Preliminary Clinical Evaluation Using A Novel Bioengineered Wound Product to Treat Lower Extremity Ulcers

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Abstract

Diabetes mellitus affects hundreds of millions of people worldwide, each of which have up to a 25% risk of developing a diabetic foot ulcer (DFU) during their lifetime. With poor DFU healing rates using standard of care, advanced treatments are introduced to attempt to close the wound. The objective of this preliminary clinical evaluation was to evaluate lower extremity ulcers treated with a novel bioengineered wound product (BWP). The BWP, a solid absorbable and conformable sheet composed of gelatin, Manuka honey, and hydroxyapatite, was applied on 12 patients with lower extremity ulcers. The patients in this evaluation spanned across 4 sites and had complicated medical histories, including little to no progression of healing with standard of care or treatment with other biomaterials. The ulcers were treated with debridement, BWP placement, dressing, appropriate compression, and offloading as necessary. Weekly follow-up visits were recommended for evaluation, debridement, and BWP reapplication. Nine patients had the BWP applied to aid in full closure. These patients achieved 100% closure within 8 weeks, with a mean closure time of 4.1 weeks. At 4 weeks, the mean percent wound closure was 94%. Three patients had the BWP applied to aid in achieving a healthy wound bed for continued treatment (eg, splitthickness skin graft) and to cover (epithelialization over) an exposed tendon. In all 12 cases, no treatment site infections were observed. The results and observations from this preliminary clinical evaluation suggest that the BWP supports rapid wound closure, a predictor of complete healing for DFUs.

Keywords

diabetic foot ulcers, chronic wounds, contour tracing, photography

Background

Diabetes mellitus has become a global epidemic, with approximately 422 million people affected worldwide, including 29 million people in the US.[1, 2] Patients with diabetes have up to a 25% risk of developing a diabetic foot ulcer (DFU) during their lifetime.[3] Currently, the standard of care (SOC) for initial treatment of DFUs is debridement, offloading, glycemic control and appropriate antimicrobial management and/or imaging when needed.[4] A meta-analysis of patients studied in controlled trials demonstrated, on average, healing rates of 31% at 20 weeks with SOC.[5] In cases in which a wound fails to decrease in size by 50% within four weeks with SOC, advanced levels of care are initiated to attempt to close the wound.[6] In this 12-patient preliminary clinical evaluation across four sites, an absorbable novel bioengineered wound product (BWP), a synthesis of gelatin (a highly purified collagen derivative), Manuka honey, and hydroxyapatite was evaluated for the management of lower extremity ulcers, mainly DFUs.

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Methods

This clinical evaluation assessed the use of a BWP (APIS®, SweetBio, Inc., Memphis, TN) per its Instruction for Use along with standard of care on patients with lower extremity chronic wounds. As this evaluation was part of a standard office visit, there were no selection criteria for patients who would have the BWP applied to their wound. However, prior to treatment with the BWP, wounds in all patients showed little to no progression after four weeks of SOC treatment and/or treatment with other products. This evaluation was retrospectively conducted, de-identified data was used, and written informed consent was provided by each subject of the case to publish the case details and associated images. The primary evaluation parameter was wound closure (complete epithelialization with no drainage) over time, with secondary endpoints such as physician observations of the wound condition (e.g. site infections and product related complications).

All patients were treated with the widely accepted approach of initial debridement and placement of product followed by a secondary dressing (non-adhering), gauze, compression bandage, and recommended offloading. The BWP was prepared per its instructions for use (hydration in sterile saline for up to two minutes) and placed directly on the debrided wound (Figure 1). Maximum contact of the BWP to the wound was achieved via bolster dressings and compression wrapping. Weekly follow-up visits were recommended for evaluation, debridement (if necessary), and BWP reapplication.

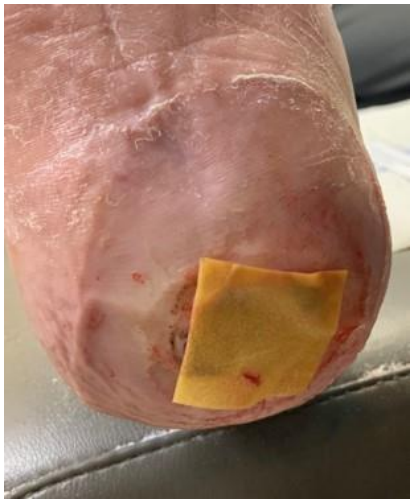

Figure 1. Hydrated BWP applied directly to a debrided DFU.

The initial visit (Day 0) and follow-up visits included a photograph of the ulcer site such that measurements of the wound could be obtained at each visit. All photographs were analyzed via ImageJ (National Institutes of Health, Bethesda, MD, USA) for wound surface area over time by setting a scale based off a known sized object (e.g. ruler) in the image and tracing the outer edges of the wound. Wound closure was defined as complete epithelialization with no drainage. Percent wound closure was determined and graphed by analyzing the above-mentioned calculated surface area at each follow-up visit compared to the original size of the wound. For weeks where the patient did not have a follow-up visit (e.g. Visit 1 Day 0 and Visit 2 Week 3), linearity of the trend of the wound size was assumed between the visits. This assumption was only used for graphical representation of wound closure over time and did not affect the endpoint of the evaluation (time to closure).

Results

In this preliminary clinical evaluation, a total of 12 patients had the BWP applied to their chronic wound along with standard of care. Patient demographics are presented in Table 1. There was a variety of comorbidities amongst the group, including Type 2 diabetes mellitus. Of the 12 patients where the BWP was used, nine patients had the BWP applied with the intention to aid in the closure of the wound, two patients had the BWP applied with the goal of aiding in the formation of granulated tissue for continued treatment (e.g. with split thickness skin graft), and one patient had the BWP applied with the purpose of aiding in the coverage of (epithelialization over) an exposed tendon. Figures 2 – 4 present the actual measured wound size over time (Figure 2), the distribution of patient's time to closure (Figure 3), and photos of the ulcers managed with the BWP intended for closure (Figure 4). Photos of the other three cases where the BWP was used to prepare a granulated wound bed or to achieve epithelialization over a tendon (herein referenced as notable cases) are presented in Figure 5.

The nine patients managed with the BWP intended for closure had a mean wound closure time of 4.1 weeks with a mean of 3.3 BWP reapplications. The shortest closure time was 2 weeks (original wound size 2.9cm^2)

and the longest closure time was 8 weeks (original wound size 4.3cm^2). At four weeks, the mean percent wound closure was 94%.

Table 1. Patient Demographics

Demographic	Value
Age (years)	
Mean ± SD	63 ± 11
Range	46 - 81
Gender, n (%)	
Male	10 (83)
Female	2 (17)
Diabetic, n (%)	11 (92)
Original ulcer size (cm^2), mean ± SD	2.8 ± 2.2
Largest	8.7
Smallest	0.5
Ulcer location, n (%)	
Plantar	8 (67)
Lateral	3 (25)
Dorsal	1 (8)

SD, standard deviation

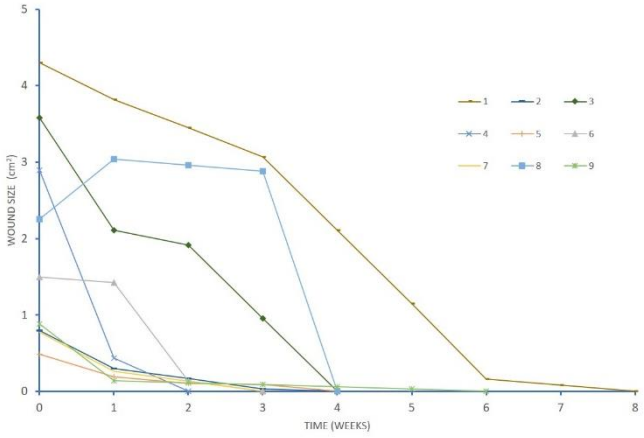

Figure 2. Wound size (cm^2) over time for the nine patients treated with BWP with the intent for closure.

Wound photos from four patients treated with the BWP for closure are presented in Figure 4. Wound location for all four patients varied. Original wound size (ranging from 0.8 – 4.3 cm^2) and visual closure over time are also presented in Figure 4.

Wound photos where the BWP was used to aid in building a granulated wound bed for continued treatment (two patients) and where the BWP was used to aid in the epithelialization over an exposed tendon (one patient), are presented in Figure 5. All wounds were located on the plantar surface of the foot and sizes were 8.5, 3.4, and 3.7 cm^2, respectively. For one patient, the BWP was reapplied every three to four days for two weeks to achieve a healthy wound bed in preparation for a split thickness skin graft.

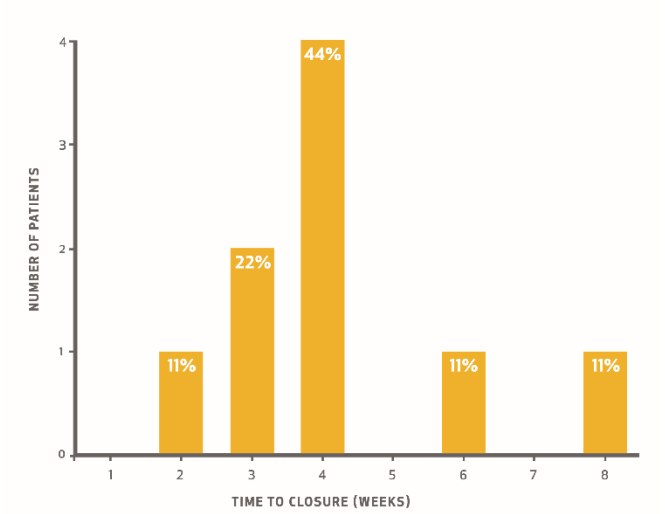

Figure 3. Number of patients with closed wounds over time. Percentages (out of nine patients) are presented at the top of each bar.

Discussion

Since lower extremity ulcers, particularly diabetic foot ulcers, are difficult to heal, there remains a need for effective wound management strategies. Once a wound becomes chronic, the changes in the environment of the wound bed make healing more challenging. Several methods of altering the wound bed, such as debridement, effective bioburden management, appropriate fluid management, and protease management, have been shown to facilitate healing.[7-10] Debridement prior to wound care product application is necessary to remove necrotic tissue and to stimulate healing.[7]

The BWP is a novel bioengineered wound product comprised of porcine-derived gelatin, Manuka honey, and hydroxyapatite, formulated into a solid sheet that is absorbable. The literature has reported an association between the BWP's ingoing materials and wound closure. Gelatin is a natural protein matrix exhibiting high biocompatibility, good biodegradability, high water absorption, and can accelerate the process of granulation and epithelialization.[11-13] Additionally, gelatin can serve as a buffer/sacrificial substrate to

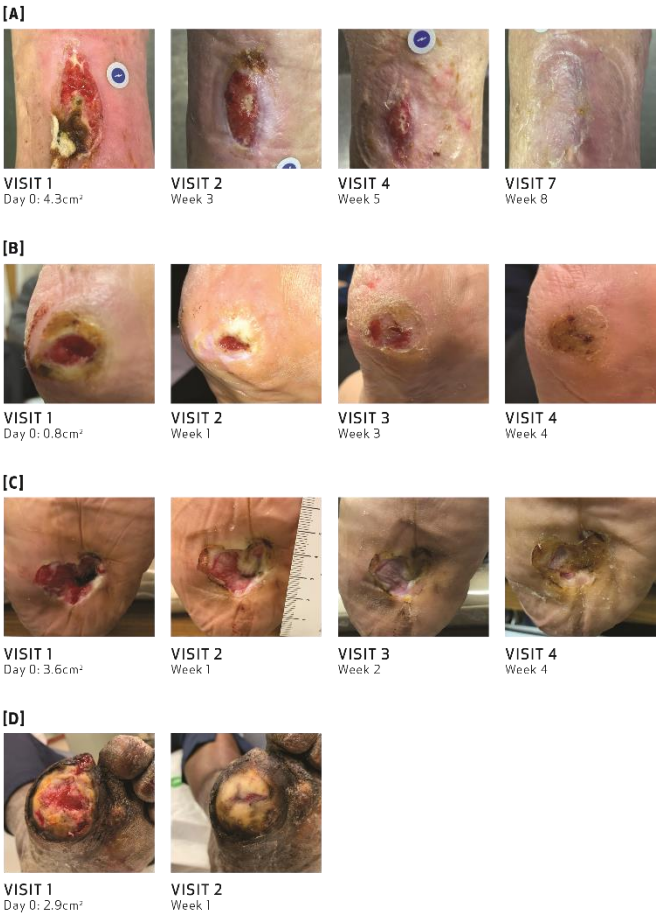

Figure 4. Photos of wound closure over time from four patients. All four patients received reapplication of the BWP at each follow-up visit. **[A]** A non-diabetic 73-year-old female with a non-pressure ulcer on the right medial foot. **[B]** A 63-year-old male with a DFU located on plantar surface of left foot. **[C]** A 60-year-old male with a DFU on the heel of the left foot. **[D]** A 46-year-old male with a DFU on the left foot.

reduce protease levels such as matrix metalloproteinases (MMP) MMP-2 and MMP-9 in the chronic wound environment.[14-16] Manuka honey can absorb and hold moisture from the environment and due to its high osmolarity, high viscosity, and low pH, has been shown to inhibit microbial growth, increase fibroblast activity and have anti-inflammatory effects.[17-22] Hydroxyapatite (calcium phosphate-based) particles provide structural reinforcement for the BWP and have been shown to promote wound healing via re-epithelialization, matrix formation, angiogenesis, and recruitment of macrophages and fibroblasts.[23-28]

This preliminary clinical evaluation summarizes the experiences of five physicians across four sites who evaluated a BWP for the management of lower extremity ulcers. This initial use of the BWP in the management of lower extremity ulcers is associated with a high incidence of wound closure and short closure times, with no cases of infection. Such facilitation of wound closure is crucial, as percent area reduction greater than 50% within four weeks has been proposed as predictors of complete healing by 12 weeks for DFUs.[6, 29] As this was a preliminary clinical evaluation of the standard application of a commercially available BWP, no specific patient selection criteria was used. However, prior to treatment with the BWP, wounds in all patients showed little to no progression after four weeks of SOC treatment and/or treatment with other products. Patient demographics are displayed in Table 1. All patients received comparable standard of care treatment with the BWP including initial debridement and recommended (weekly) follow-ups with BWP reapplications and secondary dressing changes. Considering the variety in patient demographics, this regime resulted in a mean wound closure time of 4.1 weeks, a mean BWP application of 3.3 times, and no need for site infection treatment (no infections incurred). At four weeks, the mean percent wound closure was greater than 50% (94%), a predictor of complete healing for DFUs.[6, 29]

Prior to application of the BWP, the majority of the patients had previously been treated for weeks to months with collagen products, Manuka honey gels/pastes, and/or offloading with little to no progress. Upon application of the BWP, rapid closure of the wound was observed. In addition, one patient was facing potential amputation if the ulcer was not closed. This patient had a history of non-insulin-dependent diabetes mellitus, obesity, peripheral edema, peripheral vascular disease, profound neuropathy, and chronic ulceration. Previously, the patient had exhaustive care including glycemic control, infection management, pressure relief (including total contact casting), and various wound care products and off-loading devices. The patient was recommended amputation and first ray resection as a definitive treatment, but having had a contralateral partial foot amputation, opted for continued wound care and the application of the BWP. This patient's wound was closed by Week 3 with weekly reapplications of the BWP and thus amputation was no longer needed. One patient presented a larger wound size at Weeks 1-3 compared to the initial BWP placement visit (see Figure 2). This wound was a non -

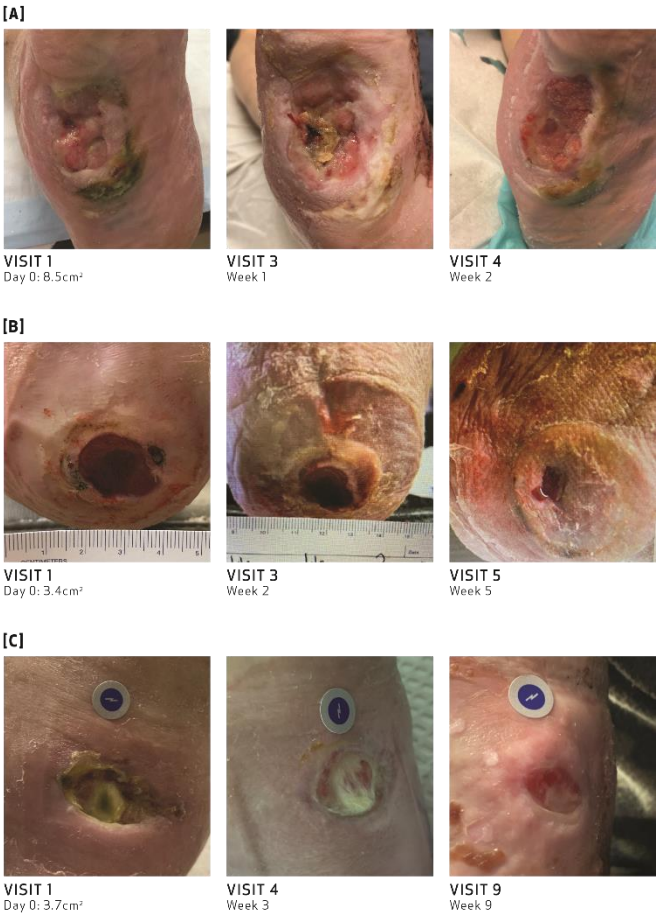

Figure 5. Notable cases. **[A]** A 70-year-old male with a DFU on plantar surface of the right foot. **[B] A** 46-year-old male with a DFU on the heel of the right foot. **[C]** A 63-year-old male with a mixed etiology ulcer on the right posterior ankle with exposed tendon.

pressure ulcer of the left lateral leg that was previously treated with collagen, foam, and covered with an Unna boot with no progression of healing. Between Weeks 1-3, the physician noted that the wound bed was eschar covered with scant drainage. The ulcer was debrided and BWP applied at each follow-up visit and by Week 4 the wound was closed.

67% of the ulcers in this evaluation were located on the plantar surface of the foot. Wounds at this anatomical location are difficult to heal because of load and shear forces which have been linked to the development and poor healing of DFUs.[30] Of the plantar wounds where the BWP was used to achieve closure, the mean closure time was 3.9 weeks.

One patient was a unique case as the BWP was used with the goal of achieving epithelialization over an exposed tendon. This patient is a 63-year-old male smoker with a history of Type 2 diabetes, diabetic neuropathy, obesity, depression, hypertension, cirrhosis, and venous insufficiency. At the initial visit, wound size was 3.7 cm^2, wound edges rolled, tendon was exposed, and slough and necrotic tissue was present in the wound bed (Figure 5C). This mix-etiology ulcer was debrided and the BWP applied and covered with foam border with multi-layer compression. After two reapplications of the BWP over three weeks, the tendon was covered, and the wound bed was granulated. Although the goal of covering the tendon was achieved, this patient is continuing treatment with the BWP and the wound is progressing towards closure.

Although SOC treatments for chronic ulcers are recognized, poor healing rates present a need for faster and more predictable closure. Products, such as cell and/or tissue-based products (CTPs), are widely used for their recognized efficacy for treating lower extremity ulcers. Specifically, randomized controlled trials using amniotic-based products have demonstrated complete closure by 12 weeks in approximately 60% of patients, a significant improvement to SOC.[31, 32] The nine patients treated with the BWP with the intent for closure, demonstrated complete closure within 3 – 8 weeks.

In vitro studies have shown that the BWP significantly reduces bacterial load (gram positive and gram negative), reduces expression of MMPs from macrophages, and increases expression of pro-regenerative cytokines from fibroblasts (data submitted for publication). *In vitro* performance may not be representative of clinical performance in human chronic wounds; however, it does provide insights into potential mechanisms of action for the results seen with the BWP treatment in this preliminary clinical evaluation. Further clinical evaluations are underway to support these findings.

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