Wound-healing Abnormalities in Diabetes and New Therapeutic Interventions

a report by

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According to the American Diabetes Association, 7% of the general population and 21% of people over the age of 60 are afflicted with diabetes in the US. One of the most outward and debilitating complications of diabetes is the development of chronic non-healing foot ulcerations, occurring in 15% of diabetics. In its most unfavorable course, diabetic foot ulceration (DFU) leads to amputation in 14–24% of afflicted individuals and is the leading cause of non-traumatic lower-extremity amputation in the US.[1] The national economic burden of DFU and amputation is correspondingly staggering, estimated near US$11 billion in 2001.[2] Traditionally, ischemia, neuropathy, trauma, and infection were considered the culprits of the recurring chronic wound.[3,4] More recently, diabetic impairment of the cutaneous wound-healing process has been recognized as a major contributor to the failure to heal, and wound healing has been appreciated as yet another biological system hindered by the metabolic, vascular, neurological, and inflammatory alterations present in both type 1 and type 2 diabetes.

Wound Healing in Diabetes

Wound healing requires the well-orchestrated integration of the complex biological and molecular events of cell migration, cell proliferation, and extracellular matrix (ECM) deposition.[5] Normal wound healing can be divided into four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. The cell types involved in each phase mediate specific events that culminate in wound closure. The first observations regarding the impact of diabetes on wound healing focused on an impaired leukocyte function related to hyperglycemia.[6,7] However, additional factors common to other chronic wounds, such as decubitus ulcers and venous ulcers, were later found to participate in impaired diabetic wound healing.[8] These abnormalities included the development of pericapillary fibrin cuffs,[9] impaired expression of ECM,[10] aberrant cellular infiltration,[11] insufficient macrophage activation,[12] impaired re-epithelialization, and impaired angiogenesis.[13,14]

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Current Therapies

Diabetic ulcers are classified as ischemic or neuropathic, and treatment relies upon the proper identification of ulcer type.[15] Ischemic ulcers are painful ulcers of the toes that appear in the setting of severe arterial insufficiency with diminished distal pulses. The primary impairment leading to ulceration is large-vessel occlusion, and prompt surgical revascularization is indicated.[16,17] Neuropathic ulcers are painless ulcers that appear on the edges or the sole of the foot in the setting of adequate distal blood flow. These ulcers result from sensory and autonomic neuropathy and current principles of treatment include debridement, pressure off-loading, infection control, judicious wound care, tight glycemic control, and optimization of cholesterol, nutrition, and other lifestyle factors.[15,18] Impaired diabetic wound healing impedes the healing process in both instances, and additional adjunctive measures are often undertaken to assist in ulcer healing.

Bioengineering and Technology

Bioengineering and technological advances have led to the development of artificial skin grafts, matrix wound dressings, and vacuum-assisted closure devices for the promotion of wound healing. Apligraf® (Graftskin; Organogenesis Inc., Canton, Mass.) is an engineered skin consisting of ECM, viable allogenic dermal fibroblasts, epidermal keratinocytes, and a stratum corneum. Studies from our group and others have shown Apligraf is effective in treating diabetic foot ulcers.[19–21] While the precise mechanism of action remains unknown, it is believed Apligraf fills the wound bed with ECM and induces the expression of growth cytokines that stimulate wound healing. A similar product, Dermagraft® (Advanced BioHealing, CA), consists of human neonatal foreskin fibroblast-derived dermal substitute composed of fibroblasts, ECM, and a bioabsorbable scaffold. Dermagraft has been shown to accelerate wound healing in neuropathic and non-neuropathic diabetic ulcers.[22–24] Matrix wound dressings are made of ECM proteins such as collagen. Recent investigations have incorporated growth factors or modulatory peptides, such as fibroblast growth factor or glycyl-histidyl-lysyl (GHL), into these matrices and have shown profound improvements in wound healing in animal studies.[25,26]

Vacuum-assisted closure (VAC) is a technique wherein sub-atmospheric pressure dressings are used to accelerate wound healing. The system uses

medical-grade reticulated polyurethane ether foam dressing with a non-collapsible evacuation tube connected to a sub-atmospheric pressure pump to allow for equal distribution of negative pressure to the entire wound surface in contact with the foam. The optimal sub-atmospheric pressure for wound healing appears to be approximately 125mmHg utilizing an alternating-pressure cycle of five minutes of suction followed by two minutes off-suction. This technique has been shown to optimize blood flow, decrease local tissue edema, and remove excessive fluid from the wound bed. These changes facilitate the removal of bacteria and alter the cytoskeleton of the cells in the wound bed, triggering a cascade of intracellular signals that increases the rate of cell division and subsequent formation of granulation tissue. Although VAC has been examined in multiple wound healing studies, only two randomized trials have been performed with diabetics, both with small numbers of patients. One of these studies demonstrated VAC-improved wound healing through a decrease in wound surface area[27] while the other showed a significant reduction in all dimensions of the wound.[28]

Surgery

Achilles tendon lengthening has been shown to be an effective strategy for promoting diabetic ulcer healing and preventing ulcer recurrence in patients with equinus deformity of the foot and limited dorsiflexion.[29,30] Poor dorsiflexion leads to increased plantar pressures that contribute to ulcer formation by causing painless trauma and skin breakdown in the setting of the insensate neuropathic foot. The percutaneous lengthening procedure can be performed under local anesthesia and consists of three hemisections of the Achilles tendon. Afterwards, the foot is placed in a total-contact cast for six weeks to allow for healing of the primary ulcer. Following ulcer resolution, significant reductions in ulcer recurrence as high as 75% after two years were shown in a randomized controlled trial comparing Achilles tendon lengthening to casting alone.[30] Biomechanical studies have demonstrated the procedure serves to off-load the foot by increasing ankle dorsiflexion with a subsequent decrease in peak forefoot pressures.[31]

Non-surgical Debridement

Maggots were used extensively in the 1930s for the debridement of wounds. Use of maggots declined with the advent of modern antibiotics and surgical debridement techniques, but experienced a resurgence in the 1990s after a study by Sherman and colleagues reaffirmed their utility.[32] The larvae of the blowfly, *Lucilia sericata*, are the most frequently used species for wound treatment as they are found to feed only on necrotic tissue.[33] Different mechanisms of wound healing by maggots have been suggested, including:[34]

- liquefaction of necrotic tissue by secretion of proteolytic enzymes;
- ingestion of necrotic tissue;
- mechanical irrigation of bacteria by the serous exudate produced by the irritating effect of maggots in the wound;
- destruction of bacteria in the alimentary tract of the maggots or by antibacterial secretions;
- alkalinization of the wound as a result of ammonia and calcium carbonate secretion;
- secretion of substances with wound-healing properties such as allantoin and urea; and
- promotion of granulation tissue by mechanical stimulation of viable tissue by the continuous crawling of the larvae.

Maggot therapy has been shown to reduce the time for wound healing in diabetic patients along with reducing the incidence of infection, but is understandably reserved for when other conventional therapies and surgical interventions have failed.[35–37]

Future Therapies

Growth Factors

Growth factors are released from platelets, macrophages, neutrophils, fibroblasts, keratinocytes, and endothelial cells and influence every phase of wound healing. Becaplermin, a recombinant human-platelet-derived growth factor (PDGF), is the only growth factor approved for the treatment of diabetic foot ulcers. PDGF is a major mitogen and acts on cells such as fibroblasts, vascular smooth muscle cells, microvascular endothelial cells, skeletal myoblasts, neurons, macrophages, and platelets/megakaryocytes and is chemotactic for neutrophils and macrophages.[38] Becaplermin is presented in a gel form (Regranex™, Janssen Cilag, High Wycombe, UK) and is currently approved for the treatment of neuropathic ulcers with adequate blood supply. Several clinical trials have validated the use of PDGF for the treatment of diabetic ulcers.[39–43]

Other growth factors currently under development for the promotion of diabetic wound healing include fibroblast growth factor,[44–47] vascular endothelial growth factor (VEGF),[48] granulocyte colony-stimulating factor,[49–51] and hepatocyte growth factor.[52–57] Although many growth factors have shown promising results in laboratory models of wound healing when used as single agents, the translation of these results to human patients has largely been unsuccessful. The complexity of the wound-healing response with multiple interacting cell types and signaling pathways may not be amenable to single-agent therapy. Combining different growth factors or using growth factors in concert with other wound-healing agents to treat chronic wounds may be a key strategy in achieving high rates of wound healing in the clinic.

Neuropeptides

The contributions of peripheral nerves and cutaneous neurobiology to normal wound healing has recently become evident, acting through a bidirectional connection between the nervous and immune systems. Signaling between the central and peripheral nervous systems together with endocrine feedback coalesce to produce complex immunomodulation.[58] This complex interaction is mediated via neuromodulators such as neuropeptides, neurotransmitters, neurotrophins, and neurohormones. These neuromodulators engage specific receptors on cutaneous cell types, including microvascular endothelial cells, keratinocytes, mast cells, fibroblasts, and immune cells, and elicit downstream signaling. In the presence of diabetic neuropathy these signaling pathways become impaired, contributing to chronic wounds and ulcers. The neuropeptides commonly implicated in impaired diabetic wound healing are substance P[59,60] and neuropeptide Y.[61,62] Current research aims to reconstitute the neuropeptide-cytokine signaling axis through neuropeptide supplementation as a future therapy to counteract wound-healing abnormalities specific to diabetes.[63]

Gene Therapy

Gene therapy studies for diabetic wound healing have primarily been limited to animal models and targeted toward the delivery of growth factors and cytokines. Growth factors hold great therapeutic potential for diabetic

wound healing. However, several hurdles complicate growth factor therapy, including the need for large amounts of purified recombinant material, the short half-life of growth factors that are easily degraded by proteases in the wound, and toxicity associated with repetitive doses. One way of overcoming these challenges is to genetically program somatic cells to synthesize and secrete growth factors. Methods commonly employed include genetically modifying autologous cells *in vitro* using replication-deficient viral vectors, liposomes, or naked DNA, and transplanting the modified cells back to the host tissue. Although promising, gene therapy has its own disadvantages, and has not been clinically approved for the treatment of wound healing. Viruses are the carrier of choice in most gene-therapy studies, but present a variety of potential problems to the patient such as toxicity, immune and inflammatory responses, and gene control and targeting issues. In addition, there is always the fear that the viral vector may recover the ability to replicate.

In a diabetic mouse study, PDGF lentiviral vectors were successfully transfected into the regenerating dermis of diabetic wounds. Although re-epithelialization was similar among the groups, there was enhanced angiogenesis and collagen deposition in the lentiviral PDGF group.[64] In a similar study, quantitative analysis showed a 3.5-fold and 2.3-fold increase in collagen content in lentiviral PDGF-treated wounds versus untreated and saline-treated wounds, respectively.[65] In another diabetic mouse study, a plasmid expressing TGF-beta-1 was injected in the wound bed followed by electroporation and electrical stimulation. Results indicated that the electrical and genetic effects worked synergistically to promote wound healing.[66] VEGF-C was overexpressed in a diabetic mouse wound bed using an adenoviral vector showing enhanced angiogenesis and lymphangiogenesis in the wound and significantly accelerated wound healing in comparison to controls.[67] Sonic hedgehog (Shh), a prototypical morphogen known to regulate epithelial-mesenchymal interaction during embryonic development, has been shown to induce angiogenesis and accelerate repair of ischemic myocardium and skeletal muscle. In diabetic mice, topical gene therapy with naked DNA encoding Shh resulted in significant local gene expression and acceleration of wound recovery with increased wound vascularity. *In vitro*, Shh promoted production of angiogenic cytokines from fibroblasts as well as proliferation of dermal fibroblasts. Furthermore, Shh directly promoted endothelial progenitor cell proliferation, migration, adhesion, and tube formation.[47]

Cell-based Therapies

Cell therapy is emerging as an exciting modality for promoting wound healing. Numerous studies have investigated the potential of stem cells, keratinocytes, and fibroblasts for the treatment of chronic wounds. However, few studies have explored these strategies in diabetics, and to date only fibroblasts have been used for the treatment of diabetic ulcers. Autologous cells limit the concern of immune rejection and therefore could be more amenable for therapy. However, a difficulty with the use of autologous fibroblasts is that fibroblasts from diabetic patients demonstrate altered phenotypes, including reduced fibroblast proliferation and response to growth factors.[10,68–72] In a study by Cavallini and colleagues, 10 diabetic patients with large leg ulcers had better wound healing outcomes when treated with autologous fibroblasts.[73] A second study using fresh allogenic fibroblasts on diabetic wounds showed similar results.[74]

Use of stem cells in diabetic wound healing has been evaluated only in animal models, but with some early promising results. Mouse diabetic wounds injected with CD34+ cells healed rapidly with a dramatic acceleration in revascularization.[75] Bone-marrow-derived endothelial progenitor cells (EPCs) are an attractive stem cell candidate as they are important for angiogenesis and revascularization of wounds.[76,77] However, similarly to fibroblasts, human EPCs from type II diabetics have been shown to exhibit impaired proliferation, adhesion, and incorporation into vascular structures; therefore, autologous EPCs could be of limited use.[78,79]

Discussion

Therapeutic interventions to combat chronic diabetic wounds are evolving, but nevertheless the problem persists. It should be underscored that the etiology of chronic diabetic wound formation is multifactorial, and hence single-agent treatment modalities could be doomed to fail. To address this problem, cell-based therapies are being designed to produce numerous growth factors and ECM proteins simultaneously. Use of stem cells and fibroblasts that can express a host of favorable wound modulators is being explored aggressively. Here the challenge is to engineer these cells with a phenotype that can sustain the hostile wound environment. Artificial skin grafts such as Dermagraft and Aplifgraf are promising, but remain second-line therapies. These skin grafts are expensive and limited by problems with graft 'take.' Of the current therapies, wound debridement and VAC have shown the most success. VAC not only helps in clearing infection by absorbing wound exudates, but also mechanically stimulates and delivers nutrients and oxygen to cells involved in wound healing stimulating proliferation. Compared with surgical debridement, maggot debridement has been shown to be more effective because maggots release digestive enzymes and selectively debride necrotic tissue. Ultimately, development of a multimodality treatment plan that is fast acting and ensures rapid and complete healing is the Holy Grail for the management of chronic non-healing diabetic wounds. ■

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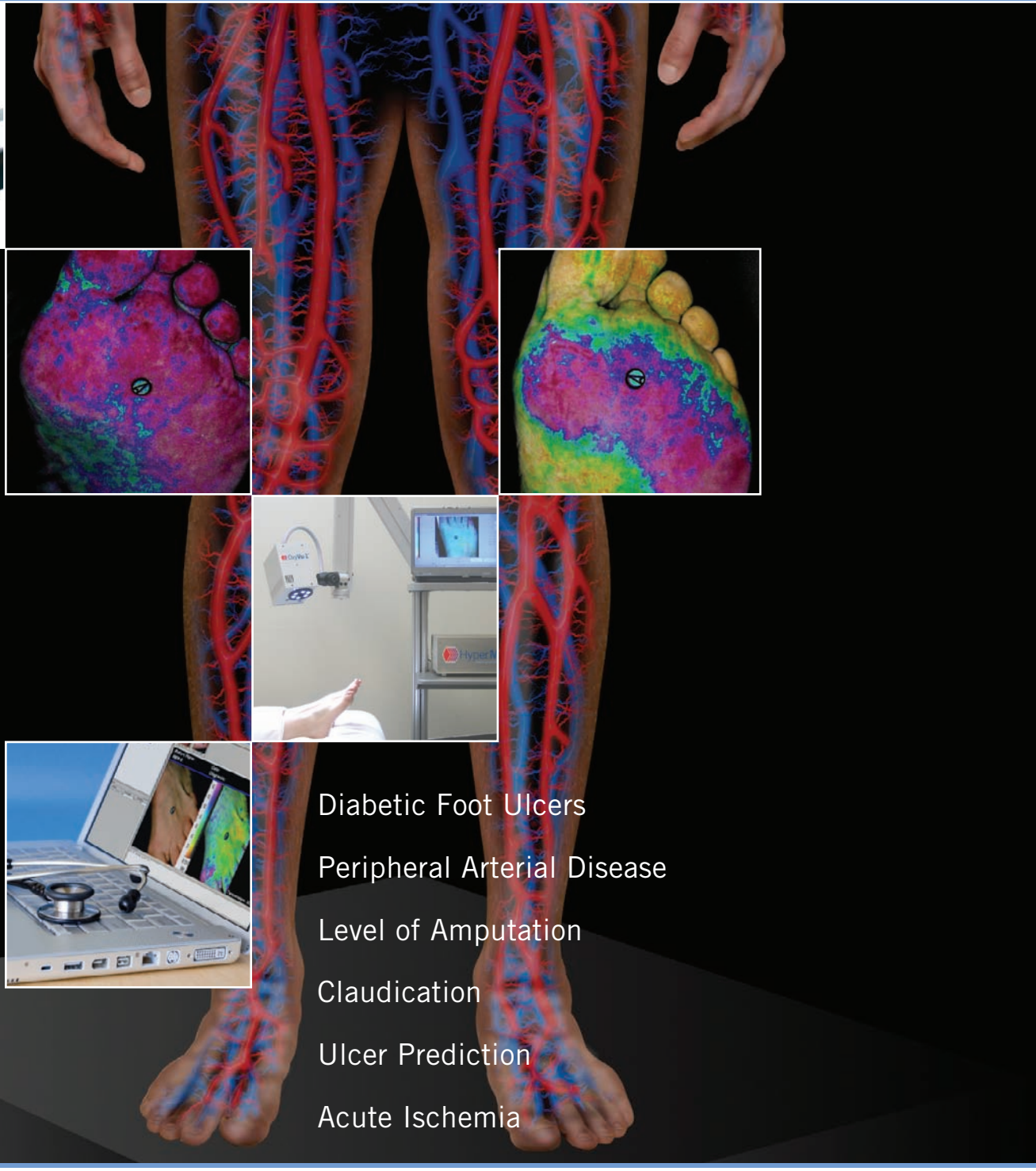

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