REVIEW

Delayed wound healing in diabetes: considering future treatments

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Practice Points

- Impaired wound healing and growth factors:
  - The diabetic foot ulcer displays characteristics of a chronic wound with diminished expression of growth factors integral to healing, such as PDGF, FGF, VEGF, EGF, NGF and GM-CSF.
- Current therapies:
  - The cornerstones of diabetic foot wound care include periodic debridement, adequate treatment of infection, treatment of ischemia, pressure off-loading and moist wound care.
  - When standard wound care fails to heal the diabetic foot ulcer, adjunctive treatment with advanced therapies such as negative pressure wound therapy, topical growth factors, hyperbaric oxygen and living skin equivalents may be necessary.
  - Adjuvant therapies have demonstrated equivocal effectiveness in scientific studies and judicious use of these modalities is encouraged, given their expense.
- Future therapies:
  - Future therapies currently under investigation for the treatment of diabetic foot ulcers include platelet-rich plasma, stem cell therapy, extracorporeal shock-wave therapy, laser therapy and topical lactoferrin.

SUMMARY Diabetic foot ulcers result from multiple risk factors including peripheral neuropathy, arterial insufficiency and foot deformities. Recent investigation has also revealed a chronic wound environment with diminished expression of growth factors and cytokines integral to the wound healing process. Current accepted standard of care for the treatment of diabetic foot ulcerations focuses on periodic debridement of the wound, appropriate topical wound therapy, pressure off-loading and treatment of infection. Owing to increased cost and equivocal effectiveness, topical growth factors, bioengineered living skin equivalents, hyperbaric oxygen therapy and negative pressure wound therapy are proposed as adjuncts to standard of care and may be added to the treatment regimen when healing of the wound has stalled. Other future therapies currently under investigation include stem cell therapy, platelet-rich plasma, extracorporeal shock-wave therapy and laser treatment. These modalities continue to be developed and tested, and may offer promise as effective therapies in the future for the chronic diabetic foot ulcer.

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Diabetic foot ulcerations will affect approximately 15% of all patients with diabetes, are the leading cause of hospitalization among all patients with diabetes, and are the most common risk factor for lower extremity amputation [1,2]. The physical, psychological and economic burden of diabetic foot ulcerations is of paramount concern to the patient, the patient's family and the healthcare system. As a result, great attention has been focused on the cause, treatment and prevention of diabetic foot ulcers in the last decade.

Diabetic foot ulcers are the result of various etiological factors and are characterized by an inability to self-repair in a timely and orderly manner [3]. Etiological factors can be categorized into intrinsic (i.e., neuropathy, peripheral vascular disease and diabetes severity) and extrinsic (i.e., wound infection, callus formation and excessive pressure to the site) causes [4].

Studies have found that the pathway to foot ulceration begins with the presence of three distinct conditions: peripheral neuropathy, foot deformities, and acute or chronic repetitive trauma. The presence of peripheral neuropathy results in an insensate foot with structural deformity vulnerable to trauma. This trauma may be presented in the form of chronic pressures from everyday activity or sudden acute trauma from the environment such as ill-fitting foot wear or stepping on a foreign object [5–7].

In addition to the triad of risk factors described, impaired wound healing, characterized by a chronic wound environment, has been implicated as another reason for poor healing exhibited in diabetic foot ulcers. Recent investigation into impaired wound healing in these chronic wounds has highlighted impairments at the microvascular level as well as abnormal expression of growth factors and other cytokines involved in the healing process.

Impaired wound healing & growth factors

Over the last decade, it has been recognized that diabetes is a disease based fundamentally on inflammation. Both Type 1 and 2 diabetes are characterized by a nonsequential release of pro- and anti-inflammatory cytokines, resulting in an imbalance that leads to impaired tissue repair and weakened cellular and humoral immune defense mechanisms [8,9].

The normal cascade of wound healing involves an orderly transition through three well-defined phases: inflammation, proliferation and remodeling. Normal wound healing involves a timely progression through these three phases, ultimately resulting in wound epithelialization. However, in the chronic wound such as the diabetic foot ulcer, the progression of healing is stalled in the initial inflammation phase and resists further progression. Central to their poor healing, diabetic foot ulcers demonstrate a decreased immune cell infiltration, with persistence of neutrophils and macrophages [10]. This diminishment in inflammatory cell recruitment ultimately results in alterations in growth factor expression **(Table 1)**.

It is well established that growth factors play an integral role in the normal wound healing cascade, and their addition to the chronic wound may serve as a catalyst to healing [11]. Growth factors influence the wound healing process both through inhibitory and stimulatory effect on the local wound environment. A multitude of growth factors are present in wound healing with the most prominent growth factors consisting of: PDGF, FGF, VEGF, EGF, NGF and GM-CSF.

PDGF plays a major role in wound healing, acting as a mitogen on fibroblasts, vascular smooth muscle cells, endothelial cells, neurons and macrophages, and as a chemotactic agent for neutrophils, macrophages and fibroblasts. PDGF also enhances proliferation of fibroblasts,

Table 1. Growth factors and their roles in normal wound healing.

PDGF	Mitogenic on fibroblasts, vascular smooth muscle cells, endothelial cells, neurons and macrophages. Enhances proliferation of fibroblasts, stimulates the production of extracellular matrix by these cells and triggers fibroblasts to acquire a myofibroblast phenotype
FGF	Stimulate angiogenesis, cell proliferation, regulate migration and differentiation of cells of mesodermal, ectodermal and endodermal origin
EGF	Regulates re-epithelialization and granulation tissue formation
VEGF	Major regulator of both vasculogenesis and angiogenesis
GM-CSF	Involved in angiogenesis and is mitogenic for keratinocytes
NGF	Essential for the development and survival of certain sympathetic and sensory neurons in both the CNS and PNS

stimulates the production of extracellular matrix by these cells and triggers fibroblasts to acquire a myofibroblast phenotype [12,13].

FGFs typically stimulate cell proliferation, regulate migration and differentiation of cells of mesodermal, ectodermal and endodermal origin. FGFs are mitogenic for several cell types present at the wound site, including fibroblasts and keratinocytes [14]. Finally, both FGF1 and FGF2 stimulate angiogenesis [15].

EGF plays an important role in re-epithelialization and granulation tissue formation. It has been shown to be mitogenic for fibroblasts and keratinocytes. VEGF has been identified as a major regulator of both vasculogenesis and angiogenesis, and its levels increase during trauma and ischemia [16]. VEGF also serves to stimulate wound angiogenesis in a paracrine manner. NGF is essential for the development and survival of certain sympathetic and sensory neurons in both the CNS and PNS. GM-CSF is involved in angiogenesis and is mitogenic for keratinocytes.

The number of growth factors identified in the normal wound healing process continues to be elucidated, offering further information on how the chronic wound differs from a wound that goes on to heal. Deficiencies in these essential growth factors can potentially diminish granulation tissue formation and maintain chronicity of the wound. Armed with this knowledge, therapies to address these deficiencies can be developed to potentiate healing.

Current therapies

The cornerstones of standard care as advocated in a consensus statement by the American Diabetes Association for diabetic foot ulcers currently consist of the following: periodic debridement, adequate treatment of infection, off-loading pressure, and evaluation and treatment of ischemia [17]. In a pivotal study, Sheehan *et al.* demonstrated that wounds that failed to show a significant decrease in size within 4 weeks of standard treatment ultimately demonstrated less than a 10% chance of being healed by week 12 [18]. As a result, a benchmark was established, where in the absence of at least 50% closure after 4 weeks of standard care, reevaluation with a new treatment approach may be necessary. This new approach typically incorporates adjunct advanced care therapies such as living skin equivalents (LSEs), hyperbaric oxygen therapy (HBOT) or negative pressure wound therapy (NPWT).

Wound debridement

Debridement of the wound incorporates the concept of wound bed preparation by controlling exudate and edema, decreasing bacterial burden, promoting healthy granulation tissue and removing necrotic tissue [19,20]. Over the last decade, the significance of wound bed preparation has evolved, given its multiple roles in wound healing. The role of wound bed preparation has been described as a dynamic concept, involving a balance between aggressive and repeated removal of all necrotic tissue followed by timely evaluation and tissue management [21].

Wound bed preparation can be performed through a variety of methods including sharp debridement, low-frequency ultrasound and enzymatic debriders. Sharp debridement is considered the gold standard as the operator can visually identify the depth and extent of tissue that needs to be removed. It can be performed in the office setting in an insensate foot with a variety of instruments, with the most commonly used being the scalpel blade.

However, when a sensate limb cannot tolerate sharp debridement, other methods of wound bed preparation must be explored. A novel alternative to sharp debridement involves the use of low-frequency (40 kHz), low-intensity (0.1–0.8 W/cm^2) ultrasound energy via atomized saline mist to the wound bed without direct contact to the wound. Benefits of low-frequency ultrasound debridement include reduction of bacterial burden to the wound, reduction in exudate and possible increase in blood flow at the microcirculation level [22].

In a prospective, randomized, double-blinded, sham-controlled multicenter study evaluating the efficacy of low-frequency ultrasound treatment in recalcitrant diabetic foot ulcers, Ennis *et al.* found that ulcers treated with the active 40 kHz ultrasound resulted in a greater proportion of wounds healed compared with sham treatment (40.7 vs 14.3%; p = 0.0366) after 12 weeks of care [22]. In addition to improved healing rates, the ultrasound-treated group demonstrated diminished exudate by week 5 compared with the sham-treated group, suggesting decreased bacterial wound bioburden.

Low-frequency ultrasound debridement has also been advocated for ischemic ulcers. In a prospective, randomized, controlled trial, Kavros *et al.* evaluated the rate of healing in nonhealing lower extremity ulcerations

complicated by critical limb ischemia. After 12 weeks of treatment, greater than 50% wound healing was achieved by 63% of low-frequency ultrasound treated wounds compared with 29% of wounds treated with standard care [23]. Of note, measured TcPO2 values were predictive of wound healing, independent of the treatment group. Those wounds with TcPO2 greater than 20 mmHg demonstrated increased wound healing compared with those wounds with less than 20 mmHG.

Finally, wound debridement can be accomplished with topical enzymatic products when sharp debridement is not possible [24]. In the face of bleeding disorders, ischemia, or significant pain associated with sharp or mechanical debridement, topical enzymatic debridements can loosen and remove adherent fibrotic and necrotic tissue. Although more time consuming than traditional sharp debridement, enzymatic debriding can be an effective method when sharp debridement is contraindicated.

Pressure off-loading

Since diabetic foot ulcers typically result from chronic repetitive trauma on the insensate foot, reducing pressure on the area of a preulcerative lesion or ulcerative lesion is paramount for healing. Various modalities are available to assist in reduction of pressures including total contact casts (TCCs), felted foam dressings, off-loading shoes, orthotics, short-leg walkers and complete nonweight bearing.

Total contact casting has long been considered the gold standard due to superior pressure reduction and wound healing rate [25]. The TCCs involves a well-molded, minimally padded plaster cast that distributes pressures evenly to the limb. Though it is widely accepted to be the best device for off-loading, Wu *et al.* found that only 1.7% of wound centers out of the 895 polled used this technique in everyday practice [26]. The most common factors given for failure to use TCCs in practice included poor patient tolerance, time needed to apply the cast and the cost of materials. In addition, inability to monitor the wound or concern of developing new wounds due to the TCCs may make healthcare providers hesitant to use TCCs in clinical practice.

As an alternative, felted foam dressings help to offload neuropathic ulcers by incorporating a cut-out of the ulcer site on a foam pad that is glued to the patient's foot with rubber cement. The cut-out of the ulcer allows the patient to inspect and apply a daily dressing. These dressings must be kept clean, dry and intact until the practitioner performs a weekly or biweekly change.

In the authors experience, the use of felted foam dressings in combination with a removal cast walker or surgical shoe has been effective in off-loading the foot ulcer to promote healing. This method is well tolerated by the patient, allows for routine inspection of the wound bed, and has minimal adverse effects.

Preventive surgery

After a prolonged conservative treatment course without satisfactory wound healing evident, attention may need to be directed towards surgically off-loading the chronic foot ulcer. This may come in the form of correcting an underlying bony deformity that is exerting pressure on the wound internally or other techniques to address any biomechanical faults. Surgical intervention has been found to be successful in reducing the need to wear cumbersome braces or footwear for deformities that might otherwise be easily corrected [27].

Topical agents

Topical dressings for the treatment of chronic wounds attempt to improve wound healing through a variety of means. These include reducing bacterial burden, maintaining a moist wound environment, delivery of growth factors and mediation of substances that inhibit wound healing.

Reduction of bacterial burden has been shown to be an important component for successful wound healing. While systemic infections should be treated with oral or intravenous antibiotics, topical delivery of antibiotics through dressings has shown a lower incidence of resistance than antibiotics [28]. Some of the most common topical antimicrobials include chlorhexidine, povidone-iodine, peroxide, silver sulfadiazine and silver nitrate.

The use of silver dressings has exploded in the treatment of chronic wounds in the last few years due to claims of reduction of infection and increased rates of healing. These dressings work by releasing ionic silver into the wound when exposed to moisture. In a recent systemic review of the medical literature, analysis of 26 randomized controlled trials found insufficient evidence to establish whether silver-containing

dressings or topical agents promote wound healing or prevent wound infection. However, the authors acknowledge that smaller, poorly designed studies did show the effectiveness of silver dressings in decreasing the risk of infection and improving healing rates [29].

Decellularized collagen dressings deliver quantities of collagen to provide a scaffold for migration of fibroblasts and keratinocytes across the wound surface. These dressings have been shown to increase fibroblast production, increase the deposition of collagen fibers, and help preserve macrophages, fibroblasts and epithelial cells. Furthermore, they may be capable of altering the chronic wound environment by actively modifying activity of growth factors and cytokines, and can protect growth factors from degradation. For example, chronic wounds have demonstrated an overabundance of matrix metalloproteases – enzymes that attack the body's natural collagen. Collagen dressings can bind to the excess matrix metalloproteases and help to promote the growth of natural collagen in the chronic wounds.

The chronic wound environment exhibited by diabetic ulcerations has demonstrated diminished levels of growth factors, thought to result in faulty wound healing. Becaplermin (rhPDGF-BB) remains the only topical growth factor approved by the US FDA for the treatment of lower extremity diabetic ulcers. However, prudent use of growth factors is necessary given a recent black-box warning from the FDA as a result of the increased mortality secondary to malignancy in patients using three or more tubes [101].

Despite considerable variation in the types of topical agents available for the treatment of the diabetic foot ulcer, wound dressings remain a cornerstone of treatment. There currently exists no consensus or consistent evidence on the type of dressing best designed to improve the velocity of wound healing. Instead, dressing selection should be individualized and factor in such variables as wound type, quantity and quality of exudates, periwound skin condition and cost.

■ Living skin equivalents

Living skin equivalents are believed to facilitate wound healing through both filling the wound with extracellular matrix and inducing the expression of growth factors and cytokines that contribute to wound healing. LSEs are bioengineered tissue developed in a lab from neonatal fibroblasts and keratinocytes. They are commercially available to supplement epidermal, dermal or composite (both epidermal and dermal) tissue. The first LSE commercially available was Apligraf® a composite graft, containing both epidermal and dermal components.

Apligraf is a living, bilayered skin substitute formed from newborn foreskin that consists of human fibroblasts impregnated into bovine type 1 collagen. It is indicated in diabetic ulcerations without exposed tendon and bone that have not responded appropriately to standard therapy after 3 weeks treatment. Edmonds performed a randomized, controlled study comparing the efficacy of Apligraft and standard therapy (wet to dry dressings and off-loading) versus standard therapy alone. By 12 weeks, 51.5% of Apligraft-treated ulcers demonstrated complete wound closure versus 26.3% of standard therapy ulcers [30].

Dermagraft® is a cryopreserved human fibroblast-derived dermal substitute composed of fibroblasts and extracellular matrix on a bioabsorbable scaffold. It is manufactured from newborn foreskin tissue where the human fibroblasts are incorporated on the mesh scaffold. It is recommended for use of chronic diabetic foot ulcers over 6-week duration. It may be performed weekly in an outpatient or inpatient setting. Marston *et al.* performed a randomized, controlled, multicenter study with 314 patients to compare the efficacy of Dermagraft versus standard therapy alone [31]. At week 12, 30% patients receiving Dermagraft treatments had complete ulcer closure versus 18.3% of control patients.

TheraSkin® is a bilayered dermal substitute processed from donated human tissue composed of 14 types of human collagen, growth factors and cytokines to help promote wound healing. Recently, Landsman *et al.* published a retrospective review of 188 patients with diabetic foot ulcers [32]. After 12 weeks of treatment, 60.38% of diabetic foot ulcerations had closed, and after 20 weeks, 74.1% of ulcerations had closed with an average of 2.03 TheraSkin allografts required for each patient.

The rampant use of LSEs for the treatment of diabetic foot ulcers has drawn criticism due to concerns of limited improvement and high associated costs. A recent systematic review by Langer and Rogowski attempted to assess the cost of tissue-engineered skin for treating chronic wounds [33]. The authors concluded

that despite high initial costs associated with the use of LSEs, the economic evidence suggests that their use may be cost effective, and in some instances cost saving, if their use is restricted to those ulcers showing poor healing response to standard treatment modalities.

In the authors' experience, LSEs can provide stimulation of healing in the stagnant wound that has been treated with a minimum of 4 weeks of good wound care. While application of the LSE does not result in immediate take with subsequent epithelialization as a traditional skin graft, it may serve as a vehicle for delivery of multiple growth factors mediated by the cellular components of the LSE.

■ Hyperbaric oxygen therapy

Hyperbaric oxygen therapy has been widely advocated in the treatment of recalcitrant diabetic foot ulcerations despite its significant cost. HBOT is postulated to increase oxygen levels in hypoxic wounds, enhancing fibroblast and leukocyte function, downregulating inflammatory cytokines and promoting angiogenesis [34]. Evidence of HBOT effectiveness has been largely anecdotal, although there has been increasing evidence of its effectiveness in the medical literature.

In a double-blinded, randomized, controlled study, Abidia *et al.* [35] showed an enhancement in the healing rates of chronic diabetic foot ulcers with hyperbaric oxygen versus control (air) after 30 sessions of therapy. However, this study was limited as the sample size was small and included only Wagner grade 1 and 2 ulcers. As a result, the authors cautioned that HBOT should be used as an adjunctive treatment and recommended larger clinical trials to examine its clinical efficacy and cost–effectiveness.

In a randomized, single-center, double-blinded, placebo-controlled clinical trial, Londahl *et al.* investigated the effectiveness of HBOT in 94 diabetic foot ulcers [36]. The authors found that 52% of patients with Wagner grade 2, 3 or 4 ulcers were healed at 1 year compared with 29% in the placebo group. To date, this is one of the largest randomized controlled studies with the added benefit of blinding and placebo control, resulting in the elimination of confounders that plagued earlier studies.

While the results of the Londahl study placed HBOT treatment on more solid footing in terms of scientific evidence, the financial burden of HBOT remains high, with the estimated cost of treatment ranging from $15,000 to $40,000 [37]. As a result of the considerable cost, it is unclear what role HBOT should play in the standard of care of the diabetic foot ulcer. In addition, questions regarding those patients who might benefit most, as well as the point in treatment at which HBOT therapy should be initiated, remain unanswered. Further studies to examine these difficult questions may be necessary before routine use of HBOT on all diabetic foot ulcers is considered [37].

■ Negative pressure wound therapy

Negative pressure wound therapy is a noninvasive treatment modality that creates a subatmospheric negative pressure wound environment to assist in creating a moist wound environment, remove waste products, reduce edema and form granulation tissue [38]. The negative suction is produced by placing a wound dressing into the wound, applying a tight suction with an adhesive dressing, and connecting tubing to the electronic machine and canister. Though the range of the pressure setting is at the discretion of the physician, -125 mmHg is the recommended amount as it corresponds to the maximum increase in blood flow at 125 mmHg [39].

Many studies have demonstrated the effectiveness of NPWT in healing diabetic foot ulcers compared with standard therapy. Blume *et al.* conducted a multicenter, randomized controlled trial with 342 patients comparing NPWT to advanced moist wound therapy with alginate or hydrogel dressings [38]. The authors found greater wound closure in ulcers randomized to NPWT treatment and concluded that NPWT is a safe and efficacious modality for improving the healing potential of diabetic foot ulcers.

Armstrong and Lavery also reported favorable findings with NPWT use following partial foot amputations. The authors reported NPWT-treated ulcers healed more frequently, healed at a faster rate, and formed granulation tissue at a more rapid pace compared with the control ulcers [40]. They concluded that NPWT treatment was a safe and effective technique for accelerating the rate of wound closure and had the potential to reduce re-amputations.

One criticism of NPWT is the increased cost associated with its use. In order to examine this criticism, Apelqvist *et al.* conducted a cost analysis of Armstrong's study patients and found

a saving of $12,800 when NPWT was used versus standard care as fewer physician visits and wound care dressings were needed [41]. The NPWT apparatus is easily changed in the outpatient setting, and advances in portability of the unit have improved patient satisfaction and are potentially improving compliance with treatment.

In a recent consensus statement by a multidisciplinary expert panel guidelines were proposed for the appropriate use of NPWT based on best available clinical evidence [42]. The authors warned against use in the presence of ischemia, active cellulitis or osteomyelitis. Regular, aggressive debridement, pressure offloading, as well as concomitant use of active wound care dressings such as acellular matrix scaffolds was encouraged in combination with NPWT.

Future therapies

Despite the advancements in technologies such as bioengineered skin equivalents and the widespread application of standard care in treating diabetic foot ulcers, it has been reported that the incidence of wound healing has remained at less than 50% [43]. This highlights the crucial need for a more practical, safe and effective therapy for nonhealing diabetic foot ulcers **(Table 2)** [43]. The majority of these therapies are currently under investigation and their use has largely been limited to clinical trials.

■ Platelet-rich plasma

Platelet-rich plasma (PRP) has found clinical application in many fields of surgery, including in the treatment of chronic soft tissue ulcers. PRP technology is concentrated blood platelets enriched with plasma and, through the degranulation process, releases growth factors that are postulated to stimulate healing. The FDA-approved method of PRP preparation requires the patient to submit autologous whole blood that then becomes centrifuged.

Driver *et al.* conducted a prospective, randomized, controlled trial of PRP gel versus a control (saline gel) evaluating both safety and efficacy in wound closure [44]. In 72 subjects, the authors found that the group treated with the PRP gel healed in an average of 42.9 days compared with 47.4 days in the saline gel treated group. In addition, complete closure was noted in 68.4% of subjects in the PRP gel group versus 42.9% in the saline gel control group by 12 weeks. However, 32 subjects were excluded from the final data analysis as a result of failure to complete treatment or protocol violations, potentially impacting the final results.

Other positive reports of PRP used for improved wound healing in the diabetic foot are primarily case studies or small pilot studies with limited generalizability regarding the results or specific methods used [45,46]. Saad Setta *et al.* evaluated the efficacy of PRP on chronic diabetic foot ulcers versus platelet-poor plasma (PPP) [47]. The study enrolled 24 subjects who were randomized into either the PRP or the PPP group, and the authors concluded that the PRP-treated group demonstrated significantly faster healing rates compared with the PPP-treated group.

As a result of the controversy and limited evidence for the use of PRP in diabetic foot ulcers, a recent systematic review of the literature found five randomized clinical trials evaluating this condition. A meta-analysis of these five studies concluded that there exists scientific evidence for PRP treatment of diabetic foot ulcers with favorable results. While this meta-analysis deemed PRP treatment beneficial, the significant cost and considerable expertise required for the procedure has prevented its widespread use [48].

Table 2. Future therapies and postulated methods of activity in improving wound healing.

Therapy	Method of activity
Platelet-rich plasma	Enriches blood plasma and, through the degranulation process, releases growth factors that help to stimulate healing
Gene therapy	Increases collagen levels and profoundly increases the production of growth factors
Extracorporeal shock-wave therapy	Increases angiogenesis by releasing vascular growth factors and proinflammatory factors
Laser therapy	Improvement of skin microcirculation through increased reactivity of arterioles
Angiotension II analog	Modulation of growth factors and cytokines
Lactoferrin	Antibacterial activity against biofilm

Stem cell research

All chronic ulcerations will ultimately require coverage in some fashion to return skin to its function of protection. Autologous skin grafting is sometimes an option, but there has been concern of infection and donor site morbidity. Recent investigation into the use of stem cell therapy as a way to obtain skin coverage without the adverse effects of skin grafting has been conducted. The cells can be derived locally with fibroblasts, skin progenitor cells and keratinocytes, or systemically from bone marrow systems [49]. In contrast to a traditional skin grafting, the coverage of skin is not immediate and may require several weeks for graft take.

Numerous rodent-based trials have demonstrated promising results in wound coverage. Kuo *et al.* found that induced diabetic wounds in rats showed increased collagen levels and profound increases in the production of growth factors (TGF-B, KGF, EGF, VEGF and PDGF) needed for wound healing [50]. Gene therapy aims to deliver a single genetically coded growth factor to wound site, which could potentially eliminate the need for repeated applications of growth factors at regular intervals. Stem cell research is proving to be promising, but more research on its *in vivo* application is needed to determine the efficacy on chronic wound healing in humans.

Extracorporeal shock-wave therapy

Extracorporeal shock-wave therapy (ESWT) is a treatment modality that consists of applying shock waves, longitudinal acoustic waves that travel at ultrasonic speed in the water of soft tissue, to the ulcer sites. These waves exert stress on the cells, which is postulated to increase angiogenesis by releasing vascular growth factors and pro-inflammatory factors to stimulate tissue healing. Blood perfusion scans in several animal studies have shown increased expression of VEGF, endothelial nitric oxide synthase and proliferating cell nuclear antigen in rats receiving ESWT compared with the control group [50,51].

In a prospective, randomized, controlled study of 30 patients with diabetic foot ulcers, Moretti *et al.* found that 53.33% of ESWT-treated ulcers healed compared with 33.3% of ulcers in the control group after 20 weeks of treatment [52]. The authors attributed the benefits of wound healing from ESWT to the pro-angiogenesis properties and also reported no observed adverse affects from treatment applied every 72 h. As a result, the authors concluded that ESWT is a useful adjunct in the management of diabetic foot ulcers.

Laser therapy

Low-intensity laser irradiation (LILI) is currently being tested for treatment possibilities in chronic, recalcitrant wounds. Though the mechanism is not fully understood, LILI has been used medically to help treat acne vulgaris, seasonal affective disorder and neonatal jaundice [53]. Rodent-based studies have found increased vascularization, granulation tissue organization, fibroblast migration and thickening of collagen after only 7 days of LILI at 660 nm [54].

Recently, a placebo-controlled, double-blinded study in humans was performed to evaluate the efficacy of broadband light sources (400–800 nm). The treatment group consisting of ten patients with 19 ulcers had a 90% healing rate, whereas the placebo group only had a 33% healing rate [55]. Laser therapy shows promise to be an exciting treatment possibility, but more evidence is needed for it to become a leading modality.

Topical agents

Armed with the knowledge of the molecular environment of the chronic wound, future treatments with topical wound agents have focused on modulation the growth factors and cytokines essential to healing. NorLeu3, an angiotension II analog, has been used to accelerate dermal healing and to reduce scar formation. Findings from a recent randomized, double-blinded, placebo-controlled trial with 170 patients showed that ulcers treated with NorLeu3 were 2.3 times as likely to heal as the ulcers treated with the placebo [56]. With the recently enacted black-box warnings from the FDA on becaplermin, NorLeu3 may prove to be more efficacious with an improved safety profile. However, more testing is needed to examine its potential effectiveness in ulcer healing.

Lactoferrin (LF) is a nonheme monomeric glycoprotein belonging to the transferrin protein family. LF, an essential component of the host innate defense system, is present in exocrine secretions such as tears, saliva, milk and colostrum. *In vitro* assays have shown that LF displays antibacterial activity against Gram positive and Gram negative bacteria, rods and cocci, facultative anaerobes and aerotolerant anaerobes [57].

In a Phase I/II clinical study, Lyons *et al.* examined the use of talactoferrin, a recombinant form of human LF, in the treatment of ulcers in patients with diabetic foot ulcers [58]. The talactoferrin was tested in two strengths (2.5% and 8.5%) with a third group receiving placebo. The groups receiving the 2.5% and 8.5% gels had twice the incidence of ≥75% reduction in ulcer size compared with the placebo group, although clinical significance was not reached.

Owing to the complexity of bacterial infections in diabetic foot ulcers, use of LF with xylitol hydrogel in combination with commercially available silver-based wound dressings has been purported to decrease the bacterial biofilm common to chronic wounds [59]. For both a single species biofilm and a dual species biofilm, the LF/xylitol hydrogel in combination with the silver wound dressing had a statistically significant reduction in biofilm viability relative to the commercially available wound hydrogel.

Conclusion

Diabetic foot ulcers remain a significant problem despite the number of treatment modalities currently available. Currently, standard of care includes periodic debridement, pressure off-loading, treatment of infection and moist wound care. Despite adequate conservative care, many of these ulcers fail to heal in a timely manner. In a meta-analysis performed by Margolis *et al.*, the authors found that less than 31% of neuropathic foot ulcers healed after 20 weeks of good wound care and a healing rate of 24% after 12 weeks of treatment [60]. As a result, future therapies aimed at addressing the deficiencies inherent to these challenging, chronic wounds continue to evolve, in the hope that a safe and reliable treatment course can be found.

Further investigation into the reasons behind the poor healing rate observed in the diabetic foot ulcer has revealed significant alterations in growth factor and cytokine expression. In addition, the wound itself appears to stall in the normal healing continuum, failing to progress past the inflammation stage. As a result, wound therapies such as topical growth factors, NPWT, LSEs, PRP and stem cell research have all attempted to address the altered biochemical milieu of the diabetic foot ulcer.

The medical literature is replete with reports of early successes of these interventions. The optimism of these reports is tempered by their design and methodology, poorly controlled, nonblinded studies with small sample sizes. Furthermore, the cost of these new therapies significantly increases the cost of treatment, currently estimated at $28,000 for each new ulcer episode in the USA [61].

Thus, it may be tempting to discount these studies on the basis of equivocal efficacy or prohibitive cost. However, judicious application of these new therapies when a wound has demonstrated poor healing potential may actually offer cost savings when used at the appropriate time in treatment [61]. A number of consensus statements have taken this approach to expensive, unproven therapies, encouraging their use only when continued evaluation of the wound suggests standard treatment will be unsuccessful [17].

Future perspective

The future treatment of diabetic foot ulcers continues to move at a rapid pace. As we learn more about the pathophysiology behind why diabetic foot ulcers develop and why they fail to heal, technology to address those issues also evolves. Examples of those technologies include HBOT, NPWT and topical growth factors.

However, technologies developed for other areas of medical research also influence how we address chronic diabetic foot ulcers. One particular area of interest is the use of stem cell therapy to test new drugs as well as a source of renewable cells and tissues for application in cell-based therapies. The development of induced pluripotent stem cell technique in 2006 raises the possibility of producing skin cells capable of transplantation with minimal immune rejection from the host [62]. While this type of technology remains in its infancy, it may revolutionize how we treat the diabetic foot and direct future therapies.

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