



Diabetic Foot Ulcer and Treatment: A Review of Progress and Future Prospects

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ABSTRACT

Chronic non-healing ulcers are a significant medical problem and the incidence of these wounds is expected to increase as the United States population ages. It was projected that approximately 1,400,000 diabetics in this country alone would suffer from Diabetic foot ulcer (DFU) in 2015. The three major challenges in the medical management of DFU are 1) reduction of microbial infection both directly and through enhancement of a productive immune response, 2) restoration of a constructive wound healing microenvironment, and 3) induction of sufficient revascularization. A recent European study showed that approximately 28% of patients with infected DFU required amputations. Although the data are challenging to interpret due to the wide range of disease severities included in the analyses, standard therapies only cure approximately 30% of DFU after 20 weeks and at best advanced modality therapies achieve ~56% healing at 12 weeks. The increasing prevalence of chronic non-healing ulcers poses significant clinical challenges to wound care, often requiring the use of potent antibiotics with undesirable side effects on wound healing. However, no current product addresses both infection and closure of chronic non-healing ulcers. There is an unmet medical need for alternative products assessed by randomized, controlled trials with well-defined and controlled manufacturing processes for the treatment of chronic cutaneous ulcers. The present review emphasizes on development of the next generation of therapeutic skin substitutes which promote wound closure.

Keywords: *Ulcers, treatment, diabetic, biological skin substitute*

INTRODUCTION

Chronic non-healing ulcers are a significant medical problem and the incidence of these wounds is expected to increase as the United States population ages [1]. Each year 2-3% of patients with diabetes will develop a foot ulcer and 15-25% will develop a foot ulcer at least once in their lifetime [2,3,4,5]. It was projected that approximately 1,400,000 diabetics in this country alone would suffer from DFU in 2015. Moreover, a DFU on the heel is associated with significantly longer healing times [6]. A recent study showed that approximately 28% of patients with infected DFU required amputations [7]. This equates to a lower limb amputation due to complications of diabetes approximately every 20 seconds. Although the data are challenging to interpret due to the wide range of disease severities included in the analyses, standard therapies only cure approximately 30% of DFU after 20 weeks and at best advanced modality therapies achieve ~56% healing at 12 weeks [8,9,10]. 85% of lower limb amputations in diabetics are due to an initial DFU [11,12]. Approximately half of all diabetic amputees will not survive 5 years, a rate comparable or worse than most malignancies [13,14]. Unmet needs in the management of DFU include stimulation of reepithelialization and neovascularization, while also reducing the bacterial bioburden in the wound, each of which are required for efficient wound closure.

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no current product addresses both infection and closure of chronic non-healing ulcers. This review focuses on current advancements, pain care management and development of the next generation of therapeutic skin substitutes which promote wound closure.

Background

Keratinocytes in Skin Function

In native skin, the epidermis is attached to the dermis via a thick basement membrane which is produced by the basal keratinocytes [15]. As basal keratinocytes divide, some undergo differentiation during which specific proteins and lipids needed to generate an epidermal permeability barrier are produced [15].

The barrier function of skin is dependent on the differentiation of keratinocytes to generate mature squames (flattened cells). This process includes the assembly of highly cross-linked proteins into a cornified envelope beneath the plasma membrane, secretion of lipids into the intercellular space, and finally keratinocyte enucleation [15]. Cells gradually die and the squames are then sloughed off by friction, cleaning, and other minor trauma. Some pathogens that attempt to invade through the skin are thus captured in and among dying cells that are subsequently shed. Additionally, the surface of the skin is populated by cutaneous microbiota many of which produce antibacterial peptides, bacteriocins, bacteriocin-like compounds, or antifungal products that are thought to limit colonization by other microbes [16]. Along with nutrient competition, the active prevention of colonization by other, potentially more pathogenic microbes has led to the theory that some members of the skin microbiota are mutualists that benefit the host even though some exhibit pathogenicity upon access to sub-epidermal tissue.

As keratinocytes differentiate, they produce host defense peptides (HDP) which are a critical component of innate defenses against wound infection. These antimicrobial peptides act locally within the epidermal and stromal tissues of skin and protect against a broad range of bacteria, fungi, and viruses, including skin flora that may act as pathogens after disruption of the epithelial barrier [17]. During injury, keratinocytes are also a rich source of chemotactic and growth factors that are crucial to the orchestration of the immune response and ultimate wound healing.

Host Defense Peptides

The epidermis serves as the first line of defense against microbial infection. Upon epidermal compromise, the cellular innate immune response works to prevent invasion of microorganisms, employing macrophage and neutrophil-mediated phagocytosis and killing through the production of reactive oxygen intermediates. Microbial infection triggers keratinocytes to increase production of potent host defense peptides (HDPs). More proximally however, keratinocytes in the epidermis produce HDP which act locally within the epidermal and stromal tissues of skin. Human cathelicidin is a multifunctional HDP possessing antimicrobial activity against a broad range of bacteria, fungi, and viruses, is a critical component of innate defenses against wound infection by enhancing leukocyte recruitment and activation, [18,19,20,21,22,23] and has been reported to promote angiogenesis [24,25,26].

Additionally, cathelicidin has been shown to promote healing by inducing neovascularization and reepithelialization at sites of skin tissue injury [24,27,28]. Though abundant in acute injuries, expression of cathelicidin is reduced in chronic cutaneous wounds.

Impaired Innate Defenses in Diabetic Foot Ulcers

In the context of DFU, portions of the innate immune system break down. Notably, there is a reduction in the amount of detectable cathelicidin in chronic wounds [27]. Additionally, neovascularization associated with wound healing is impaired in these chronic ulcers [24]. The standard of care for chronic, infected, non-healing diabetic wounds is debridement of infected and nonviable tissue followed by antibiotic treatment until infection is no longer clinically significant. Maintenance of wound hydration is also important to promote wound closure. Because commonly encountered bacterial strains can develop antibiotic resistance, especially in the chronic wound, the clinician is often limited to more potent antibiotics which can have deleterious effects on the viability and migration of keratinocytes. As a result, the need to use these antibiotics actually prolongs wound healing [29,30]. This, coupled with the growing concern over emerging new multidrug-resistant strains of bacteria, underscores the need for innovative approaches to supplement antibiotic treatment regimens used in open wound therapy.

Overview of Medical Management of Diabetic Foot Ulcers

The current management of DFU includes initial wound assessment, restoration of blood flow as needed, identification and treatment of infection, cleansing and debridement of devitalized tissue, dressing selection, pressure relieving strategies, and diabetes control. Debridement is critical to the healing of DFU by promoting granulation tissue formation [31,32,33]. The gold standard is the “sharp” method, involving the removal of callus, necrotic, nonviable and/or infected tissue by scalpel, scissors and/or forceps, thus exposing a healthy, bleeding ulcer bed while improving drainage.

Topical antibacterial products are often used in conjunction with dressings, as bacterial colonization of DFU is common and can impair wound healing [34,35]. Metronidazole, Neomycin, Gentamycin, Mupirocin and silver-impregnated dressings are examples of topical antimicrobial products used to treat, prevent, or control infection in the treatment of DFU [34]. Wound location, wound surface and peri-wound skin, amount of exudate, and compatibility with other therapy such as off-loading devices must be considered when determining which dressing to use [36]. It has been shown that maintaining a moist wound environment promotes the healing of chronic wounds by inducing proliferation of keratinocytes and fibroblasts and enhancing collagen synthesis [37]. Hydrogel dressings, hydrocolloid dressings, polyurethane foam, alginate dressings, and honey-impregnated dressings are among the many varieties commonly used to promote a moist wound environment and contribute other functions such as absorption of exudates [34,37]. These dressings are used in conjunction with appropriate off-loading methods to relieve pressure from the wound and improve healing time [9].

Negative-pressure wound therapy or vacuum-assisted closure is a popular device-based adjuvant which includes the use of a pump to apply an intermittent or continuous sub-atmospheric pressure to a debrided DFU. It has been shown to increase blood flow, remove exudate and bacteria, and potentially improve the rate of healing [38]. Challenges with this therapy include periwound maceration, increased incidence of candidiasis, and the requirement for prolonged connection to the device which greatly limits ambulation and negatively impacts patient compliance [39].

Summary of Current FDA-cleared, -approved, or -regulated Products

Currently, standard of care therapies are able to cure only ~30% of DFU after 20 weeks [8]. The addition of advanced therapies in the treatment of non-healing DFU has shown significant advantage beyond traditional treatment alone [40]. Options include topical therapies, devices, systemic therapies, as well as bioengineered skin tissue substitutes.

None of the current, commercially available, FDA-cleared, -approved, or regulated products for the treatment of chronic wounds, including CellerateRx®, Integra®, PriMatrix®, AlloDerm®, Graftjacket®, and EpiFix®, have achieved sufficient efficacy to be the therapy of choice. CellerateRx (Wound Management Technologies, TX) is comprised of hydrolyzed bovine collagen alone, whereas Integra (Integra, NJ) is a mixture of bovine collagen and shark cartilage glycosaminoglycan, and includes a silicone membrane covering. PriMatrix (TEI Biosciences, MA) is an acellular matrix created from fetal bovine dermis. PriMatrix Ag is a derivative of PriMatrix that incorporates silver ions to reduce bacterial colonization of the dermal substitute. This is the only marketed product enhanced to address bacterial colonization, a known impediment to the healing of chronic wounds. With each of these products, there is concern regarding the transmission of bovine spongiform encephalopathy (BSE). AlloDerm and Graftjacket (Acelity, TX) are made from decellularized human cadaveric skin tissues. These products bear a significant risk of disease transmission in addition to variable product performance since each product lot is derived from a different cadaveric tissue donor. As a result of harvesting and limited testing, this type of allogeneic product has been associated with safety concerns including disease transmission and manufacture recalls due to suspect sterility results and donor screening procedures [41,42,43].

EpiFix Amniotic Membrane Allograft (MiMedx, GA) is a composite tissue of human amniotic membrane and underlying chorion that has been processed to retain an intact extracellular matrix and is then dehydrated and sterilized [44]. This product does not contain viable cells. In a multicenter trial evaluating treatment of DFU, healing promoted by EpiFix was more rapid than either standard of care or a bioengineered skin substitute (Apligraf®) [45]. Challenges of this product include the need for regular sourcing of human tissue which introduces variability in product performance and necessitates adventitious agent testing for each new lot. The use of EpiFix is contraindicated on infected wounds.

In contrast to products based on acellular or dehydrated dermal analogs, Dermagraft® (Organogenesis, MA) and Grafix® (Osiris, MD) contain living human cells. First generation cultured skin substitutes have shown efficacy as second-line therapies in the treatment of non-infected chronic wounds and their use for these indications is gaining widespread acceptance [46,47]. Dermagraft contains human foreskin fibroblasts within a polyglactin mesh scaffold. Dermagraft is approved for use as a second-line therapy in chronic DFU and contraindicated for use in infected wounds. Grafix is derived from human placental membranes and although it showed promising clinical results in patients with DFU, [48] the FDA recently reviewed this product and determined it does not meet all of the criteria in 21 CFR 1271.10(a)(4)(ii) because Grafix is dependent upon the metabolic activity of living cells for its primary function, and is not intended for autologous use [49]. Therefore, Grafix is not solely regulated as a human cellular and tissue-based product under section 361 of the Public Health Service Act and 21 CFR Part 1271. As a result, Grafix now requires a valid biologics license to be in effect prior to making claims of promotion of healing [48]. Currently, Grafix has an IND application but not an approved biologics license application.

Bioengineered skin substitutes composed of human keratinocytes grown on dermal analogs containing living human fibroblasts reproduce many of the structural and biological features of intact human skin. First generation cultured skin substitutes have shown efficacy as second-line therapies in the treatment of non-infected chronic wounds and their use for these indications is gaining widespread acceptance [47,50]. Apligraf® (Organogenesis, MA), indicated as a second-line treatment for chronic skin wounds, is the only currently-available skin substitute comprised of both dermal and epidermal components that is approved for use in the United States. Challenges of this product include the need for periodic sourcing of cells from human tissue, which introduces variability in product performance and necessitates adventitious agent testing for each new bank. Furthermore, infection was a major AE in clinical trials on venous stasis ulcers; 29.6% of patients receiving Apligraf had a suspected wound infection versus 14.0% in the control [51]. Importantly, because the shelf life of Apligraf is only 15 days [52], it is released for clinical use prior to the completion of sterility tests on the final product.

Currently marketed skin substitutes, composed of viable or nonviable biomaterials, have been designed to

replace or compensate for nonfunctioning skin. Most do not address the major challenges in the management of diabetic skin wounds: 1) productive reepithelialization, 2) sufficient re-vascularization, and 3) reduction of microbial infection. In fact, some of these marketed products have been associated with higher rates of infection and all are contraindicated for the treatment of infected wounds.

Conclusion and Future prospects

The advances and regulatory challenges experienced with tissue-engineered products have been the subject of several reviews [53,54]. Due to the limitations of the first- and second-line therapies discussed above, there is significant medical need for the development of innovative, second generation therapeutic skin substitutes that reduce bacterial bioburden, stimulate wound reepithelialization, promote vascularization, and reduce time to wound closure for use in the treatment of diabetic skin ulcers. The identification of NIKS® cells as a continuous, genetically uniform source of human keratinocytes that can be genetically modified by stable integration of non-viral DNA expression fragments presented the opportunity to create optimized cell-based human skin substitutes with improved wound-healing properties relative to current products and therapies.

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