A Prospective, Randomized, Multicenter, Controlled Evaluation of the Use of Dehydrated Amniotic Membrane Allograft Compared to Standard of Care for the Closure of Chronic Diabetic Foot Ulcers

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Abstract: Delayed closure of foot ulcers is a primary factor leading to lower extremity amputation in patients with diabetes, creating great demand for products or therapies to accelerate the rate of wound closure in this population. This study (ClinicalTrials.gov Identifier: NCT02209051) was designed to evaluate dehydrated amniotic membrane allograft (DAMA) (AMNIOEXCEL, Derma Sciences Inc, Princeton, NJ) plus standard of care (SOC) compared to SOC alone for the closure of chronic diabetic foot ulcers (DFUs). *Materials and Methods*. This prospective, open-label, randomized, parallel group trial was implemented at 8 clinical sites in the United States. Eligibility criteria included adults with type 1 or type 2 diabetes mellitus who have 1 or more ulcers with a Wagner classification of grade 1 or superficial 2 measuring between 1 cm^2 and 25 cm^2 in area, presenting for more than 1 month with no signs of infection/osteomyelitis; ABI > 0.7; HbA1c < 12%; and serum creatinine < 3.0 mg/dL. Eligible subjects were randomized (1:1) to receive either SOC alone (n = 14) or DAMA+SOC (n = 15) until wound closure or 6 weeks, whichever occurred first. The endpoint was the proportion of subjects with complete wound closure (defined as complete reepithelialization without drainage or need for dressings). *Results*. Thirty-three percent of subjects in the DAMA+SOC cohort achieved complete wound closure at or before week 6, compared with 0% of the SOC alone cohort (intent-to-treat population, $P = 0.017$). There was a more robust response noted in the per protocol population, with 45.5% of subjects in the DAMA+SOC cohort achieving complete wound closure, while 0% of SOC-alone subjects achieved complete closure ($P = 0.0083$). No treatment-related adverse events were reported. *Conclusion*. The results suggest DAMA is safe and effective in the management of DFUs, but additional research is needed.

Key words: diabetic foot ulcer, dehydrated amniotic membrane allograft, wound closure

In 2014, the global prevalence of diabetes was estimated to be 9% among adults.[1] In 2013, 382 million people worldwide suffered from diabetes, and this number is expected to rise to 592 million by 2035.[2] Patients with diabetes face a variety of health concerns, not the least of which is the peripheral sensory neuropathy associated with the development of diabetic foot ulcers (DFUs). The lifetime risk of a person with diabetes developing a DFU is approximately 25%,[3] and delayed healing of these ulcers is a primary factor leading to lower extremity amputations in patients with diabetes.[4] Amputations have life-altering repercussions for patients and represent a considerable burden for the health care industry as a whole.[3]

With the well-being of millions of patients at stake, there is a great need for products or treatments that bring DFUs to closure as quickly as possible. The current standard of care (SOC) regimen involves maintaining a moist wound environment, debriding nonviable tissue, relieving pressure with an offloading device, and preventing or managing wound infection.[5] Even with a good SOC, DFUs are notoriously slow to close, creating a demand for drugs and techniques to enhance closure.[6]

Human amniotic membrane has been used for wound healing purposes since the early 20th century.[7] The numerous potential applications of this tissue are being investigated and include DFUs and other chronic skin wounds[5,6,8,9] for which it is an attractive cellular and/or tissue product (CTP) due to myriad beneficial characteristics. Studies have demonstrated that amniotic membrane has anti-inflammatory effects,[7] is antimicrobial,[10] demonstrates antiscarring and antiadhesive activity,[7,11] is nonimmunogenic with low antigenicity,[10] has analgesic properties,[12] and promotes reepithelialization.[10,11,13]

Dehydrated amniotic membrane allograft (DAMA) is one commercially available amniotic membrane allograft (AMNIOEXCEL, Derma Sciences, Princeton, NJ). The product is provided in multiple geometric configurations to be applied directly to clean, debrided wounds where bacterial burden and offloading have been addressed. Additionally, adequate vascular status and perfusion must also exist for DFUs to heal in a timely and orderly fashion. Dehydrated amniotic membrane allograft is processed in compliance with US Food and Drug Administration Code of Federal Regulations (CFR) Title 21 Part 1271 and Section 361 of the Public Health Service Act and regulated as a human cell and tissue product (HCT/P). The base material for DAMA is collected from live, healthy, planned cesarean section births of appropriately screened women, per American Association of Tissue Banks requirements. The collected amniotic tissue is then washed, dehydrated, cut, and packaged for commercial distribution.

This study was designed to describe the natural history

Table 1. Eligibility Criteria

Inclusion criteria

An eligible study candidate will:

1. be an ambulatory person of at least 18 years of age at the time of informed consent.
2. have type 1 or type 2 diabetes mellitus.
3. have an HbA1c of < 12%.
4. have at least 1 wound that:
   a) is Wagner grade 1 or superficial 2 (ie, without bone, tendon, or joint exposure),
   b) has a duration of at least 1 month,
   c) has no clinical signs of infection or osteomyelitis,
   d) is between 1 cm^2 and 25 cm^2 in area,
   e) closed < 30% in area during the screening period, and
   f) is located on the foot, distal to malleolus.
5. have adequate circulation to the affected extremity as demonstrated by an ankle brachial index between 0.7 and 1.2, or triphasic or biphasic Doppler arterial waveform at the ankle of the affected leg, or dorsum transcutaneous oxygen test ≥ 30 mm Hg.
6. have a serum creatinine of < 3.0 mg/dL or CrCl > 30 mL/min.
7. have the ability and willingness to understand and comply with study procedures and give written, informed consent prior to enrollment in the study or initiation of study procedures.

Exclusion criteria

Potential study candidates will be excluded if they:

1. have participated in another clinical trial within 30 days prior to consent.
2. have an active Charcot deformity of the study foot (ie, foot is erythematous, warm, edematous, and is actively remodeling).
3. are receiving radiation or chemotherapy of any kind.
4. have a known or suspected malignancy of a current ulcer.
5. are pregnant or breast feeding.
6. have an active malignant disease.
7. are receiving hemodialysis or peritoneal dialysis.
8. have sickle cell anemia or Raynaud's syndrome.
9. have a diagnosis of autoimmune connective tissue disease.
10. have received a biologic agent, growth factor, xenograft, or skin equivalent to the ulcer 30 days prior to consent.
11. have exposed bone, tendon, or joint capsule in the study ulcer.
12. are taking medications considered to be immune system modulators.

of DAMA+SOC compared to SOC alone in subjects with chronic grade 1 or 2 DFUs.

Methods

In this prospective, randomized, multicenter, open-label, parallel group study conducted with 29 subjects from 8 clinical study sites, study candidates with open chronic wounds of the foot were assessed for eligibility as listed in Table 1. The study period consisted of a 2-week screening period, the baseline visit with randomization of qualified subjects, and a treatment period of 6 weeks, a timeframe modeled after Zelen et al.[6,8] During each weekly study visit, the ulcer bed was assessed for epithelialization, granulation, and necrosis as well as any clinical signs of infection, the ulcers were photographed and measured, and all adverse events collected. Data were recorded in a web-based electronic case report form and stored in an electronic database used to perform all the statistical assessments (XTrials Research, Somerset, NJ).

Standard of care for all subjects. All subjects enrolled in this study received SOC throughout the trial, which included debridement of necrotic/nonviable tissue and hemostasis (no silver nitrate sticks or styptic pencils were permitted), moist wound dressings, offloading where appropriate with a DH Walker boot (Össur, Foothill Ranch, CA), and infection surveillance and management. All subjects returned to the clinic every week for dressing changes, wound inspection, debridement, and application of DAMA (as per randomization allocation and per the investigator's discretion).

Screening period. After eligible candidates provided informed consent, target DFUs were cleaned, debrided, photographed, and measured. Candidates were provided with dressing materials and an offloading device and were instructed to return to the clinic in 2 weeks for assessment. If, upon return, the target DFU had closed less than 30% in area during the 2-week screening period, the subject was randomized (1:1) to 1 of 2 treatment groups: DAMA+SOC or SOC alone. Randomization occurred through a module within the electronic case report form and was stratified by ulcer area (1 cm^2-10 cm^2 vs 10.1 cm^2-25 cm^2) and clinical site.

Standard of care alone cohort. For subjects randomized to receive SOC alone, in addition to the SOC outlined above in "Standard of care for all subjects," XTRASORB Foam Non-Adhesive Dressing (Derma Sciences,

Table 2. Demographic and baseline characteristics, intent to treat population.

	Standard of Care (N = 14)	Dehydrated amniotic membrane allograft + Standard of Care (N = 15)	Statistic (*P* value)
Age (years) (Mean ± SD) (range)	 58.6 ± 6.97 (48-71.2)	 57.9 ± 12.49 (34-85)	0.855
Male	13 (92.9%)	12 (80%)	0.5977
Race Caucasian Black or African American American Indian or Alaska Native Other	 11 (78.6) 2 (14.3) 0.0 1 (7.1)	 8 (53.3) 3 (20.0) 1 (6.7) 3 (20.0)	 0.2451 1.000 1.000 0.5977
Ethnicity Hispanic or Latino	 2 (14.3)	 4 (26.7)	 0.6513
Weight (pounds) (Mean ± SD) (range)	 242.4 ± 53.1 (166-345)	 234.5 ± 38.2 (180-292)	0.648
Height (inches) (Mean ± SD) (range)	 69.8 ± 4.4 (61-75)	 68.9 ± 5.6 (52.5-75)	0.638
Body mass index (Mean ± SD) (range)	 35.1 ± 8.1 (24.9-55.7)	 34.9 ± 5.9 (28.2-50.2)	0.944

Denominator percentage is the number of subjects in the column. Body mass index = kg/m^2 body surface area.

Princeton, NJ) was applied to the ulcer after debridement (when necessary) and, once hemostasis was achieved, the wound was wrapped with Duform Synthetic Conforming Bandage (Derma Sciences, Princeton, NJ) and lightly secured. A compression dressing of Duban Cohesive Bandage (Derma Sciences, Princeton, NJ) wrap was applied as a cover dressing.

Dehydrated amniotic membrane allograft + standard of care cohort. For subjects randomized to receive DAMA in addition to SOC as outlined above (n = 15), the ulcer was debrided, hemostasis achieved, and DAMA that had been cut to fit the wound bed was applied. The ulcer was dressed with Adaptic (Systagenix, Gatwick, UK) and covered with the foam nonadhesive dressing. The area was wrapped with the conforming bandage and lightly secured. A compression dressing of the cohesive bandage wrap was applied as a cover dressing. Subjects were instructed not to change their dressings. Reapplication of DAMA could occur as often as once per week, based upon the physician's judgment.

Primary endpoint and statistical analyses. The primary endpoint of the study was the proportion of subjects with complete wound closure prior to or on week 6 after initiation of treatment. Complete wound closure was defined as 100% complete skin reepithelialization without drainage or dressing requirements.

The proportion of subjects with complete wound closure at or before 6 weeks was analyzed using Fisher's Exact test, and Kaplan-Meier methodology was used for time-to-event analyses. Data were, at a minimum, summarized using mean, standard deviation, medians, and/or proportions, as appropriate.

The protocol, informed consent form, and any appropriate related documents were reviewed and approved by the following Institutional Review Boards (IRB): Western IRB, Beth Israel Deaconess-Plymouth IRB, Duke University Health System IRB, and Wayne Memorial Hospital IRB. The study was conducted in adherence to Good Clinical Practice guidelines as required by the Principles of the World Medical Association Declaration of Helsinki 2013; the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) E6 Guideline for Good Manufacturing Practice (Committee for Proprietary Medicinal Products/ICH/135/95) of the European Agency for the Evaluation of Medicinal Products; and the US Food and Drug Administration CFR Title 21 regarding clinical studies, including Part 50 and Part 56 concerning informed

Table 3. Target ulcer characteristics.

	Standard of Care (N = 14)	Dehydrated amniotic membrane allograft + Standard of Care (N = 15)	Statistic (*P* value)
Lower extremity with target ulcer, n (%) Right foot	8 (57.1)	10 (66.7)	0.597
Side of foot, n (%) Plantar	9 (64.3)	10 (66.7)	1.000
Position to midline, n (%) Midline Lateral Medial	2 (14.3) 6 (42.9) 6 (42.9)	2 (13.3) 5 (33.3) 8 (33.3)	0.842
Ulcer in part of foot, n (%) Forefoot Hindfoot Metatarsals Midfoot Phalanges	6 (42.9) 3 (21.4) 1 (7.1) 4 (28.6) 0.0	9 (60.0) 2 (13.3) 0.0 3 (20.0) 1 (6.7)	0.572
Ulcer width cm (Mean ± SD) (range)	1.9 ± 0.92 (0.7-4.5)	1.8 ± 0.9 (0.7-3.5)	0.769
Ulcer length cm (Mean ± SD) (range)	3.4 ± 3.28 (0.9-13.2)	2.2 ± 1.56 (1-5.7)	0.214
Area of wound cm^2 (Mean ± SD) (range)	6.9 ± 6.75 (1.1-21.1)	4.7 ± 5.43 (1.2-16.5)	0.340

subject consent and IRB regulations and applicable sections of US 21 CFR Parts 312 and 1271.

Results

In total, 49 patients were screened and 29 patients were randomized. Of the 20 screening failures, 4 (20%) had clinically infected ulcers, 4 (20%) had ulcers decrease > 30% in area during the screening period, 2 (10%) had ulcers < 1cm^2 in area, 2 (10%) experienced protocol deviations during screening, and 8 (40%) failed 1or more of the other eligibility criteria (eg, exclusionary lab values, noncompliance, cancer diagnosis).

Twenty-nine subjects (intent-to-treat population [ITT], Table 2) were randomized to receive DAMA+SOC (n = 15) or SOC alone (n = 14).Target ulcer characteristics are provided in Table 3.There were 4 early withdrawals in each group, leaving 10 in the SOC cohort and 11 in the DAMA cohort to complete the trial (per protocol [PP] population;Table 4). In the SOC alone cohort, withdrawals were due to withdrawn consent (50%), ulcer infection (25%), and protocol violation (25%). In the DAMA+SOC cohort, withdrawals were due to ulcer infection (25%), protocol violation (50%), and loss to follow-up (25%).

In the ITT population, 33% of subjects in the DAMA+SOC cohort achieved complete wound closure at or before week 6, while 0% of SOC alone subjects achieved complete wound closure (P = 0.017) (Table 5), with a statistically significant 95% confidence interval (CI) of responder ratio (25.0%, 46.4%; P = 0.0407). In the PP population, 45.5% of subjects in the DAMA+SOC cohort achieved complete wound closure, while 0% of SOC alone subjects achieved complete wound closure (P = 0.0083) (Table 6), with a statistically significant 95% CI of responder ratio (32.9%, 58.0%; P = 0.0137). Further, subjects in the DAMA+SOC achieved wound closure more rapidly than did those allocated to SOC alone (P < 0.0001) based upon the Kaplan-Meier analysis (Figure 1).A series of photographs of a representative subject that received DAMA is provided in Figure 2.

The frequency of DAMA application was determined by the investigator based upon ulcer appearance and clinical judgement. The 15 subjects who were random-

Table 4. Disposition of all randomized subjects.

Disposition Reasons n (%)	Standard of Care (N = 14)	Dehydrated amniotic membrane allograft + Standard of Care (N = 15)
Randomized Subjects n (%)	14 (100.0)	15 (100.0)
Intent-to-treat subjects	14 (100.0)	15 (100.0)
Per-protocol subjects	10 (71.4)	11 (73.3)
Safety subjects	14 (100.0)	15 (100.0)
Study Completion n (%)		
Completed study	10 (71.4)	11 (73.3)
Early withdrawal	4 (28.6)	4 (26.7)
Reasons for early withdrawal n (%)		
Completed study	10 (71.4)	11 (73.3)
Lost to follow-up	0	1 (6.7)
Withdrawal of consent	2 (14.3)	0
Adverse event (infection)	1 (7.1)	1 (6.7)
Other	1[a] (7.1)	2[b] (13.4)

Denominator of the percent is the number of subjects in each treatment group.
[a]Protocol violation; [b]Prostate cancer history (1); Protocol violation (1)

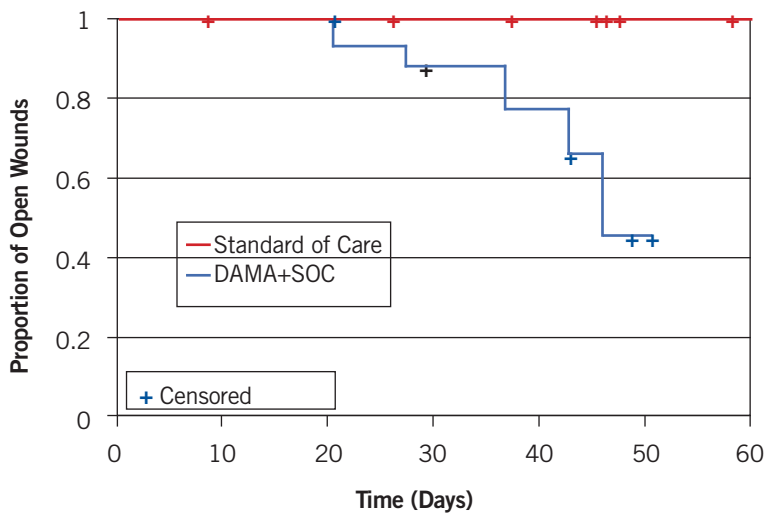
Table 5. Proportion of intent-to-treat subjects with complete wound closure at or before week 6, primary efficacy endpoint.

Statistics	Standard of Care (N = 14)	Dehydrated amniotic membrane allograft + Standard of Care (N = 15)	Test *P* value
Number of responders (Ratio %)	0 (0.0)	5 (33.3)	0.0170
95% CI of responder ratio	(0.0, 0.0)	(25.0, 46.4)	0.0407

CI: confidence interval

ized to receive DAMA had a total of 4.3 ± 1.7 (Mean ± SD) pieces applied, with 1 piece applied weekly (7.3 ± 0.6 days). There was no difference in DAMA usage between the subjects who achieved wound closure (4.6 ± 1.34 pieces) compared to those who did not (4.2 ± 1.93 pieces).

Six subjects in the SOC alone cohort and 4 subjects in the DAMA+SOC cohort (ITT population) experienced treatment-emergent adverse events (Table 7). In the DAMA+SOC cohort, these events included wound infection, localized infection, osteomyelitis, prolonged bleeding, cellulitis, and atrial flutter. Treatment-emergent adverse events observed in the SOC-alone cohort included tendon injury, skin ulcer, diabetic foot infection, cellulitis, and deep vein thrombosis. The incidence of adverse events was not different between the groups and, given the nature of the underlying diabetic disease and associated comorbidities, these events were not unexpected.



DAMA+SOC: Dehydrated amniotic membrane allograft + standard of care

Figure 1. Kaplan Meier plot of time to complete wound closure in the intent-to-treat population; primary efficacy endpoint.

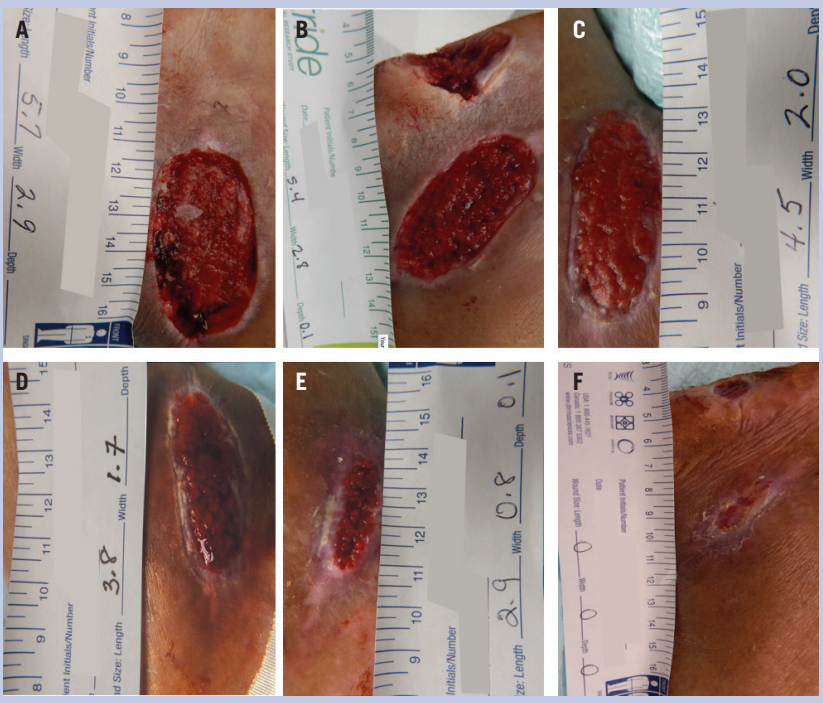


Figure 2. Closure of wound following treatment with dehydrated amniotic membrane allograft. A) Baseline; B) week 1; C) week 2; D) week 3; E) week 5; and F) week 6.

Discussion

When considering the clinical utility of amniotic membrane in the management of DFUs, an understanding of the characteristics of this tissue is useful. The human placenta is comprised of the inner amniotic membrane and the outer chorion.[14] At an ultrastructural level, amniotic membrane is a thin, tough, transparent, avascular tissue composed of 5 major layers: the epithelium, basement membrane, compact layer, fibroblast layer, and spongy layer. Hodde[9] postulated that amniotic membrane is unique among the naturally occurring extracellular matrices (ECM) because it includes the basement membrane, the layer from which all cutaneous cells arise, and might therefore have

Table 6. Proportion of per-protocol subjects with complete wound closure at or before week 6, primary efficacy endpoint.

Statistics	Standard of Care (N = 10)	Dehydrated amniotic membrane allograft + Standard of Care (N = 11)	Test *P* value
Number of patients evaluated	10	11	
Number of responders (Ratio %)	0 (0.0)	5 (45.5)	0.0083
95% CI of responder ratio	(0.0, 0.0)	(32.9, 58.0)	0.0137

CI: confidence interval

Table 7. Summary of treatment-emergent adverse events by body system and preferred term in the intent-to-treat population (safety analysis population).

System Organ Class Preferred Term[a]	Standard of Care (N = 14)	Dehydrated amniotic membrane allograft + Standard of Care (N = 15)
Total number of subjects, n (%)	3 (21)	4 (27)
Cardiac disorders n (%)	**0 (0.0)**	**1 (6.7)**
Atrial flutter	0 (0.0)	1 (6.7)
Infections and infestations n (%)	2 (14)	3 (20)
Wound infection	0 (0.0)	1 (6.7)
Osteomyelitis	0 (0.0)	1 (6.7)
Localized infection	0 (0.0)	1 (6.7)
Diabetic foot infection	1 (7.1)	0 (0.0)
Cellulitis	1 (7.1)	1 (6.7)
Vascular disorders n (%)	1 (7.1)	0 (0.0)
Deep vein thrombosis	1 (7.1)	0 (0.0)

[a]The order of preferred term is ordered by the number of total incidences of all groups.

advantage over other ECMs in wound-healing applications. Further, amniotic membrane provides a matrix for cellular migration and proliferation, is nonimmunogenic, reduces inflammation, reduces scar tissue, has antibacterial properties, reduces pain at the site of application, provides a natural biological barrier, and contains a number of essential growth factors and cytokines.[7]

Future research may focus on the comparison of DAMA to other amniotic membrane products. However, few of these products have published prospective, randomized human trials demonstrating clinical outcomes. Zelen et al[8] compared a cultured tissue (Apligraf, Organogenesis Inc, Canton, MA), to a dehydrated human amnion/chorion (EpiFix, MiMedx, Marietta, GA), and SOC (an alginate dressing) for the closure of DFUs, demonstrating a significantly higher rate of closure as well as a shortened time to closure using the dehydrated human amnion/chorion. Lavery et al[15] compared the efficacy of a cryopreserved human amniotic membrane (Grafix, Osiris Therapeutics Inc, Columbia, MD) to standard wound care in patients with DFUs; treatment with this cryopreserved human amniotic membrane resulted in a higher rate of complete wound closure, improved median time to healing, and reduced rate of infection, leading investigators to conclude the cryopreserved human amniotic membrane is safer and more effective than standard wound therapy in the treatment of DFUs.

The dearth of prospective, randomized clinical trials and the varied eligibility criteria makes a clinician's choice of product challenging. It is important to thoroughly assess the available data, including the subjects studied in each trial. In this study, subjects underwent a 2-week run-in period with good SOC and if upon their return the ulcer had closed ≥ 30% in area, the subject was excluded from participation in the study.This criteria differed from the Zelen study[6] with its lack of a screening period, the ≥ 20% area decrease in 2 weeks eligibility criteria of the second Zelen study,[8] as well as the ≥ 30% closure in 1 week of the cryopreserved human amniotic membrane Lavery study.[15] Given the effectiveness of SOC alone,[16] and the demonstration that a 50% closure at 4 weeks was a robust predictor of ultimate closure at 12 weeks,[17,18] the authors believe a screening period was necessary to ensure that only subjects who would not successfully close with SOC alone are included.

Finally, DAMA is ready to use off the shelf, does not

require refrigeration or thawing prior to use, and has no "sided-ness," so either side may be applied to the wound. The hydrophilic nature of this dehydrated tissue causes it to immediately adhere to the moist wound bed, sometimes seeming to disappear due to the intimacy of contact between tissues and eliminating any need to fix the allograft to the wound.

Conclusion

In conclusion, this study demonstrated a statistically significant advantage of DAMA+SOC as compared to SOC alone in facilitating closure of chronic DFUs. Forty-five percent of subjects in the DAMA+SOC cohort achieved complete wound closure, while 0% of SOC alone subjects achieved complete wound closure within 6 weeks (PP population, $P = 0.0137$). Further, there appears to be no increased rate of adverse events associated with use of DAMA in these wounds. Thus, DAMA in combination with SOC, including debridement, well-controlled offloading, management of bacterial burden, and adequate perfusion, is more likely to lead to complete wound closure, to accelerate the rate of wound closure, and presents no additional safety risks when compared to SOC alone in the treatment of DFUs. The biggest limitation of this study is the small sample size, which decreases the generalizability. Additional prospective studies are needed.

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