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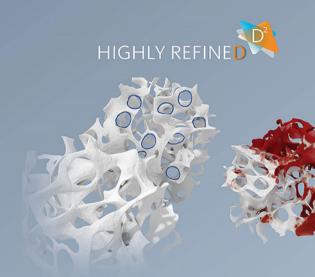**Geistlich Derma-Gide® – Advanced Matrix**

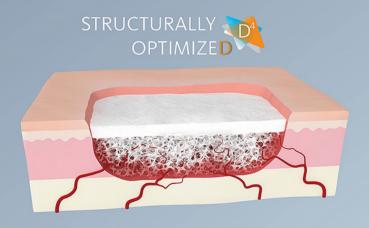Featuring 4-Dimensions of product design . . .

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ORIGINAL ARTICLE

Multi-centre prospective randomised controlled clinical trial to evaluate a bioactive split thickness skin allograft vs standard of care in the treatment of diabetic foot ulcers

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Abstract

Diabetic foot ulcers (DFUs) pose a significant risk for infection and limb loss. Advanced wound therapies including human skin allografts have shown promise in resolving these challenging wounds. The primary objective of this randomised, prospective study was to compare the response of 100 subjects with non-healing DFUs of which 50 were treated with a cryopreserved bioactive split thickness skin allograft (BSA) (TheraSkin; Misonix,Inc., Farmingdale, NY) compared with 50 subjects treated with standard of care (SOC, collagen alginate dressing) at 12 weeks. Both groups received standardised care that included glucose monitoring, weekly debridement's as appropriate, and an offloading device. The primary endpoint was proportion of full-thickness wounds healed at 12 weeks, with secondary endpoints including differences in percent area reduction (PAR) at 12 weeks, changes in Semmes-Weinstein monofilament score, VAS pain, and w-QoL. The result illustrated in the intent-to-treat analysis at 12 weeks showed that 76% (38/50) of the BSA-treated DFUs healed compared with 36% (18/50) treated with SOC alone (adjusted $P = .00056$). Mean PAR at 12 weeks was 77.8% in the BSA group compared with 49.6% in the SOC group (adjusted $P = .0019$). In conclusion, adding BSA to SOC appeared to significantly improve wound healing with a lower incidence of adverse events related to treatment compared with SOC alone.

KEYWORDS
bioactive split thickness skin allograft, complete wound healing, diabetic foot ulcer, randomised controlled trial, standard of care

Key Messages
- diabetic foot ulcers are more likely to heal within 12 weeks when treated with a bioactive split thickness allograft as compared with standard of care alone
- the mean PAR was significantly improved in the first 12 weeks of treatment with a bioactive split thickness allograft as compared with SOC alone

1 | INTRODUCTION

One of the most dangerous complications of diabetes is the development of foot ulcerations. Up to 34% of people with diabetes will develop a diabetic foot ulcer (DFU).[1-3] Treatment of these difficult wounds is further complicated by other risks factors such as neuropathy and peripheral arterial disease,[4] which bring additional burdens and difficulties to the treatment process. The development of an ulcer can be an early indicator of a more serious problem. Indeed, at least half of people with DFUs will develop a foot infection. Foot ulcers, infections and amputation dramatically increase the risk for hospitalisation and death.[5,6] Indeed, people with DFUs carry a 2.6-fold greater risk for death in the year following the development of a DFU[7] and a greater than 30% 5 year mortality. People receiving high-level amputation have 5-year mortality of greater than 50%. This rivals all but the most aggressive cancers.[8]

Treatments for diabetic ulcers tend to revolve around several fundamental principles. Although the events leading up to the development of an ulcer may be quite varied, the treatments remain focused on several unifying factors. Wounds require adequate blood flow to achieve healing. This is critical for the exchange of nutrients and various growth factors, as well as oxygen. In the presence of marginal blood flow, patients become much more susceptible to other factors that limit their ability to heal. Biofilms that collect bacteria on the surface of a wound become much more entrenched when wounds are not debrided properly. Furthermore, patients with neuropathy are much more susceptible to developing ulcers and infections, particularly when repetitive mechanical loads continue to degrade the skin and worsen the wound.[1,9,10]

When blood flow is adequate, infection is under control, and mechanical factors have been controlled, it is still common for diabetics with foot ulcers to have a difficult time achieving wound closure.

Chronic wounds are defined as wounds that fail to proceed through the normal phases of healing in an orderly and timely fashion.[11] Most diabetic ulcers that fail to achieve a significant level of healing after 4 weeks are considered chronic, and require a more in depth reassessment of the underlying pathology and potentially an advanced therapeutic treatment to stimulate the healing process.[11]

Advanced therapeutic treatments encompass a broad cross section of treatment options including growth factors, collagen, anti-bacterial agents, protease inhibitors, and anti-inflammatories as well as angiogenic stimulators and a variety of chemo attractants. Not surprisingly, the variety of materials range from topical pharmaceutical agents to laboratory engineered tissue components to cryopreserved skin allografts.

There have been numerous published studies that compare the healing rates of DFUs treated with advanced therapeutic materials to standard of care (SOC; ie, regular debridement with protection from mechanical forces, and simple dressings such as saline-moistened gauze), and these have demonstrated on many occasions that wounds treated with an advanced therapeutic agent is much more likely to close in 12 weeks then those treated with SOC alone. A meta-analysis by Gordon et al considered 25 studies that met their criteria and found that wounds treated with advanced therapeutic materials were 1.67 times more likely to heal by 12 weeks as compared with SOC.[12] Furthermore, evaluation of more than 900 000 patients with diabetes and chronic wounds found that use of advanced therapy led to a dramatic reduction in risk for minor amputations, major amputations, emergency department visits ($P < .0001$), and hospital readmissions ($P < .0001$) compared with those not receiving advanced therapy.[13]

Although as a class, advanced therapeutics are known to enhance wound healing in patients with DFUs, the variety of treatment options is so broad that testing of individual materials is necessary to gauge the therapeutic value of a specific product, and determine if the therapeutic gains justify the added cost and treatment regime associated with the product. The goal of the current study is to examine a widely used bioactive split thickness skin allograft (BSA) for the treatment of DFUs in a large, randomised, prospective clinical trial, and compare its efficacy to SOC.

Human split-thickness skin allografts have been used for many years for temporary wound coverage due to the preservation of naturally occurring growth factors and collagen[14] as well as in some instances for infection

control of chronic wounds. The BSA chosen for this study was TheraSkin, which is marketed and distributed by Misonix, Inc., Farmingdale, NY, and processed by Life-Net Health, Virginia Beach, VA (Figure 1). It is a cryopreserved split-thickness human skin allograft that is procured from consented donors who have been thoroughly screened and found to be free of communicable diseases. The consented donor may also donate other organs and tissues in addition to skin tissue, such as hearts, lungs, kidneys, bone, and tendon. Following a proprietary process that includes a cleansing process with antibiotics and other agents, BSA is carefully cryopreserved to preserve the living cells and the other relevant characteristics of human skin tissue related to
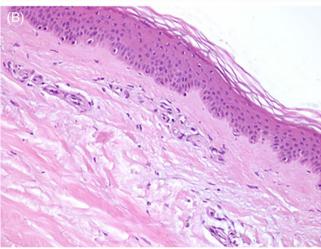

FIGURE 1 (A) BSA (TheraSkin) is a split-thickness skin allograft placed on a plantar first ray wound. (B) TheraSkin stained with H&E at ×200. Cell nuclei are stained dark purple/blue and the cytoplasm and other tissue constituents are stained various shades of red/pink. Note that TheraSkin contains the epidermal layer where there is a dense cell population. The large number of cells per mm³ in TheraSkin can be attributed to the presence of this layer

wound healing of skin tissue. A study by Landsman et al[15] validated the survival of the living cells in BSA through the procurement, cryopreservation, and thawing process, and proved that the native collagen and growth factors remained intact.

Henn et al, proved that BSA promotes angiogenesis and dermal regeneration.[16] Their study showed survival of a substantial portion of the living cells in BSA, as well as preservation of the native collagen structure on the cellular level. When compared with a decellularized human dermal allograft (HDA), the BSA stimulated significantly more vascularization of the murine dermis. They also found that there was a much higher degree of fibrosis with the HDA grafts, whereas the BSA grafts preserved the characteristic basket weave pattern of native dermis. Collectively, these studies demonstrate the advantages of using BSA to promote wound healing.

Numerous clinical studies have demonstrated the efficacy of BSA. A large consecutive retrospective study looked at 188 subjects treated with BSA for either DFUs or venous leg ulcers, and found that 60% of the subjects' wounds were completely closed after 12 weeks, and 74% after 20 weeks.[17] Similarly, in a randomised prospective clinical trial by DiDomenico et al,[18] the BSA closed 67% of DFUs in 12 weeks, as compared with 41% closure of DFU's treated with a tissue cultured skin substitute (Apligraf; Organogenesis, Canton, MA). Sanders et al[19] also performed a randomised prospective study comparing BSA to a cryopreserved tissue cultured dermis (Dermagraft; Organogenesis, Canton, MA), and found a 64% closure rate for DFUs treated with the BSA, as compared with 33% of wounds treated with the tissue cultured dermis. Furthermore, in the Sanders study, the average number of BSA grafts required to achieve closure was 4.36, as compared with 8.92 with cryopreserved tissue cultured dermis.

Two very large matched cohort studies published in 2020 also demonstrated the efficacy for treating difficult wounds with the BSA. The study by Gurtner et al,[20] used matched-cohort data on 3994 subjects from 644 different sites with a variety of wounds including DFUs and venous leg ulcers, and showed closure of wounds treated with the BSA in 68% of the cases. Even in cases in which deep structures were exposed, such as muscle, tendon, or bone, the closure rate of wounds treated with the BSA remained a very robust 64%. Another large, matched cohort study by Barbul et al,[21] focused exclusively on DFUs in 1556 subjects drawn from 470 different sites, and found a 67% closure rate for wounds treated with the BSA, with an average of 2.9 grafts used.

The primary objective of this study is to further examine the healing potential of BSA in subjects with chronic DFUs, as compared with treatment with SOC alone. In

this study, 100 subjects were randomised for treatment with both BSA and SOC, or with SOC alone. Study participants were treated for 12 weeks to determine the wound healing rate as well as the PAR, and any changes in pain, neuropathy, and incidence of adverse events.

2 | MATERIALS AND METHODS

The study was pre-registered on clinical trials.gov; NCT04040426, and submitted and approved by Western Institutional Review Board (WIRB #20191776). Subjects in both groups had to meet the inclusion and exclusion criteria listed in Table 1. All study participants signed an informed consent, approved by the IRB. Enrollment was considered to have occurred once the informed consent form was signed. A patient allocation system was employed based on a random sequence of block sizes of 10 designed to achieve a balanced design. The system used concealed envelopes at each site with allocation on a slip of paper within each envelope. All subjects were randomised if they continued to meet inclusion/exclusion

TABLE 1 Inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
• At least 18 y old.	• Index ulcer(s) deemed by the investigator to be caused by a medical condition other than diabetes.
• Presence of a DFU, Wagner 1 extending at least through the dermis provided it is below the medial aspect of the malleolus.	• Index ulcer, in the opinion of the investigator, is suspicious for cancer and should undergo an ulcer biopsy to rule out a carcinoma of the ulcer.
• The index ulcer will be the largest ulcer if two or more DFUs are present with the same Wagner grade and will be the only one evaluated in the study. If other ulcerations are present on the same foot they must be more than 2 cm distant from the index ulcer.	• Osteomyelitis or bone infection of the affected foot as verified by x-ray within 30 d prior to randomisation. (In the event of an ambiguous diagnosis, the Principal Investigator will make the final decision.)
• Index ulcer (ie, current episode of ulceration) has been present for greater than 4 wk prior to study screening and less than 1 y, as of the date the subject consents for study.	• Presence of diabetes with poor metabolic control as documented with an HbA1c >12.0 within last 90 d of randomisation.
• Index ulcer is a minimum of 1.0 cm^2 and a maximum of 25 cm^2 at screening and first treatment visits.	• Subjects on any investigational drug(s) or therapeutic device(s) within 30 d preceding SV1.
• Adequate circulation to the affected foot as documented by a dorsal transcutaneous oxygen measurement (TCOM) or a skin perfusion pressure (SPP) measurement of ≥30 mmHg or an Ankle Brachial Index (ABI) between 0.7 and 1.3 within 3 mo of screening using the affected study extremity. As an alternative, arterial Doppler ultrasound can be performed evaluating for biphasic dorsalis pedis and posterior tibial vessels at the level of the ankle or a TBI (Toe Brachial Index) of >0.6 is acceptable.	• Subjects with a history of more than 2 wk of treatment with immune-suppressants (including systemic corticosteroids >10 mg daily dose), cytotoxic chemotherapy, or application of topical steroids to the ulcer surface within 1-month prior to first SV1, or who receive such medications during the screening period or who are anticipated to require such medications during the course of the study.
• The target ulcer has been offloaded for at least 14 d prior to randomisation.	• Index ulcer has been previously treated or will need to be treated with any prohibited therapies.
• Females of childbearing potential must be willing to use acceptable methods of contraception (birth control pills, barriers or abstinence) during the course of the study and undergo pregnancy tests.	• Presence of any condition(s) which seriously compromises the subject's ability to complete this study or has a known history of poor adherence with medical treatment.
• Subject understands and is willing to participate in the clinical study and can comply with weekly visits.	• History of radiation at the ulcer site (regardless of time since last radiation treatment).
	• Subject is pregnant or breast-feeding.
	• Subjects taking a selective COX-2 inhibitor, such as Celecoxib, for any condition.
	• Subject with end stage renal disease as evidenced by a serum creatinine ≥3.0 mg/dL within 6 months of randomisation.
	• Index ulcer has reduced in area by 20% or more after 14 d of SOC from SV1 to the TV1/randomisation visit.

criteria to one of the two arms, both of which received the following routine procedures as part of the SOC:

- Evaluation on a weekly basis
- Offloading of the DFU (CAM boots or total contact casting [TCC] if the subject's foot is too large for a CAM)
- Appropriate sharp or surgical debridement
- Infection management (systemic antibiotics only in conjunction with debridement)

Fifty subjects were assigned to the BSA arm, and 50 additional subjects were assigned to the SOC arm. The BSA subjects received the BSA weekly, followed by a non-adherent dressing, 3-layer dressing comprised of 4x4 gauze pads, soft roll and compressive wrap (Dyna Flex 3M Maplewood, MN) or equivalent. SOC subjects received calcium alginate (Fibracol Plus, 3M Maplewood, MN) dressing followed by a non-adherent dressing and a padded 3-layer dressing comprised of 4x4 gauze pads, soft roll and compressive wrap (Dyna Flex or equivalent). The calcium alginate dressing (Fibracol Plus, 3M Maplewood, MN) is a wound dressing composed of collagen and calcium alginate fibres and is designed to form a soft absorbent and conformable topical wound dressing. It maintains a physiologically moist microenvironment at the wound surface that is conducive to granulation tissue formation and epithelization. In a meta-analysis performed in 2013, six studies including 375 subjects found that there was no statistically significant difference between alginate dressings and basic wound contact dressing (ie, saline moistened gauze), foam dressings, or anti-microbial (silver) hydrocolloid dressings.[22] Thus, the investigators felt that this was a good comparator for SOC.

Data collection was performed during weekly sessions and included basic demographic information, as well as a complete medical history with emphasis on medical conditions related to diabetes and/or foot ulcerations.

Foot ulcer history included the duration of the current DFU, type and duration of offloading system, prior DFU treatments utilised during the previous year, number of DFU's, history of DFU recurrence, prior amputations, skin or fungal conditions, and any significant foot deformities (ie, Charcot).

In addition to a comprehensive physical exam, vital signs were collected, and females of childbearing potential were given pregnancy tests. Circulation to the foot was confirmed with one of the following, in accordance with the inclusion/exclusion criteria for the study (Table 1): transcutaneous oxygen measurement (TCOM), skin perfusion pressure (SPP) measurement, Ankle Brachial Index (ABI) or Arterial Doppler.

Quality of life measures used the w-QoL, a short form questionnaire specific for patients with wounds, and a visual analog pain scale (VAS) was used to assess related pain levels.

Ulcers were assessed prior to debrement by measuring the %Granulation, %Non-viable tissue, and % Epithelised skin (total to 100%). Exudate was also assessed based on volume and type. Wounds were deemed infected if they met three or more of the criteria proposed by Woo and Sibbald.[23] (Table 2).

Wound healing was assessed initially by the investigator and digital images were captured at each study visit. Wounds were deemed closed only when there was 100% re-epithelization and absent drainage. Patients with closed wounds were asked to return to clinic in 2 weeks to assess durable healing and continued closure. A blinded adjudication panel performed wound assessments of the images to confirm investigator's assessment of closure. All subjects were followed until 2 weeks after closure, if before the 12-week mark, or until 14 weeks, to allow for confirmation of closure for those wounds that closed at the 12-week visit.

All enrolled subjects were included in the data analysis. Subjects could be withdrawn if they were significantly non-compliant with the requirements of the protocol, became pregnant, had revascularization surgery on the study limb, experienced deterioration of the ulcer site to the point where bone was exposed, or developed an infection lasting more than 2 weeks, that was unresponsive to allowable treatments. In addition, if subjects failed to heal by 50% after 6 weeks of treatment, they were exited and allowed to seek alternative care.

Data analysis included both quantitative and qualitative data and utilised wound healing measures. All testing for endpoints was two-sided with alpha set at .05 level of significance for demographic data as well as primary and secondary endpoints. The primary study hypothesis was that the proportion of wounds healed at 12 weeks, after up to 12 weeks of SOC with the BSA and SOC alone, will be equal for Groups 1 and 2. Formally,

TABLE 2 Ulcer infection assessment. Three or more of the following signs or symptoms are present

- Increased surface area
- Increased peri-wound margin temperature by more than 3°F difference between two mirror image sites
- Exposed bone or can be probed to the bone
- New areas of breakdown or satellite lesions
- Presence of swelling or reddened skin in peri-wound area
- Increased wound drainage
- Unpleasant, sweet or sickening odour present

H0: I1-I2 = 0; HA: I1-I2 = D1 ≠ 0, where I1 is the proportion of wounds healed in Group 1, I2 is the same metric for Group 2, D1 is the difference[11,12]; assuming the alternative hypothesis and statistical test used is chi square/Fisher exact test. Analysis may be adjusted using generalised linear modelling based on available variables at baseline known to affect wound healing.

Group sequential trials with sample sizes of [50] and [50] at the final look achieve 85% power to detect a difference of 0.30 between a treatment group proportion of 0.65 and a control group proportion of 0.35 at the 0.045 significance level (alpha) using a two-sided Z-Test (Pooled).

The populations defined for analysis included the intent-to-treat (ITT) and per protocol (PP). The ITT and safety populations comprised randomised patients who received at least 1 treatment. All analyses used the ITT approach. Missing data were imputed according to the

SAP. Study variables were summarised as means and ±SDs for continuous variables as well as medians/interquartile ranges for non-normal data. Categorical

TABLE 3 Comparison by treatment group for key subject-related variables

Variable	BSA	SOC	P
Patient age (y)	61.2 (12.55)	60.0 (10.99)	.62
BMI	31.1 (5.69)	34.5 (6.91)	**.01**
Gender			
Male	26 (52)	27 (54)	.84
Female	24 (48)	23 (46)	
Race/ethnicity			
White/non-Hispanic	35 (70)	38 (76)	.37
Black/African	10 (20)	5 (10)	
American	3 (6)	6 (12)	
Hispanic	2 (4)	1 (2)	
Other			
Alcohol use/history			
Current use	20 (40)	19 (38)	.96
Former use	7 (14)	8 (16)	
Never use	23 (46)	23 (46)	
Smoking use/history			
Current use	10 (20)	9 (18)	.59
Former use	18 (36)	14 (28)	
Never use	22 (44)	27 (54)	
Major foot deformity	20 (40)	19 (38)	.84
Blood glucose (mg/dL)	180 (76.42)	215 (108.05)	.14
Creatinine (mg/dL)	1.2 (0.51)	1.2 (0.51)	.92
HbA1c (%)	7.1 (1.65)	7.9 (1.86)	**.029**

Note: Continuous variables are reported as means (SD) with median/IQR additionally reported for key non-normally distributed continuous variables, and categorical variables as counts (percentage). Bold values are statistically significant (P < .05).

TABLE 4 Comparison by treatment group for key wound-related variables

Variable	BSA	SOC	P
Wound area (cm^2)[a]	4.2 (7.8)	3.9 (5.22)	.70
	Median: 2.2; IQR: 3.4	Median: 1.9; IQR: 3.1	
Depth (mm)			
1	26 (52)	30 (60)	.35
2	15 (30)	8 (16)	
>2	9 (18)	12 (24)	
Wound age (wk)[a]	17.5 (11.06)	15.5 (13.20)	.056
	Median: 13.5; IQR: 18	Median: 8.5; IQR: 13	
DFU location			
Plantar	35 (70)	45 (90)	**.023**
Dorsal	15 (30)	5 (10)	
DFU location			
Toe	8 (16)	5 (10)	.76
Forefoot	18 (36)	22 (44)	
Midfoot	15 (30)	17 (34)	
Heel	7 (14)	5 (10)	
Ankle	2 (4)	1 (2)	
Concurrent DFUs	13 (26)	7 (14)	.13
Lifetime DFU count	4.3 (3.87)	5.0 (4.54)	.49
	Median: 3; IQR: 4	Median: 4.5; IQR: 5	
Minor amputation	19 (38)	19 (38)	1.0
Major amputation	2 (4)	0 (0)	.50
Offloading type			
None	5 (10)	2 (4)	.30
Felt	4 (8)	8 (16)	
Shoe	6 (12)	8 (16)	
Shoe + felt	6 (12)	7 (14)	
Camboot or equivalent	22 (44)	23 (46)	
TCC	4 (8)	0 (0)	
Wheelchair	3 (6)	2 (2)	

Note: Continuous variables are reported as means (SD) with median/IQR additionally reported for key non-normally distributed continuous variables, and categorical variables as counts (percentage). Bold values are statistically significant (P < .05).
Abbreviations: DFU, diabetic foot ulcer; IQR, interquartile range; TCC, total contact cast.
[a]At randomisation.

variables were presented as counts and proportions or percentages. Statistical testing between treatment groups at baseline was carried out to examine the success of randomisation. For categorical variables, chi square or Fisher exact tests were performed and for continuous variables independent t tests or Mann-Whitney tests were used (depending on variable normality) to test for statistical differences.

The primary endpoint (proportion of wounds healed) between treatment groups was analysed using chi square (unadjusted results) and logistic regression (adjusted results). Secondary endpoints included differences in PAR at 12 weeks; and Semmes-Weinstein score, VAS pain, and w-QoL (between baseline and ESO visits). The PAR for the index wound at X weeks was calculated as ([AI−AXW]/AI)*100, where AI is the area of the index wound at randomisation and AXW the area at X weeks.

Two-sided P values <.05 were considered significant for testing between groups at baseline. Because an interim analysis was performed, alpha was set to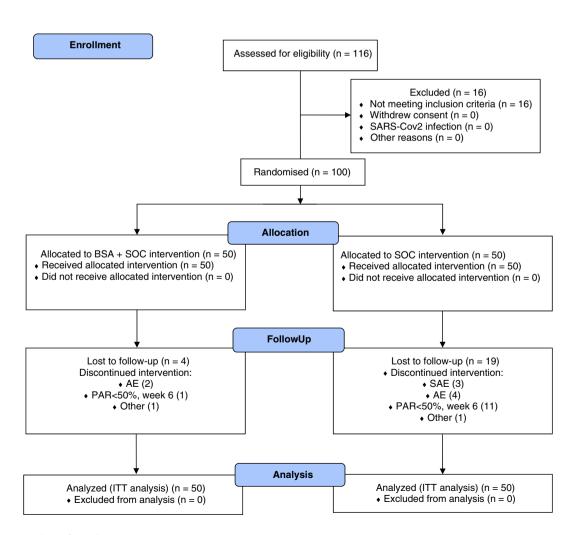 0.045 for the primary and PAR secondary endpoints. PASW 28 (IBM, Chicago, IL) was used to perform all statistical testing.

Time to heal is the first date that the wound is considered healed (completely epithelialised, 0 cm^2 area, with no drainage.

Secondary analysis included: 1. Time to heal within 12 weeks using Kaplan-Meier analysis; 2. PAR at 12 weeks (Group 1 vs Group 2) using the Mann-Whitney test; 3. Mean difference between Groups 1 and 2 (baseline and 12 weeks) of change in peripheral neuropathy score surrounding the DFU using the Semmes-Weinstein monofilament procedure; 4. Mean difference between Groups 1 and 2 of change in W-QoL score based on difference between baseline and 12 weeks using t test if data are normal or Mann-Whitney test if data are non-normal.

Secondary endpoint p values were adjusted for multiplicity of statistical testing using the step-up Hochberg procedure.

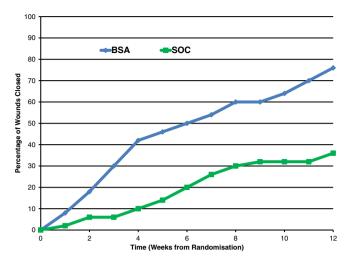Analysis of tertiary endpoints did not use comparative statistical analysis.

FIGURE 2 Subject flow chart

FIGURE 3 Weekly healing rates for both treatment groups

Control of the familywise error rate (all endpoints) was achieved through the Holm step-down procedure at both interim and final analysis.

Every effort was made to obtain required data at each scheduled evaluation from all subjects who have been randomised and treated. Subjects who were lost to follow-up were included in the ITT analysis of primary and secondary endpoints using Last Observation Carried Forward principles to impute missing data.

3 | RESULTS

A pool of 116 eligible study subjects were screened to successfully enrol 100 subjects. Exclusions were due to a history of more than 2 weeks of treatment with immunosuppressants, subjects on investigational drug(s)

Variable	B	P	OR	95% CI
BSA[1]	1.91	**.00015**	6.74	2.52-18.02
Ethnicity[2]	0.007	.92	1.08	0.28-4.14
African American	1.72	**.045**	5.57	1.04-29.83
Other[a]				
Area at randomisation (cm^2)	−0.13	**.014**	0.88	0.79-0.97
BMI	−0.071	.094	0.93	0.86-1.01
Constant	3.54	**.017**	34.33	

TABLE 5 Logistic regression, dependent variable of healed or not, and independent variables as listed

Note: Reference groups: [1]SOC; [2]Caucasian. Bold values are statistically significant ($P < .05$).
Abbreviations: CI, confidence interval; OR, odds ratio.
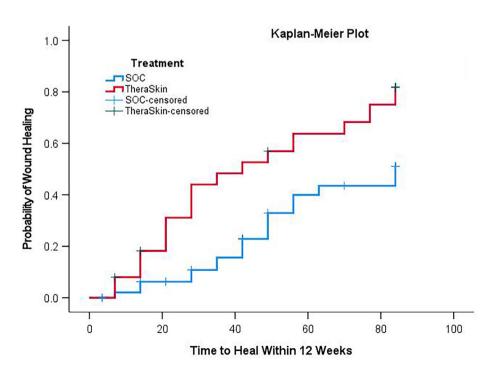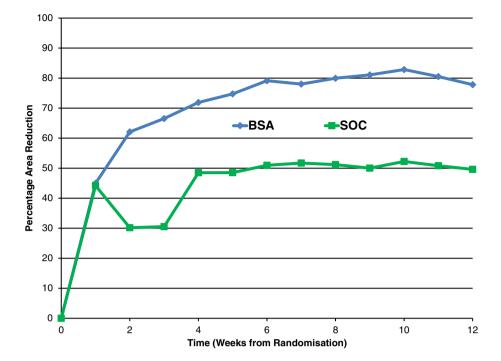[a]Includes Hispanic, Asian, Middle-Eastern, and Native Hawaiian races/ethnicities.

FIGURE 4 Kaplan-Meier plot of wound healing probability for both treatment groups

FIGURE 5 Weekly PAR values for both treatment groups. Bioactive split thickness skin allograft (BSA): Mean: 77.8%; SD: 62.5. Standard of care (SOC): Mean: 49.6%; SD: 97.6. $P = .0019$

or therapeutic device(s) within 30 days preceding study visit 1, ulcers previously treated with any prohibited therapy, osteomyelitis or bone infection of the affected foot as verified by x-ray within 30 days prior to randomisation, HbA1c >12.0 within last 90 days of randomisation, and index ulcer reduced in area by 20% or more after 14 days of SOC between screening and randomisation visits.

A comparison by treatment group for key subject variables (Table 3) and wound-related variables (Table 4) showed that variables were well balanced between groups with the exception of BMI (higher statistically in the SOC group) and HbA1c (statistically higher in the SOC group) compared with the BSA group for patient-related variables, and a statistical higher proportion of plantar DFUs in the SOC group compared with the BSA group. Wound age as well as wound size were slightly higher in the BSA group compared with the SOC group.

During the course of the study, 23 subjects were withdrawn. In the BSA group, 4 subjects were withdrawn or lost to follow-up:

- 1 subject was removed for not meeting the ≥50% PAR rule at 6 weeks post-randomisation
- 1 subject was removed due to worsening condition of the wound, but was not an AE
- 2 subjects were removed due to adverse events (osteomyelitis; SARS-Cov2 infection)

In the SOC group, 19 subjects were withdrawn or lost to follow-up:

TABLE 6 Changes in quality of life, pain, and Semmes-Weinstein scores from baseline to end of study

	BSA	SOC	*P*
w-QoL			
Baseline	1.4 (SD: 1.02)	1.2 (SD: 0.92)	.67
End of study	0.2 (SD: 0.98)	0.3 (SD: 0.47)	
VAS			
Baseline	0.9 (SD: 1.90)	1.3 (SD: 2.6)	.67
End of study	0.6 (SD: 1.68)	1.3 (SD: 2.22)	
S-W score			
Baseline	0.9 (SD: 1.73)	1.0 (SD: 2.18)	.67
End of study	1.2 (SD: 2.29)	1.3 (SD: 2.87)	

- 11 subjects were removed for not meeting the ≥50% PAR rule at 6 weeks post-randomisation
- 1 subject was removed because the wound reopened at the healing confirmation visit
- 3 subjects were removed due to SAEs:
  ○ Study foot abscess, osteomyelitis, and cellulitis
  ○ Infection of the non-study foot
  ○ Congestive heart failure
- 4 subjects were removed due to AEs (infection of non-study venous leg ulcer; osteomyelitis of the study foot; osteomyelitis of the non-study foot; SARS-Cov2 infection)

An overview of the enrollment and analysis process is seen in Figure 2; Consort Diagram.
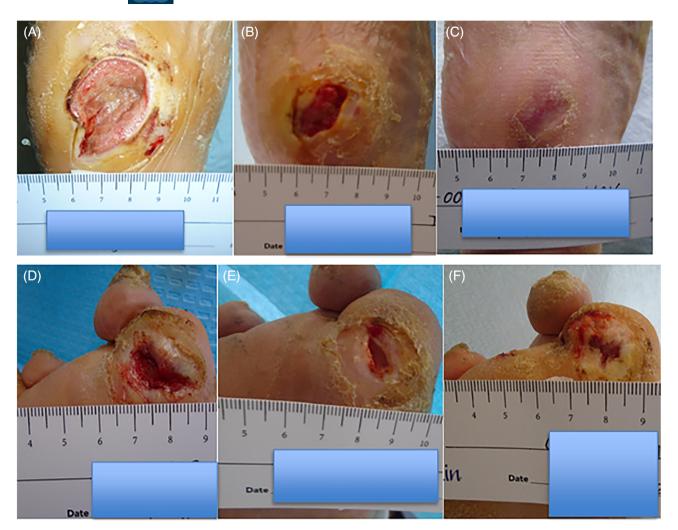
FIGURE 6 (A) Diabetic neuropathic non-healing ulcer for 9 weeks with a HgA1c of 7.0 and serum creatinine of 1.01 mg/dL measuring 7.44 cm² at study enrollment; receiving bioactive split thickness skin allograft (BSA) and standard of care (SOC). (B) After 6 weekly graft applications measuring 2.28 cm². (C) At healing confirmation after 10 grafts complete healing with durable healing at healing confirmation. (D) Diabetic neuropathic non-healing ulcer for 10 weeks with a HgA1c of 8.1 and serum creatinine of 1.25 mg/dL measuring 1.08 cm² at study enrollment; receiving SOC. (E) After 5 weeks of SOC treatment measuring 0.55 cm². (F) After 12 weeks of treatment with SOC measuring 0.48 cm² wound was able to achieve slightly over 50 PAR at end of study with SOC treatment

Evaluation of the primary endpoint, complete wound healing, was 76% (38/50) in the BSA group, as compared with 36% (18/50) in the SOC group ($P = .00056$) (Figure 3). Adjusted results utilised a main effects logistic model constructed using methodology specified in SAP. Model fit parameters were Nagelkerke R2: 0.36, Hosmer & Lemeshow fit test: 0.21, C statistic: 0.80. The model met all logistic regression assumptions. BMI was kept in the model even though it is non-significant to avoid over-dispersion issues.

The results of the logistic regression analysis are shown in Table 5. The treatment (BSA) dominates the other variables and increases the odds of healing by 6.74 ($P = .00015$). Having a higher wound area at randomisation lowered the odds of wound healing, while having a race/ethnicity of other (Hispanic, Asian, Middle Eastern,

Native Hawaiian) was very protective (protective means that the odds of wound healing increased). Rates of healing for both groups are shown in Figure 3.

Secondary endpoint analysis demonstrated strong differences between the groups. The probability of a wound healing over time is shown for each group in Figure 4. The average time for closure within the 12-week period for BSA was 46.9 days (95% CI: 38.7-55.1) vs closure with SOC was 65.3 days (95% CI: 57.7-72.9). This difference was statistically significant ($P = .0019$).

The percentage area reduction (PAR) is shown for the two groups on a weekly basis in Figure 5. On average, the PAR was 77.8% (SD: 62.5) for BSA, as compared with 49.6% (SD: 97.6) for SOC at 12 weeks. These differences were statistically significant as well ($P = .0019$).

Quality of life (*w-QoL*), pain scores (VAS), and Semmes-Weinstein scores were evaluated, and showed insignificant changes in both groups from baseline, with an insignificant improvement in w-QoL and reduction in VAS scores in the BSA group, as compared with the SOC group. Similarly, there was an insignificant improvement in the BSA group for Semmes-Weinstein score from baseline. These results are summarised in Table 6. Two representative cases are illustrated in Figure 6 of non-healing diabetic foot wounds enrolled into treatment with either weekly application of BSA with SOC or SOC alone.

4 | DISCUSSION

DFUs can be one of the most challenging types of wounds to heal. Standards of care have evolved to include periodic debridement, regulation of moisture, reduction of infection, optimization of blood flow, and reducing mechanical loading factors, in addition to systemic control of the disease itself. In an effort to facilitate healing, clinicians have been supplementing the healing process with various 'biologics' including growth factors, cytokines, collagen, and living cell treatments.

In the consensus recommendations by Snyder et al,[24] a panel of thought leaders in wound care suggested that biologics should be included as the new SOC due to the enhancement in healing rates demonstrated in numerous studies.

A meta-analysis conducted by Santema et al[25] also supports the advantages of using an advanced biologic. They reviewed a large number of randomised controlled clinical trials, and their meta-analysis reported closure rates on 1961 collected patients treated with an advanced biologic to be approximately 48.6% (range from 35.4% to 82.6%) over 6-20 weeks, as compared with approximately 27.3% for subjects treated over 6-16 weeks with SOC.

In the current study, the value of BSA, an advanced biologic material, is demonstrated. Closure rates and PAR, were significantly superior to SOC alone. Furthermore, the closure rate of 76% demonstrated here, during the first 12 weeks is consistent with prior studies[17-21] using the same BSA, in which the closure rate ranged from 67%-93%, with an average closure rate of 65.2% over 12 weeks. Although these previous studies differed in study design (eg, retrospective with matched cohort, prospective, case series), the types of wounds found in the current study remain consistent. Furthermore, the subjects in the current study were very typical of patients seen in wound clinics. Tables 1 and 2 demonstrate a few critical elements including HbA1c in the mid-7 range, BMI in the low 30 range, and average wound size of approximately 4 cm^2 in both groups.

An examination of the adverse events and serious adverse events observed in the current study was lower for the BSA group, as compared with the SOC group. Although this difference is likely multifactorial, it is logical that the longer a wound remains open, the more likely it is for adverse events to occur.

Based on the multi-variant regression analysis, it is most likely that the reason for the significantly higher rate of successful treatment in the BSA group is attributed to the use of BSA. Previously, a characterisation study[15] of this BSA demonstrated that approximately 67% of the cells found in natural skin would survive the harvesting, cryopreservation and thawing process, and remain viable upon application. Furthermore, the natural collagen structure is maintained throughout. This BSA allograft is comparable in every way to a typical split-thickness skin autograft, in that it retains most of the signalling molecules, living cells, and extracellular matrix naturally found in the autograft.

When assessing the value of the current study, the strengths include a robust trial design, multiple investigative sites, standardisation of SOC methods, strong statistically sound methodology, and ITT analysis. The study limitations include, lack of investigator blinding, which would not be possible due to the difference in the tissue allograft and calcium alginate. In addition, adding a third cohort with a comparative biologic or tissue graft could have provided additional strength to the study. Furthermore, although the study cohorts had no statistically significant differences among the key wound-related variables, the strength of the comparison could have been improved if a more comprehensive examination of comorbidities could have been included. Future studies should consider or may allow for enrollment of wounds of greater complexity, perhaps including wounds with exposed deeper structures.

In conclusion, this prospective randomised study demonstrated that wounds treated with BSA in addition to SOC were more likely to close during the first 12 weeks of treatment. The subjects who received BSA experienced less adverse events and were more likely to show progression of healing based on PAR over time.

from PERI to serve as Co-Principal Investigator/Study Chair for this trial and to design and administrate the trial, review study photos and also assist with the writing and review of the manuscript. Dennis P. Orgill, MD, PhD received research funds to serve as a validating/adjudicating plastic surgeon to review study photos and assist with the writing and review of the manuscript. Paul M. Glat, MD received research funds to serve as a validating/adjudicating plastic surgeon to review study photos and assist with the writing and review of the manuscript. Marissa J. Carter, PhD received research funds to provide the statistical analysis plan, and provide the statistical analysis for this trial and assist with writing of the result section of the manuscript. Lawrence A. Didomenico, DPM is the medical director of LEIRT and his company received research funds to serve as a site investigator for this trial and to assist with the writing and review of the manuscript. Alexander M. Reyzelman, DPM received research funds and served a site investigator for this trial and assisted with the writing and review of the manuscript. Charles M. Zelen, DPM is the medical director and president of the PERI and his company received research funds to administrate the clinical trial and write the paper for publication. There is no other conflict of interests with any of the authors in relationship to this study, or with regard to Misonix, Inc. IRB conflict of interest statements are on file with PERI.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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