A multicenter, randomized, controlled clinical trial evaluating the use of dehydrated human amnion/chorion membrane allografts and multilayer compression therapy vs. multilayer compression therapy alone in the treatment of venous leg ulcers

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ABSTRACT

Venous leg ulcers produce significant clinical and economic burdens on society and often require advanced wound therapy. The purpose of this multicenter, randomized, controlled study is to evaluate the safety and efficacy of one or two applications of dehydrated human amnion/chorion membrane allograft and multilayer compression therapy vs. multilayer compression therapy alone in the treatment of venous leg ulcers. The primary study outcome was the proportion of patients achieving 40% wound closure at 4 weeks. Of the 84 participants enrolled, 53 were randomized to receive allograft and 31 were randomized to the control group of multilayer compression therapy alone. At 4 weeks, 62% in the allograft group and 32% in the control group showed a greater than 40% wound closure ($p = 0.005$), thus showing a significant difference between the allograft-treated groups and the multilayer compression therapy alone group at the 4-week surrogate endpoint. After 4 weeks, wounds treated with allograft had reduced in size a mean of 48.1% compared with 19.0% for controls. Venous leg ulcers treated with allograft had a significant improvement in healing at 4 weeks compared with multilayer compression therapy alone.

Chronic leg wounds due to venous hypertension are emerging as a major clinical care and public health challenge.[1] Costs associated with chronic wounds are significant. Annually in the United States, direct costs for treatment of chronic wounds are approximately $25 billion per year.[2] Venous ulceration is the most common type of lower extremity wound, as approximately 80% of leg ulcers have a venous component.[3] Between 500,000 and 2 million persons annually in the United States are affected with chronic venous leg ulcers (VLUs).[4,5] The prevalence of VLUs will continue to increase due to the aging population and increasing incidence of risk factors such as obesity and congestive heart failure.[2]

Chronic VLUs are associated with considerable morbidity and impaired quality of life with healing being a long and painful process.[6] Even under the best of circumstances, these ulcers require weeks or months to heal. The natural history of the disease is a continuous and frustrating cycle of slow healing and recurrent breakdown. Not uncommonly wound care specialists see patients who have suffered for years or face amputation of the limb as their only option to alleviate the pain.

The therapeutic mainstay in management of venous leg ulceration is graduated compression bandaging. Healing rates in VLUs are significantly improved by the application of compression therapy,[7,8] although overall healing rates and time to healing vary greatly.[9] Given that VLUs often have a prolonged trajectory of healing, early identification of patients unlikely to heal with standard compression therapy allows for more expedient modification of the plan of care to include more advanced wound care products and potentially reduce a patient's morbidity and suffering. Additional economic and clinical research benefits are also derived from being able to tell early in the treatment process whether a therapy is working. Evaluating the efficacy of new VLU treatments is often difficult given the protracted study duration required before an endpoint of complete wound epithelialization can be achieved, thus intermediary outcomes may be reasonable allowing for more rapid evaluation of treatment safety and potential benefits. In patients receiving standard

dHACM	Dehydrated human amnion/chorion membrane
MLCT	Multilayer compression therapy
VAS	Visual analog scale
VLUs	Venous leg ulcers

wound care, it has been shown that the percent change in wound area of a VLU at the third or fourth week of care can serve as an important surrogate marker of complete wound healing after 12 or 24 weeks of care.[9–12]

Amniotic membrane is a unique material, and its composition contains collagen types IV, V, and VII. Amniotic membrane is a structural extracellular matrix (ECM), which also contains specialized proteins, fibronectins, laminins, proteoglycans, and glycosaminoglycans. In addition, amniotic membrane delivers well-known essential wound healing growth factors like epidermal growth factor (EGF), transforming growth factor beta (TGF-β), fibroblast growth factors (FGFs), and platelet-derived growth factors (PDGFs) to the wound surface. In their natural state, these growth factors increase cell signaling and promote epithelialization of the wound bed.[13]

Recently, with advances in preparation and preservation techniques, a dehydrated human amnion/chorion membrane (dHACM) allograft (EpiFix, MiMedx Group, Inc., Marietta, GA) has become commercially available as a biologic material for chronic and acute wound care management. The purpose of the present study is to evaluate the safety and efficacy of dHACM in addition to multilayer compression therapy (MLCT) vs. MLCT alone in the treatment of VLUs.

MATERIALS AND METHODS

We conducted a multicenter, randomized, controlled open-label study designed to evaluate the safety and efficacy of dHACM allograft (either one application or two applications) and MLCT vs. MLCT alone in the healing of VLUs. The study population consisted of patients with VLUs receiving care from physicians specializing in wound care and podiatric specialists in eight outpatient wound care centers geographically distributed in the United States (Pennsylvania, Massachusetts, Florida, Oklahoma, Indiana, and Texas). The study was conducted under direction of a primary investigator (Dr. Thomas Serena). Consent was obtained prior to any study-related procedures, and all patients signed an Investigational Review Board (IRB)-approved informed consent form. In obtaining and documenting informed consent, the Investigator complied with applicable regulatory requirements and adhered to Good Clinical Practice. This study was conducted in accordance with the provisions of the Declaration of Helsinki. Additionally, all study products used in this study were manufactured, handled, and stored in accordance with applicable Good Manufacturing Practices. The study was reviewed and approved by Liberty IRB or each sites local IRB and preregistered in Clinical Trials.gov (NCT01552447). Confidentiality was maintained with all patient records.

Patient screening and eligibility

The study population was comprised of patients presenting for care of a VLU. Patients were eligible for inclusion if they were willing to participate in the clinical study and agreed to comply with the weekly visits and follow-up regimen. The study consisted of two phases: screening and treatment. The screening period was designed to determine whether subjects were eligible to proceed to the treatment period of the study. Inclusion and exclusion criteria are listed in Table 1. During this screening period, a series of assessments was conducted

Table 1. Inclusion and exclusion criteria

Inclusion criteria
Age 18 or older
ABI >0.75
Presence of a VLU extending through the full thickness of the skin but not down to muscle, tendon, or bone
VLU present for at least 1 month
VLU is a minimum of 2 cm² and a maximum of 20 cm²
VLU has been treated with compression therapy for at least 14 days
Ulcer has a clean, granulating base with minimal adherent slough

Exclusion criteria
Ulcer caused by a medical condition other than venous insufficiency
Exhibits clinical signs and symptoms of infection
Uncontrolled diabetes (HbA1c >10%)
Suspicious for cancer
History of more than 2 weeks treatment with immunosuppressant's, cytotoxic chemotherapy, or application of topical steroids within 1 month
Investigational drug(s) or therapeutic device(s) within 30 days
History of radiation at ulcer site
Undergone 12 months of continuous high strength compression therapy over its duration
Known history of AIDS or HIV
Previously treated with tissue engineered materials (e.g., Apligraf or Dermagraft) or other scaffold materials (e.g., Oasis, Matristem) within the last 30 days
Requiring negative pressure wound therapy or hyperbaric oxygen
NYHA Class III and IV congestive heart failure (CHF)
Ulcers on the dorsum of the foot or with more than 50% of the ulcer below the malleolus
Pregnant or breast feeding
Allergic to gentamicin and streptomycin

to determine eligibility and included: demographics, medical history, assessment of concomitant medications, vital signs, physical examination, pain assessment using visual analog scale (VAS), leg ulcer history, assessment of signs and symptoms of clinical infection of the study ulcer, and ankle-brachial index measurement.

At the first screening visit, the investigator assessed the study ulcer. In the situation where a patient had more than one VLU, the largest VLU that met the eligibility criteria was selected as the study ulcer. Patients whose target ulcer had been treated with MLCT for at least 2 weeks were eligible to enter the treatment phase immediately once all of the inclusion and exclusion criteria were met. If the ulcer had not

received MLCT, the patient was placed in compression and enrolled in the study after 14 days of MLCT. The study ulcer was cleaned and debrided if applicable. The wound area was calculated by multiplying the width and length. A digital photo of the ulcer was taken. An MLCT bandage was applied, and the next visit was scheduled.

Study treatment

The treatment phase began with a series of assessments designed to confirm the patients' continued eligibility. Subjects who continued to meet study inclusion criteria after the screening period were randomized to one of three groups: (1) one application of dHACM and MLCT; (2) two applications of dHACM and MLCT; or (3) MLCT alone, which was considered standard of care for this study. Neither patients nor clinicians were blinded to group assignment. The randomization schedule was balanced and permuted in blocks of 15. When a patient was ready for randomization, the study site called a representative from the sponsor who then opened a sequentially numbered opaque envelope to disclose the group assignment, thus ensuring allocation concealment. During the 4-week treatment phase, patients were reevaluated on a weekly basis. The dHACM was applied once in the one dHACM application treatment group at day zero and applied twice in the two dHACM applications treatment group at day zero and week 2. The MLCT bandage (Coban2, 3M St. Paul, MN) was used in both groups and applied at every visit according to the manufacturer's suggested technique.

During the four weekly follow-up visits after randomization, assessments were performed in the following order: pain assessment using VAS; assessment of existing compression bandage; review of concomitant medications, changes in the subject's health and occurrence of adverse events defined as any unfavorable or unintended sign, symptom, or disease that occurred or was reported by the patient to have occurred, or a worsening of a preexisting condition; and study ulcer closure assessment. If the study ulcer was 100% reepithelialized, no other study procedures were completed at this visit, and the patient was scheduled for a follow-up visit after 1 week to verify healing. If complete healing was not observed, an assessment for signs of clinical infection was performed. If clinical diagnosis of infection was made, treatment with topical antimicrobials or oral antibiotics was permissible, but not topical antibiotics. After infection assessment, the ulcer was cleaned, photographed, and debrided at the discretion of the investigator to obtain a clean, granulating ulcer base with minimal adherent slough. MLCT was then reapplied, and the patient was instructed to keep the bandaging dry and to call or visit the study site if the bandage became soiled or removed.

Study completion

Patients completed the study 4 weeks after the first treatment visit. In addition, patients whose study ulcer closed prior to the 4-week visit were considered as having completed the study. Complete healing of the study ulcer was defined as 100% reepithelialization without drainage. At any point during the treatment period, patients could refuse to participate or withdraw from the study without prejudice. If a patient withdrew from the study, their last available wound measurement was carried forward and used to calculate change in wound size and their final outcome.

Study outcomes

The primary study outcome was the proportion of wounds achieving 40% closure at 4 weeks in patients treated with dHACM and MLCT vs. MLCT alone. Secondary outcomes included: (1) proportion of 40% wound closure at 4 weeks in patients receiving two applications of dHACM vs. MLCT; (2) proportion of 40% wound closure at 4 weeks in patients receiving one application of dHACM vs. MLCT; and (3) proportion of 40% wound closure at 4 weeks in patients receiving one application of dHACM vs. two applications of dHACM.

Statistical methods

The null hypothesis is that the proportion of dHACM-treated subjects (one or two treatments) who reach 40% wound closure at 4 weeks is the same as the proportion of MLCT only (allocation ratio overall of 1:1:1, which is 2:1 dHACM/MLCT only). If this hypothesis is rejected, then either one treatment or two treatments can be considered superior.

Sample sizes of 30 in each group were calculated to achieve a power of 81% when the difference between proportions healed at 4 weeks was 0.30 and the proportion healed in the MLCT group was 0.2. The test statistic used was the two-sided likelihood ratio test with a significance level of 0.047. An interim analysis was planned by the study sponsor after enrollment of 60 patients to determine adequacy of sample size.

An intent-to-treat analysis was used including all patients as originally allocated after randomization. For missing observations, the last known value was carried forward. Study variables were summarized as means and standard deviations for continuous variables and proportions/percentages for categories.

Chi-square test was used to compare groups in regard to study outcomes with statistical testing of outcomes in prespecified order (see Study Outcomes) to control for the global familywise error rate (i.e., a closed gatekeeping testing sequence applied to all primary/secondary endpoints). Alpha was set to 0.05 with all tests performed as two sided. SAS 9.4 (SAS institute, Inc., Cary, NC) was used to perform statistical testing.

RESULTS

Of 141 patients presenting with VLUs initially screened for eligibility, 88 patients entered the screening phase of the study between March 2012 and March 2014. Of these 88 patients, three patients no longer met eligibility requirements at their randomization visit, and one patient withdrew consent prior to randomization, thus four patients were not randomized into the treatment phase. Of the 84 patients enrolled in the treatment phase, 26 were randomized to the one dHACM application group, 27 were randomized to two dHACM applications group, and 31 were randomized to receive MLCT only (Figure 1). At study enrollment, no differences were observed in patient characteristics, wound size, or wound duration between those receiving either one or two applications of dHACM and those receiving MLCT only. Patient characteristics for the study groups are presented in Table 2.
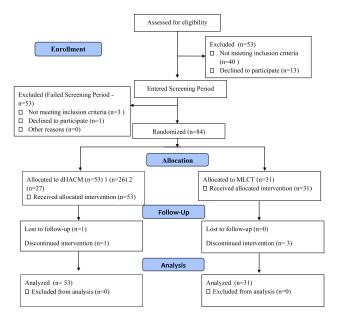
Figure 1. Flow diagram of study enrollment, intervention allocation, follow-up, and data analysis.

Figure 2. Mean percent reduction in wound size during the 4-week study period.

Study outcomes

The primary study outcome was proportion of patients with ≥40% reduction of wound size at 4 weeks for those receiving dHACM vs. those receiving MLCT only. Reduction in wound size of ≥40% occurred in significantly greater numbers of patients receiving dHACM vs. those receiving MLCT only (33/53 [62%] vs 10/31 [32%]; $p = 0.005$). Within the dHACM group, proportions of wound reduction ≥40% after 4 weeks were similar for those patients receiving one vs. two dHACM applications (62% [16/26] and 63.0% [17/27], respectively,

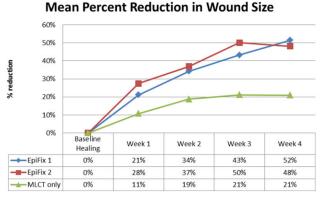not statistically significant), but comparison of these proportions to the MLCT group showed statistically significant proportions in favor of two applications or one application of dHACM: $p = 0.019$ and 0.027, respectively.

Patients receiving dHACM in addition to MLCT had a mean reduction in VLU size over the 4-week study period of 48.1% compared with 19.0% in the MLCT only group. Percent of wound reduction was similar for those receiving one or two dHACM applications at 51.9% and 44.4%. Mean percent reduction in VLU size at each week during the study period are presented in Figure 2. Note the increased rates of healing in both dHACM groups compared with those patients receiving only MLCT during the 4-week study period. Within the dHACM group, wound area was reduced by a mean of 2.28 ± 3.04 cm^2 during the study period (from randomization to end of study). For those patients receiving MLCT only, wound area was not reduced as much during the study period with a mean difference of only 0.41 ± 2.68 cm^2 between

Table 2. Characteristics of study sample

	dHACM ($n = 53$)		MLCT only ($n = 31$)	
	Mean (SD) or Percentage	Median (IQR)	Mean (SD) or Percentage	Median (IQR)
Age (y)	59.0 (17.75)	60.0 (18.72)	62.6 (13.53)	61.1 (13.9)
≥65 years	39.6%	—	35.5%	—
Male	58.5%	—	48.4%	—
Body mass index	37.6 (14.09)	34.4 (16.75)	37.0 (10.30)	36.6 (14.62)
Obese	69.8%	—	74.2%	—
VLU duration (months)	13.8 (20.83)	4 (10.5)	13.0 (16.40)	5.5 (15)
VLU duration >12 months	24.5%	—	25.8%	—
VLU size (cm^2)	6.0 (4.33)	4.4 (4.3)	6.3 (5.27)	4.1 (5.9)
VLU Size >10 cm^2	18.9%	—	19.4%	—
VAS pain score (baseline)	3.9 (3.01) ($n = 49$)	3.7 (5.5)	2.9 (2.85) ($n = 30$)	2.15 (5.71)

Data presented as mean ± SD, or percentage as indicated.
dHACM, dehydrated human amnion/chorion membrane; MLCT, multilayer compression therapy; VAS, visual analog scale; VLU, venous leg ulcer.

Figure 3. Case example of healing between baseline observation and 4-week study endpoint for one patient receiving dHACM.

randomization and the 4-week visit. In the 4-week study period, six patients in the dHACM group and four patients in the MLCT group had complete wound closure. Examples of wound closure during the 4-week study period for patients receiving dHACM are presented in Figure 3.

Pain scores were collected at randomization and week 4 for 49 patients in the dHACM group and 28 patients in the MLCT only group using a VAS. Of those with recorded pain scores (44/49 [89.8%]) in the dHACM group reported VLU pain at randomization and in the MLCT group (21/28 [75.0%]) reported VLU pain. During the study period, 35/44 (79.5%) of patients in the dHACM group reported a reduction in pain from the randomization visit when dHACM was applied and the 4-week visit. For patients receiving MLCT only, 11/21 (52.4%) reported reduced VLU pain in the study period.

Study completion

Five patients, two in the dHACM group and three in the MLCT only group, failed to complete the study. Of the two in the dHACM group, one withdrew after two visits due to a wound abscess and one was lost to follow-up after three visits with 67.5% wound closure. In the MLCT only group, two patients withdrew after one visit and one patient withdrew following randomization as they were unhappy with their group assignment.

Adverse events

There were 14 adverse events reported from 12 patients. In the dHACM group, seven patients reported nine adverse events. Five of the nine events consisting of falls, worsening COPD, syncope, and cellulitis of nonstudy leg were unrelated to treatment. There were two reported cases of cellulitis on the affected extremity, one wound infection, and one wound with increased drainage and abscess. There were five patients with adverse events in the MLCT group including bronchitis, febrile confusion, maceration around the wound with increased drainage, and two wound infections.

DISCUSSION

Previous studies have established that weekly or every other week application of dHACM is an effective treatment for chronic diabetic foot ulcers.[14–16] This current study is unique as it is the first randomized trial to evaluate the efficacy of dHACM allograft as a treatment for VLUs. It is also unique in its use of

a surrogate endpoint to measure results of healing with an advanced wound care product. Our results show that VLUs treated with dHACM in addition to MLCT heal in a significantly more rapid fashion than those treated with MLCT alone.

Although human amniotic membrane in its natural state has been used as a wound covering for over 100 years, there are often issues relative to obtaining, preparing, and storing the tissue for use in clinical practice, along with potential for infectious disease transmission.[17] The dHACM used in this study is a commercially available allograft (EpiFix, MiMedx Group Inc.).[18] The proprietary PURION® Process (MiMedx Group, Inc.) safely and gently separates placental tissues donated from screened and tested women undergoing Cesarean delivery, cleans and reassembles various layers, and then dehydrates the tissue.[19] PURION Processed dHACM retains its biological activities related to wound healing, including the potential to positively affect four distinct and pivotal physiological processes intimately involved in wound healing: cell proliferation, inflammation, metalloproteinase activity, and recruitment of progenitor cells.[19] The dHACM material has a stable shelf life of 5 years at ambient conditions. It is available in multiple sizes that allow the clinician to utilize a wound appropriate–sized graft and therefore minimize waste. The dHACM has been shown to contain many growth factors that help in wound healing, including PDGF-AA, PDGF-BB, bFGF, TGF-β1, EGF, vascular endothelial growth factor, and placental growth factor (PPlGF).[20] In addition to growth factors, cytokines including anti-inflammatory interleukins (IL-1ra, IL-4, and IL-10) and the tissue inhibitor of metalloproteinases (TIMPs) (TIMP-1, TIMP-2, and TIMP-4), which help regulate the matrix metalloproteinase (MMP) activity, are also present in dHACM.[19] Results from both in vitro and in vivo experiments clearly established that dHACM contains one or more soluble factors capable of stimulating mesenchymal stem cell migration and recruitment.[19] In addition to these important regenerative molecules, other components of dHACM, specifically the collagen-rich ECM, could act to reduce MMPs or provide a scaffolding substrate for cellular ingrowth, and these activities could be part of the mechanism for the product's effectiveness for wound healing.

The ultimate goal of treating VLUs is to achieve complete healing, yet less than two-thirds (62%) of all VLUs heal by 24 weeks with standard care.[20] Given the long healing period, intermediate endpoints such as percent change in wound area by the fourth week of treatment have been shown to be important surrogate markers of complete wound healing by 12 or 24 weeks.[10] Surrogate endpoints that can predict the ultimate outcome of treatment are beneficial for researchers of new wound healing products or techniques allowing for more rapid evaluation of potentially promising innovations When surrogate endpoints are used, clinical trials can be more efficiently executed with less follow-up time needed to complete the study and a lower required sample size.[10] Resources can then be directed toward those products or techniques that show potential as an effective treatment, saving time and money while mitigating patient risks. Operationally, a shorter study duration reduces the risk for noncompliance, loss to follow-up, and missing data, resulting in more accurate observations. From both a clinical and ethical standpoint, surrogate outcomes allow for patients with nonresponding wounds to seek alternative treatment and possibly reduce their suffering.

A strength of our study is its randomized, multicenter design, although care givers were unable to be blinded as to

group assignment. Objective measurements were used to determine wound size, and all assessments were conducted in a specific sequence during the study to reduce bias. Lack of long-term follow-up data does not allow us to validate the surrogate endpoint against ultimate rates of healing or durability of healed wounds, although retrospective follow-up is currently underway. Further studies are needed to determine how healing rates with PURION Processed dHACM compare with other advanced therapies. Because those patients receiving dHACM only received one or two allografts during the study period, we do not know if more frequent application of dHACM during a protracted healing period is beneficial. These study results may not be generalized to other amniotic membrane products, as it is unknown how differences in preservation techniques and membrane configurations influence product effectiveness. As all patients received care in a wound care center and received MLCT, we do not know the generalizability of our results in other settings or when MLCT is not utilized.

Use of a surrogate endpoint allowed us to identify that VLUs treated with dHACM in addition to MLCT had significantly reduced wound size within 4 weeks after one or two dHACM applications compared with MLCT therapy alone. Although further studies are needed to determine ultimate healing rates and ideal frequency of dHACM application, the results of this first clinical trial support the use of dHACM as an efficacious treatment for VLUs.

ACKNOWLEDGMENTS

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