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A Prospective Multicenter Study of a Weekly Application Regimen of Viable Human Amnion Membrane Allograft in the Management of Nonhealing Diabetic Foot Ulcers

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Background: Diabetic foot ulcers (DFUs) pose a significant clinical challenge for providers and patients, and often precede devastating complications such as infection, hospitalization, and amputation. Therefore, advanced treatment options are needed to facilitate the healing of chronic DFUs and improve outcomes in this high-risk population. Cryopreserved viable human amnion membrane allograft (vHAMA) has shown great promise in the treatment of recalcitrant DFUs as a supplement to standard of care (SOC). Placental grafts are rich in extracellular matrix proteins, growth factors, and cytokines, which can induce angiogenesis and dermal fibroblast proliferation, resulting in accelerated healing.

Methods: In this prospective, multicenter single arm trial, 20 patients with nonhealing DFUs received weekly application of vHAMA, in addition to SOC, for up to 12 weeks. The primary study endpoint was proportion of healed wounds at 12 weeks. Secondary endpoints included proportion of wounds healed at 6 weeks, time to heal, and percentage area wound reduction. Subjects were evaluated for ulcer healing and assessed for adverse events at every treatment visit.

Results: At study conclusion, 85% of patients receiving vHAMA healed. Ten wounds healed (50%) by 6 weeks, and 17 wounds (85%) healed by 12 weeks. The mean time to heal was 46.6 days (95% CI: 35.1–58.0), and the average number of vHAMAs used was 5.4 (SD: 3.25). The mean PAR was 86.3% (SD: 40.51).

Conclusions: Aseptically processed, cryopreserved vHAMA should be considered as a safe and effective option for DFUs refractory to SOC therapy. *(Plast Reconstr Surg Glob Open 2023; 11:e5291; doi: 10.1097/GOX.0000000000005291; Published online 6 October 2023.)*

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INTRODUCTION

Diabetic foot ulcers (DFUs) pose a significant clinical challenge for providers and patients, and often precede devastating complications such as lower extremity infection, hospitalization, and limb amputation. As the worldwide diabetes epidemic shows no signs of abating, treatment of the diabetic foot is increasingly common, costly, and complex. Globally, according to the latest data, the number of adults living with diabetes is 537 million, and is expected to reach 783 million by 2045.[1] The lifetime incidence of a DFU is up to 34%, and between 9.1 and 26.1 million people with diabetes develop a foot ulcer every year.[2] Around the world, a DFU now occurs every 1.2 seconds, and every 20 seconds, a limb is amputated.[3] In the United States, the annual direct cost for diabetes care

Disclosure statements are at the end of this article, following the correspondence information.

is $176 billion, with as much as one-third related to care of the lower extremity, such as DFUs.[4,5]

Furthermore, the 5-year mortality rate after a major lower extremity amputation (above the level of the ankle), is 56.6%, and, when compared with cancer, is only second to lung cancer (80%).[6,7] Following a diabetic amputation, 19% of patients within 1 year, and 37% within 5 years, will undergo another amputation.[8] Thus, given the substantial cost and morbidity associated with diabetic foot complications, the need to accelerate wound healing in chronic DFUs is critically important.

For over a century, human amniotic membrane grafts have been used as biomaterials in reconstructive surgery.[9–18] The grafts, derived from placental membranes, contain large numbers of extracellular matrix proteins, growth factors, and cytokines, which promote wound healing by inducing angiogenesis and proliferation of dermal fibroblasts, as well as recruiting mesenchymal stem cells involved in repair and regeneration.[19–22] Although most of the clinical trials involving chronic ulcers and amniotic grafts have utilized amniotic membranes that are processed via dehydration with terminal sterilization,[23–28] in a study conducted by DiDomenico et al,[29] the authors reported a significant difference in the healing rate of chronic DFUs treated with aseptically processed dehydrated human amnion and chorion allograft (dHACA) versus standard of care (SOC). At 12 weeks, 85% of the wounds receiving dHACA healed.

Another form of amniotic graft, cryopreserved viable human amnion membrane allograft (vHAMA), may also provide advantages in the management of nonhealing DFUs in terms of preserving the amniotic membrane matrix architecture and cellular trafficking (Fig. 1). Regulski et al reported on wounds that received aseptically processed vHAMA, which also demonstrated success in the treatment of chronic, nonhealing wounds of various etiologies, in older patients (aged >65 years) with multiple comorbidities[30] Therefore, the goal of this study was to further prospectively evaluate the efficacy of aseptically processed vHAMA as an adjunctive treatment to SOC in the treatment of chronic DFUs.

Fig. 1. Cryopreserved viable human amnion membrane allograft (vHAMA).

Takeaways

Question: Identifying the best and most expeditious methods for healing diabetic foot ulceration.

Findings: This study was a prospective trial of twenty patients with nonhealing diabetic foot wounds receiving weekly application of viable human amniotic membrane allograft (vHAMA) in addition to standard of care for up to 12 weeks. At study conclusion, 85% of patients receiving vHAMA healed. The mean time to heal was 46.6 days (95% CI: 35.1–58.0), and the average number of vHAMAs used was 5.4 (SD: 3.25). The mean PAR was 86.3% (SD: 40.51).

Meaning: Amniotic membrane grafts that are aseptically processed and cryopreserved are an effective method for treating recalcitrant diabetic foot wounds

METHODS

A prospective, single arm, multicenter clinical trial was conducted without a control arm to evaluate the safety and efficacy of AmnioBand Viable Membrane (MTF Biologics, Edison, N.J.), a cryopreserved vHAMA that is aseptically processed and not subject to terminal sterilization, for the treatment of chronic DFUs. In the study, 20 patients with a history of a chronic DFUs were treated with weekly application of vHAMA, in addition to SOC, for up to 12 weeks, or until complete epithelialization of the wound was noted. The study was conducted in two outpatient wound centers and was approved by the Western Institutional Review Board (sponsor protocol #MTF-DFU-ABD-002, WIRB protocol #20182210).

Patients with nonhealing DFUs, up to and including full thickness, but not extending beyond the subcutaneous layer, and corresponding to University of Texas grade 1A or Wagner grade 1, were included in the study. Patients with wounds present for more than four weeks, but less than 1 year, and refractory to SOC therapies were eligible for participation. Adequate circulation to the affected foot as determined by a dorsum transcutaneous oxygen measurement, a skin perfusion pressure measurement of 30 mm Hg or more, or an ABI between 0.7 and 1.3 or greater within 3 months of the first screening visit, or arterial Doppler with biphasic or triphasic flow to the affected extremity, was required for study inclusion. Subjects with an ulcer extending beyond the subcutaneous layer on either foot (ie, University of Texas grade 2 or Wagner grade 2 or higher), osteomyelitis involving the affected foot as assessed by X-ray within 30 days of study entry, and active Charcot arthropathy were excluded. Baseline demographics, including age, height, weight, gender, race, and comorbidities were obtained from the medical record.

Upon signing informed consent, all patients underwent a two-week screening process in which SOC, consisting of offloading of the DFU with a removable cast walker, appropriate sharp or surgical debridement, infection management as indicated, and use of an appropriate wound dressing, was performed. The appropriate wound dressing included calcium alginate (Fibracol; 3M Corporation Minneapolis, Minn.) and a secondary dressing that

consisted of a padded multilayer dressing and Dynaflex (3M Corporation Minneapolis, Minn.), changed weekly. Upon meeting the inclusion and exclusion criteria and not improving by at least 20% within the screening period, the patient was permitted to enroll. Wounds were then treated with weekly application of vHAMA, in addition to SOC, for up to 12 weeks, or until complete wound healing was noted. During every treatment visit, wounds were sharply debrided, cleaned, digitally photographed, and measured based on ruler measurement and acetate tracing. Acceptable SOC primary dressings used in the study included a nonadherent dressing (Adaptic Touch; 3M Corporation Minneapolis, Minn.). The secondary dressing consisted of a padded multilayer dressing (Dynaflex; 3M Corporation Minneapolis, Minn.), changed weekly. Subjects were evaluated for ulcer healing and assessed for adverse events at every treatment visit. In the event of infection involving the study ulcer, treatment involved aggressive sharp debridement, if appropriate; systemic antibiotics; and local antimicrobial dressings. Wound culture, if indicated, was taken post debridement. Subjects were withdrawn from the study if percentage surface area reduction (PAR) was less than 50% at 6 weeks.[31] A healing confirmation visit was conducted after complete wound closure occurred.

Statistical Analysis

The intent-to-treat (ITT) and safety populations comprised patients who received at least one treatment. All analyses used the ITT approach. The last observation carried forward principle was used in regard to missing observations. Study variables were summarized as means and SDs (±SDs) for continuous variables as well as medians for nonnormal data. Categorical variables were presented as counts and proportions or percentages. Statistical testing with the treatment group (wound area) used a paired *t* test. Two-sided *P* values of less than 0.05 were considered significant. PASW 25 (IBM, Chicago, Ill.) was used to perform all statistical testing. The PAR for the index wound at 12 weeks was calculated as $[(A_I - A_{XW})/A_I]*100$, where A_I is the area of the index wound at randomization and A_{XW} the area at 6 or 12 weeks. Summation of number of grafts per patient was based on one graft at each visit until the wound healed or an event occurred (withdrawal of patient).

RESULTS

A total of 20 patients who passed screening with a chronic DFU and adequate arterial perfusion were included in this single arm, multicenter trial. After the screening process, each patient received weekly application of vHAMA, as an adjunctive treatment to SOC, for up to 12 weeks, or until complete epithelialization of the ulcer was noted. Wounds included in the study were recalcitrant to SOC for at least 4 weeks, but no longer than 1 year, before beginning treatment.

Patient demographics are detailed in Table 1. The mean subject age was 64.5 years (SD: 10.37), and average BMI was 33.4 (SD: 5.07). The HbA1c at screening was 6.7 (SD: 1.45). There was an even distribution of men and women, and all patients (100%) had a history of hypertension.

Table 1. Key Subject-related Variables

Variable	Value
Patient age (y)	64.5 (10.37)
BMI	33.4 (5.07)
Gender	
Male	10 (50%)
Female	10 (50%)
Race/ethnicity	
White	19 (95%)
African American	1 (5%)
Non-Hispanic	20 (100%)
HbA1c (screening)	6.7 (1.45)
HbA1c (end of study)	6.9 (1.30)
Serum creatinine	1.2 mg/dL (0.41)
Smoker	5 (25%)
Nonsmoker	15 (75%)
Hypertension	20 (100%)

BMI: Body mass index.
Continuous variables are reported as means (SD) and categorical variables as counts (percentage)

Wound characteristics are shown in Table 2. The baseline wound area was 3.0 cm^2 (SD: 2.74) with an average ulcer age of 11.7 weeks (SD: 11.93). Most ulcers were located on the midfoot (50%) and plantarly (85%).

Overall, 85% of patients receiving weekly application of vHAMA healed. Ten wounds healed (50%) by 6 weeks, and 17 wounds (85%) healed by 12 weeks. Representative case examples are shown in Figures 2–4. Three patients did not heal, including one who demonstrated less than 50% PAR by week 6. The other two patients were withdrawn due to an adverse event (cellulitis) and serious adverse event (UTI and hospitalization), respectively, that the principal investigator determined were not related to the use of vHAMA. There were no major or

Table 2. Key Wound-related Variables

Variable	Value
Wound area (cm^2)	3.0 (2.74)
	Median: 1.8
	IQR: 2.6
Wound aspect	
Plantar	17 (85%)
Dorsal	2 (10%)
Heel	1 (5%)
Wound location	
Toe	6 (30%)
Forefoot	3 (15%)
Midfoot	10 (50%)
Heel	1 (5%)
No. vHAMAs used	5.4 (3.25)
	Median: 4.5
	IQR: 6.0

IQR: interquartile range.
Continuous variables are reported as means (SD) with median/IQR additionally reported for key nonnormally distributed continuous variables, and categorical variables as counts (percentage).

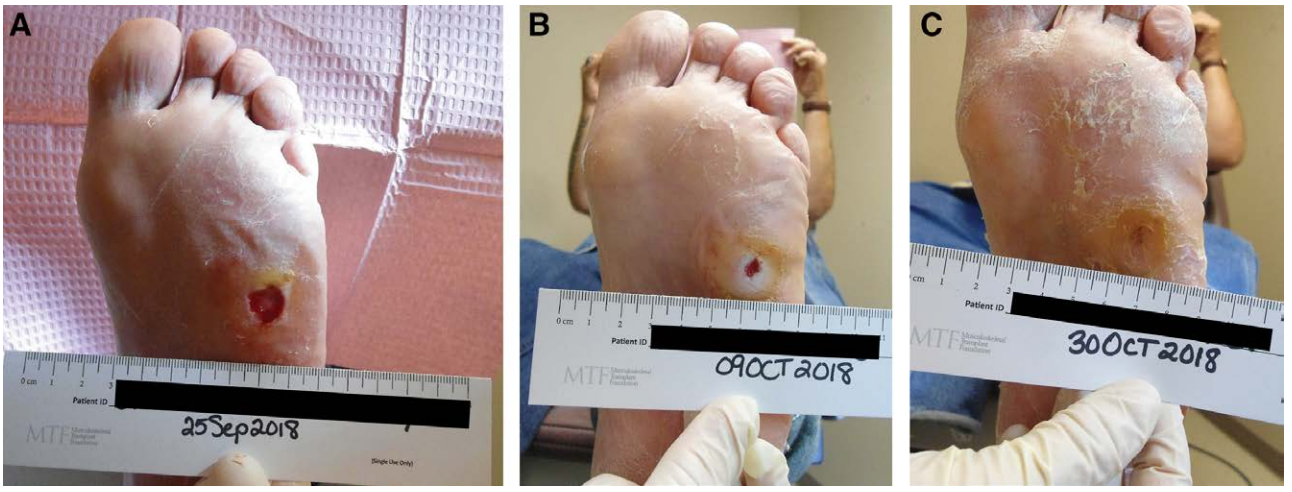

Fig. 2. A 60-year-old man with a chronic midfoot DFU, present for 50 weeks. A, At baseline, the ulcer measured 1.20 cm^2 and the patient had HgA1c of 10.5 and creatinine of 1.40. B, After two graft applications, the wound measured 0.20 cm^2, representing a 90% improvement. C, The ulcer demonstrated complete epithelialization following four graft applications.

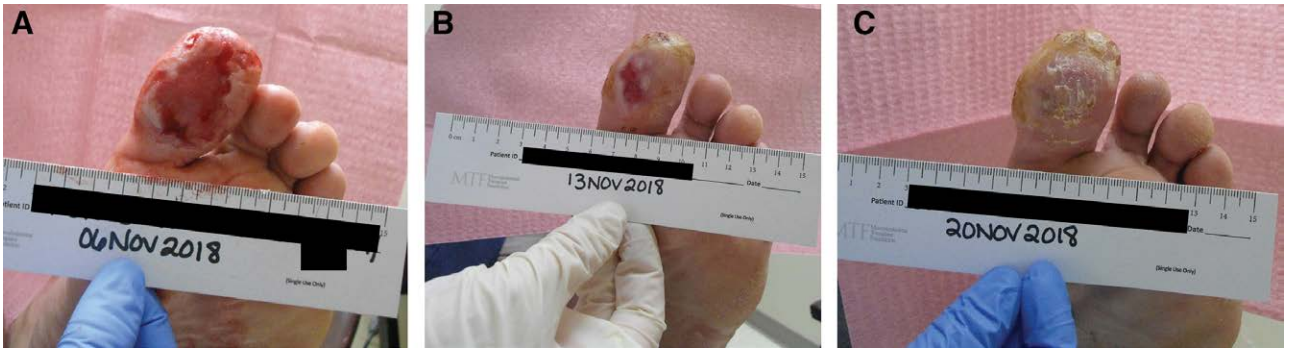

Fig. 3. A 66-year-old patient with chronic hallux DFU, present for 28 weeks. A, The ulcer measured 10.5 cm^2 at baseline. B, After application of one graft, the wound exhibited 90% healing. C, The wound healed following two graft applications.

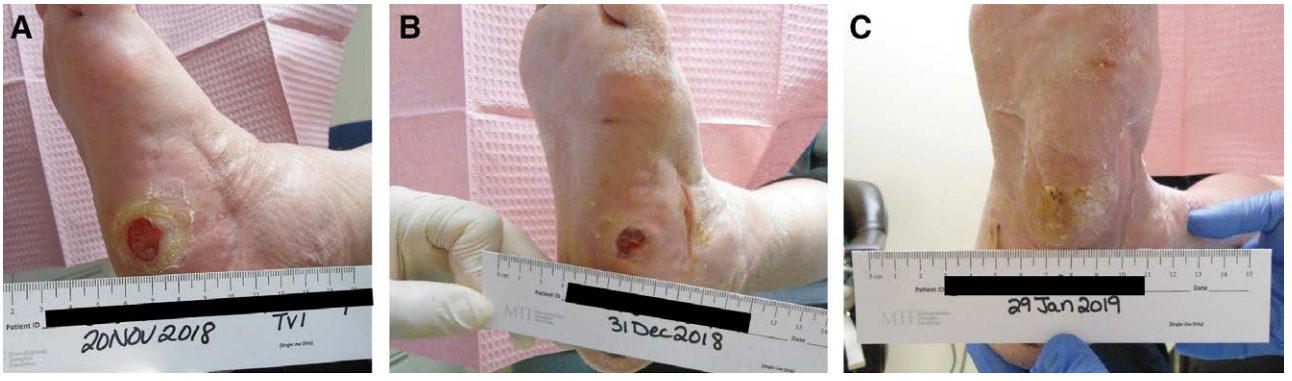

Fig. 4. A 59-year-old patient with chronic midfoot DFU. A, The ulcer measured 1.70 cm^2 at baseline. B, Following six graft applications, the ulcer was greater than 50% closed. C, The wound demonstrated complete healing following nine graft applications.

minor amputations in this cohort. The three patients who did not heal went on to receive various other treatments, including surgical debridement for the patient having infection as well as secondary closure and split thickness skin grafting.

The mean time to heal in these wounds was 46.6 days (95% CI: 35.1–58.0), and the average number of vHAMAs used was 5.4 (SD: 3.25). The percentage of wounds healed by week is shown in Figure 5. The mean PAR was 86.3% (SD: 40.51), as shown in Figure 6.

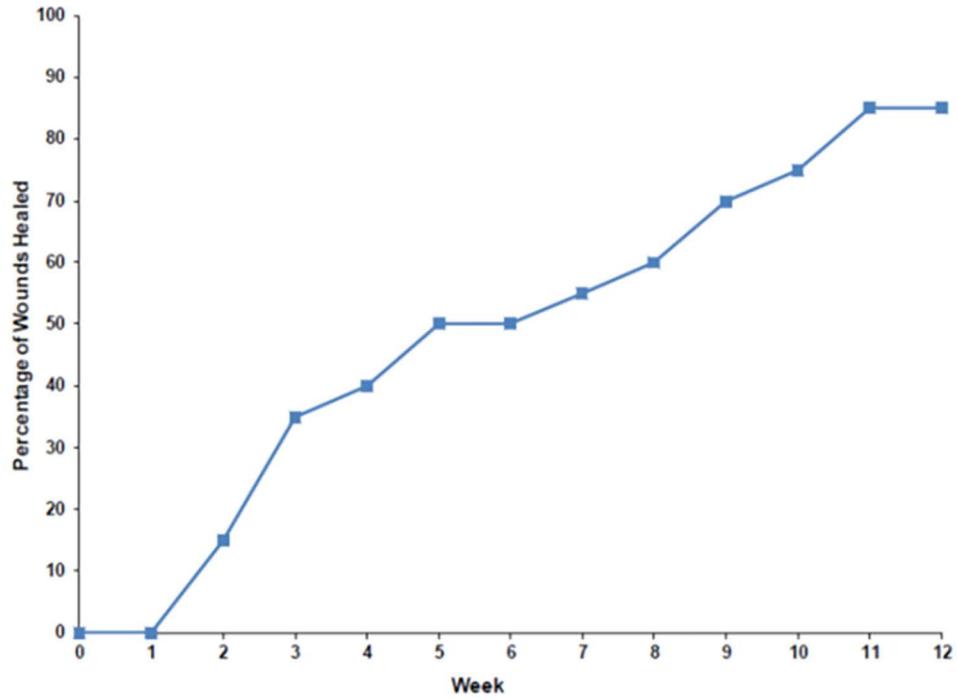

Fig. 5. Percentage of wounds closed by week.

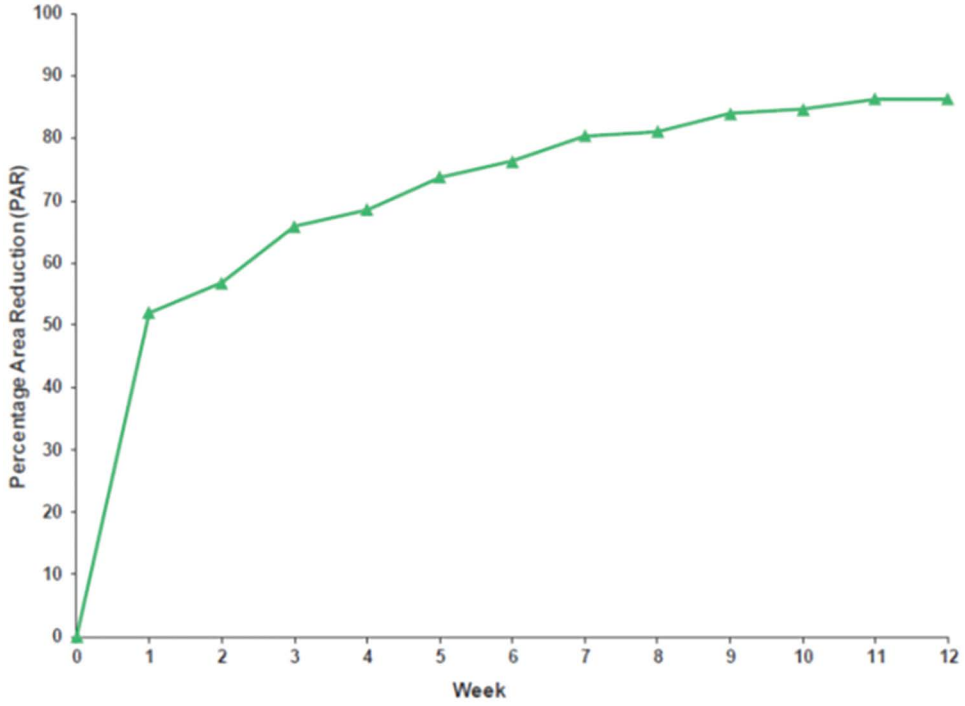

Fig. 6. Percent wound area reduction (PAR) over 12 weeks.

DISCUSSION

Diabetic foot complications continue to wreak havoc on the global health system in terms of overall cost and patient morbidity, and chronic DFUs often lead to infection and amputation. In our cohort of subjects, most had failed modalities including hydrogels, debridement agents, antibiotic creams, silver and standard alginate, and negative pressure wound management did not result in successful healing. The use of placental membrane grafts, rich in extracellular matrix proteins,

growth factors, and cytokines, have been shown to accelerate healing in chronic DFUs. Furthermore, vHAMA has shown tremendous promise in the treatment of DFUs recalcitrant to SOC. The purpose of this study was to evaluate the effectiveness of vHAMA as an adjunctive treatment to SOC in the treatment of chronic DFUs that do not extend through subcutaneous tissue at 12 weeks. There were three failures per protocol; each of those subjects showed improvement in wound size during the study period, and in fact, two of the subjects exited due to adverse events not related to the vHAMA treatment. So certainly, if you exclude the patients whose treatment did not fail due to use of the vHAMA, the healing rate would be as high as 17 of 18 patients, or 94%. For comparison, in a randomized controlled study conducted by Lavery and coworkers,[26] patients receiving a human viable wound matrix demonstrated a significantly higher proportion of wounds healed and faster healing rate compared with SOC [(62%) compared with controls (21%, $P = 0{\cdot}0001$)].

Using an aseptically processed dehydrated human amnion and chorion allograft (dHACA), DiDomenico et al reported that among 80 patients with chronic DFUs, 85% of dHACA-treated wounds healed. The mean healing time was significantly faster for the dHACA- treated group (37 days) compared with SOC (67 days), and the mean number of grafts used per ulcer healed was 4.0. The mean cost of tissue to heal a DFU was $1771.[32] The proportion of wounds healed is consistent with the findings of our study, in which 85% of patients receiving weekly application of vHAMA healed, and far superior to the healing rate of their SOC group (33%). Moreover, Glat and colleagues[33] conducted a randomized controlled trial in which dHACA was compared with a commonly used tissue-engineered skin substitute, and found that the aseptically processed amnion and chorion graft provided superior healing rates and a lower cost than the tissue-engineered skin substitute. These results further demonstrate the versatility of aseptically-processed placental allografts in various forms for the mitigation of nonhealing DFUs. Both dHACA and vHAMA are aseptically processed; the dHACA contains both amnion and chorion in the graft, whereas vHAMA contains only the amnion layer, with both tissues showing healing in stalled DFUs. The application regimen of both grafts is identical, with the only exception being that the cryopreserved graft requires thawing in a warm water bath and a saline lavage before application, but dHACA is ready for use off the shelf and stored at room temperature. Further preclinical and scientific evaluations are ongoing by the primary author to better understand the underlying mechanisms of action of the two tissue forms related to their clinical outcomes. There is no significant difference in the cost of the two allografts.

There are many strengths in this prospective, multicenter trial, including a robust trial design, appropriate screening procedures, a standardized approach to SOC, ITT analysis, and appropriate adjustment for multiple statistical testing. Study weaknesses include the fact that there was only one treatment arm and no comparator and a small sample size. Further randomized controlled models against SOC alone or a well-established advanced wound care modality with a larger sample population can be considered to validate these initial satisfactory results. Further, an economic analysis on costs of the graft and SOC should be considered in larger trials. Finally, expanding and reporting on any additional comorbidities and changes in medical management during the study period could add additional insight into how the subjects responded to treatment.

CONCLUSIONS

The results of this prospective, multicenter clinical trial with aseptically processed vHAMA confirms previously reported positive outcomes in chronic wounds of various etiologies, and supports its use as an effective treatment option for DFUs as a supplement to SOC. Although the number of patients was limited and the criteria were aligned with those of larger randomized trials rather than real world registries, the high healing rate clearly shows the value of the amniotic tissue for the treatment of stalled DFUs. Future larger multicenter randomized controlled trials and real world trials and registries will help confirm these positive findings.

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DISCLOSURES

Robert Galiano is a consultant for MTF Biologics and receives research funding through grants to Northwestern University School of Medicine. Dennis Orgill is a consultant for MTF Biologics and receives research funding through grants to Brigham and Women's Hospital. Paul Glat is the medical director and owner of Dr. Glat PC and receives research funding from MTF biologics. David G. Armstrong has no financial interest to declare in relation to the content of this article. Marissa Carter receives funds as a consultant to PERI and MTF Biologics to conduct the statistical analysis for this trial. Charles M. Zelen, DPM is the medical director and president of the Professional Education and Research Institute and received funds from MTF Biologics to conduct this clinical trial. This study was supported by MTF Biologics, Edison, New Jersey.

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