound healing the great advances in the basic sciences, embryology, regeneration, tissue engineering, and stem-cell biology. These developments will also take advantage of the encouraging breakthroughs in correcting the metabolic abnormalities of diabetes. There is good reason to believe that the near future will be marked by therapeutic approaches increasingly rooted in scientific advances from the laboratory and the bedside.

Conflict of interest statement

In the past 3 years, I have received honoraria and research grant support from Smith & Nephew, Novartis, Organogenesis, and Johnson & Johnson.

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