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ORIGINAL ARTICLE

A confirmatory study on the efficacy of dehydrated human amnion/chorion membrane dHACM allograft in the management of diabetic foot ulcers: A prospective, multicentre, randomised, controlled study of 110 patients from 14 wound clinics

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A randomised, controlled multicentre clinical trial was conducted at 14 wound care centres in the United States to confirm the efficacy of dehydrated human amnion/chorion membrane allograft (dHACM) for the treatment of chronic lower extremity ulcers in persons with diabetes. Patients with a lower extremity ulcer of at least 4 weeks duration were entered into a 2-week study run-in phase and treated with alginate wound dressings and appropriate offloading. Those with less than or equal to 25% wound closure after run-in were randomly assigned to receive weekly dHACM application in addition to offloading or standard of care with alginate wound dressings, for 12 weeks. A total of 110 patients were included in the intent-to-treat (ITT) analysis, with $n = 54$ in the dHACM group and $n = 56$ in the no-dHACM group. Of the participants, 98 completed the study per protocol, with 47 receiving dHACM and 51 not receiving dHACM. The primary study outcome was percentage of study ulcers completely healed in 12 weeks, with both ITT and per-protocol participants receiving weekly dHACM significantly more likely to completely heal than those not receiving dHACM (ITT—70% versus 50%, $P = 0.0338$, per-protocol—81% versus 55%, $P = 0.0093$). A Kaplan–Meier analysis was performed to compare the time-to-healing performance with/without dHACM, showing a significantly improved time to healing with the use of allograft, log-rank $P < 0.0187$. Cox regression analysis showed that dHACM-treated subjects were more than twice as likely to heal completely within 12 weeks than no-dHACM subjects (HR: 2.15, 95% confidence interval 1.30–3.57, $P = 0.003$). At the final follow up at 16 weeks, 95% of dHACM-healed ulcers and 86% of healed ulcers in the no-dHACM group remained closed. These results confirm that dHACM is an efficacious treatment for lower extremity ulcers in a heterogeneous patient population.

KEYWORDS

advanced wound care, amniotic membrane, dehydrated human amnion/chorion membrane, diabetic ulcers, lower extremity ulcers

1 | INTRODUCTION

Diabetic foot complications, such as ulcerations, infections, and gangrene, are a common cause of hospitalisation, amputations, and disability among diabetic patients. Routine ulcer care, treatment of infections, amputations, and hospitalisations cost billions of dollars every year in the United States alone and place tremendous burdens on health care systems worldwide.[1–3] Over their lifetime, up to 34% of people with diabetes may develop lower extremity ulcers, and an estimated 12% of those patients will ultimately require lower extremity amputation.[4,5] Concomitant conditions associated with diabetes, such as peripheral vascular disease, neuropathy, and poor blood glucose controls, as well as other often-present comorbidities, contribute to the slow healing rates and high rates of recurrence of these wounds. An estimated 40% of diabetic patients have a recurrence within 1 year after ulcer healing.[4] Slow healing of lower extremity ulcers increases the risk of infection and potential for amputation. More than 80, 000 amputations are performed each year on diabetic patients in the United States.[1] Ulcers precede 85% of lower extremity amputations in persons with diabetes, and it is estimated that up to 85% of these amputations may be preventable.[6]

Successful treatment of a lower extremity ulcer in a person with diabetes requires teamwork between a skilled and observant clinician and a compliant and motivated patient. Standard first-line management of diabetic foot ulcers (DFUs) includes moist dressings, debridement, offloading of weight-bearing ulcers, infection prevention, and patient education on proper foot care, yet even with the best conservative care, many months of treatment may be required before an ulcer heals.[7] Guidelines suggest that advanced wound therapies be incorporated into the treatment plan if an ulcer does not reduce in size by at least 40% after 4 weeks of standard wound therapy.[8]

Clinicians seek advanced treatments that encourage rapid and complete healing of wounds in order to reduce the risk for infection and associated morbidities. Advanced treatment for diabetic lower extremity ulcers includes collagen, biological dressings and skin equivalents, platelet-derived growth factors (PDGF), platelet-rich plasma (PRP), silver products, negative pressure wound therapy (NPWT), and hyperbaric oxygen therapy (HBO_2).[5] There is little consensus as to which advanced therapy provides the greatest benefit in rates of complete healing and time to wound closure. Indeed, a systematic review of randomised controlled trials published in 2013 found a discouragingly low strength of evidence regarding the effectiveness and comparative effectiveness of advanced wound care therapies for treatment of lower extremity ulcers.[5] Yet, since the publication of that systematic review, there have been multiple publications with high-level evidence reporting the results of randomised controlled studies on the use of a dehydrated human amnion/chorion membrane (dHACM) allograft (EpiFix, MiMedx Group Inc., Marietta, Georgia) for the treatment of lower extremity ulcers.[9–11]

Key Messages

- level 1 evidence examining the efficacy of dehydrated human amnion/chorion membrane (dHACM) as a treatment for diabetic foot ulcers (DFUs), in addition to wound offloading, has reported statistically significant superior rates of complete healing and a more rapid time to healing when dHACM is incorporated into the treatment plan, compared with standard wound care and offloading alone
- the purpose of this study is to further confirm the efficacy of using the dHACM allograft as a treatment for chronic diabetic lower extremity ulcers when administered by a wider variety of clinicians at multiple locations with a more heterogeneous patient population
- both intent-to-treat and per-protocol participants receiving weekly dHACM were significantly more likely to completely heal than those not receiving dHACM (ITT—70% versus 50%, $P = 0.0338$, per-protocol—81% versus 55%, $P = 0.0093$); adjusting for covariates associated with healing, Cox regression analysis showed that subjects treated with dHACM were more than twice as likely to heal completely within 12 weeks than those not receiving dHACM

Level 1 evidence examining the efficacy of dHACM as a treatment for DFUs, in addition to wound offloading, has reported statistically significant superior rates of complete healing and a more rapid time to healing when dHACM is incorporated into the treatment plan, compared with standard wound care and offloading alone.[9] Two additional Level 1 comparative effectiveness studies have shown the superiority of dHACM, when compared with another bioengineered skin substitute (BSS) and a standard care group, in healing at 6 weeks and at 12 weeks.[10,11] While the results of the previous Level 1 dHACM studies were impressive, there were only four treating clinicians and study sites over a limited geographic area. The purpose of the present study is to further confirm the efficacy of using the dHACM allograft as a treatment for chronic diabetic lower extremity ulcers when administered by a wider variety of clinicians at multiple locations with a more heterogeneous patient population.

2 | MATERIALS AND METHODS

A prospective, randomised, controlled, multicentre clinical trial was conducted to evaluate healing outcomes in diabetic patients with chronic lower extremity ulcers treated with weekly application of dHACM as an adjunct to standard wound care and offloading or standard wound care and offloading alone. The study population consisted of patients

TABLE 1 Major inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age 18 or older	Current participation in another clinical trial
Type 1 or Type 2 diabetes	Index wound duration of >52 weeks without intermittent healing
Able and willing to provide consent and agrees to comply with study procedures and follow-up evaluations	Index ulcer probing to tendon, muscle, capsule, or bone
Ulcer size ≥1 cm^2 and < 25 cm^2	Currently receiving radiation or chemotherapy
Ulcer duration of ≥4 weeks, unresponsive to standard wound care	Known or suspected malignancy of current ulcer
No clinical signs of infection	Diagnosis of autoimmune connective tissue disease
Serum creatinine <3.0 mg/dL	Use of biomedical/topical growth factor within previous 30 days
HgA1c < 12%	Pregnant or breast feeding
Adequate circulation to the affected extremity as demonstrated by dorsum transcutaneous oxygen test (TcPO2) ≥ 30 mm Hg, ABI between 0.7 and 1.2, or triphasic or biphasic Doppler arterial waveforms at the ankle of affected leg	Taking medications considered to be immune system modulators
	Allergy or known sensitivity to gentamicin or streptomycin
	Wounds improving greater than 25% over the 2-week run-in period of the trial using standard of care dressing and Camboot offloading.
	Patient taking Cox-2 inhibitors.
	Planned use of Dakin's solution, Mafenide acetate, scarlet red dressing, Tincoban, zinc sulfate, povidone-iodine solution, Mafenide acetate, Polymyxin/nystatin, or chlorhexidine during trial.

with diabetes receiving care from clinicians specialising in wound care at 14 outpatient centres in the United States. All patients signed an Institutional Review Board (IRB)-approved informed consent form prior to any study-related procedures in compliance with applicable regulatory requirements. The study was conducted under the guidelines of Good Clinical Practice (GCP) and in accordance with the provisions of the Declaration of Helsinki. Tissue products used in the study were manufactured, handled, and stored in accordance with applicable Good Tissue Practices (GTP). The study was reviewed and approved by Chesapeake IRB or a site's local IRB and was pre-registered in ClinicalTrials.gov (NCT01693133). Study records were retained in locked files at each study site, and patient confidentiality was maintained. Only minimally necessary data were collected on study subjects, and subject identifiers were limited.

2.1 | Patient screening and eligibility

The study population was comprised of patients diagnosed with Type 1 or Type 2 diabetes presenting to a study site for care of a lower extremity ulcer below the level of the ankle. Willing participants in the clinical study who agreed to comply with the weekly visits and follow-up regimen were eligible for study inclusion. Study inclusion and exclusion criteria listed in Table 1 were used to determine patients eligible to enter a 2-week study run-in period prior to study enrolment and randomisation. The 2-week run-in period was used to identify subjects who were eligible to proceed to the treatment phase of the study. During the 2-week run-in period, patients were instructed on proper techniques for dressing changes as needed and wound offloading. They were provided with alginate dressings, absorbent non-adhesive hydropolymer secondary dressings, gauze, and an offloading device (cam walker, offloading boot, shoe, or complete contact cast) as determined appropriate by the primary investigator for their wound location and expected level of compliance. During the run-in period, patients were seen weekly at the clinic for wound cleansing, sharp debridement, and wound measurements. At the conclusion of the run-in period, only those patients whose wounds had reduced in size by no greater than 25% were allowed to enter the treatment phase of the study.

2.2 | Treatment phase of study

After the run-in period, subjects demonstrating a reduction in wound size of 25% or less who still met all study inclusion/exclusion criteria were enrolled in the treatment phase of the study. At enrolment in the treatment phase, patients were randomised in a 1:1 ratio to receive a weekly application of dHACM (dHACM group) directly to the wound, in addition to absorbent non-adhesive hydropolymer secondary dressings and gauze, or to receive only standard wound dressings with alginate dressings, absorbent non-adhesive hydropolymer secondary dressings, and gauze, (no-dHACM group). Wounds continued to be offloaded in both study groups. Randomisation was conducted at each study site through sequentially numbered and sealed opaque envelopes. Group assignments were verified and monitored by the study sponsor (MiMedx Group Inc.). Neither the treating physician nor the patient was blinded to group assignment, but study adjudicators who examined photographic images for validation of healing at completion of the study were blinded as to group assignment.

Study visits during the treatment phase occurred weekly for up to 12 weeks. At each study visit, the subject was assessed for adverse events. The study ulcer was cleansed and sharp debridement performed (if wound had not healed). In the dHACM group, a wound size-appropriate allograft was applied to the debrided wound bed and then hydrated with several drops of sterile saline (if necessary). The wound was then dressed with a non-adherent silicone dressing and an absorbent non-adhesive hydropolymer secondary dressing and wrapped with gauze. Subjects in the no-dHACM group had their wounds cleansed, debrided, and dressed with a standard alginate dressing and an absorbent non-adhesive hydropolymer secondary dressing and wrapped with gauze. Dressings were changed weekly at the study site unless they became wet or soiled. If additional dressing changes were required in the treatment group, only the outer dressings were changed. In both study groups, wounds were similarly offloaded during all phases of the study. Ulcer measurements were performed after cleansing and debridement using the SilhouetteStar camera and Silhouette Connect system.

2.3 | Validation of healing

Complete reepithelialisation of the wound without drainage or need for dressing was used as the definition for complete healing. For statistical analysis, final study outcomes regarding wound reduction, time to healing, and healing status were determined via adjudication. At study completion, after the final visit at week 16, all photographic images taken after debridement with the Silhouette system, blinded to group assignment, study sites, and treating clinician, were reviewed by three independent physicians specialising in wound care who did not enrol patients into the study. The adjudication was performed as a group, and the determination of timing of complete epithelialisation was reached by consensus of the adjudicators. During the adjudication process, the level and consistency of debridement was also evaluated.

2.4 | Study outcomes

Primary study outcome was the incidence of complete wound closure at 12 weeks. Secondary outcomes included time to healing and incidence of ulcer recurrence at the site of the study ulcer during the follow-up phase.

2.5 | Statistical analysis

The nQuery Advisor 7.01 was used for to determine the number of study subjects to provide sufficient statistical power for the primary endpoint. The sample size calculation was based on the assumption that there is a difference of 35% between the two treatment groups in the percentage of healed subjects.

Under the above assumptions, at least 35 subjects per treatment group were required to meet the Type I error rate (P-value) of 0.05 and 85% power of a total of 70 subjects for the study. To accommodate for potential discontinuations and study dropouts and to make the study more clinically relevant, we sought to enrol a minimum of 100 subjects.

The study hypothesis tested was that the use of dHACM offers a statistically significant advantage over standard care alone for the treatment of lower extremity ulcers in patients with diabetes. Study variables were summarised as means and SDs for continuous variables unless the data were non-normal, in which case medians were also reported. Proportions/percentages were reported for categorical variables. Parametric and non-parametric tests were used as appropriate. Student's t-test, analysis of covariance (ANCOVA), or the Kruskal–Wallis test was used to test for differences in continuous variables. For categorical variables, χ^2 or Fisher's exact tests were performed to test for statistical differences. Kaplan–Meier analysis and Cox proportional hazards regression modelling was performed, with two-sided P-values < 0.05 considered significant. Regression modelling included fixed effects for treatment, as well as patient demographics, medical history, and ulcer characteristics as covariates. SAS 9.4 (SAS Institute, Inc., Cary, North Carolina) was used to perform statistical testing. A modified intent-to-treat (ITT) analysis was performed. Subjects were only excluded from analysis if they were deemed ineligible after randomisation or never started the assigned treatment. Subjects not completing the study because of early withdrawal or lost to follow up had their last observation carried forward. In order to better assess differences in treatment outcomes, additional analysis was performed on the population completing the study per protocol.

3 | RESULTS

Study subjects were enrolled at 14 study sites in the United States. Study sites were located in California, Virginia, Ohio, Texas, Massachusetts, Oregon, and Alabama, and both hospital-based and private clinic settings in urban and rural areas were represented. As indicated in the CONSORT flow diagram (Figure 1), a total of 218 subjects were screened and entered the study for the 2-week run-in period between October 2014 and June 2017. At the conclusion of the run-in period, there were 92 patients no longer eligible for randomisation because of >25% reduction in wound size or no longer meeting study inclusion/exclusion criteria. A total of 126 subjects were randomised, 63 to the dHACM group and 63 to the standard of care, no-dHACM group. Sixteen randomised subjects were excluded prior to data analysis because of identification of factors, which should have precluded randomisation, including a wound smaller than 1 cm^2 at initial screening, wound infection/osteomyelitis/cellulitis/necrosis at enrolment, or charcot abnormality. After these exclusions, there were 110 subjects meeting study inclusion criteria included in the ITT analysis. Fifty-four had been randomised to receive dHACM (dHACM group), while 56 had

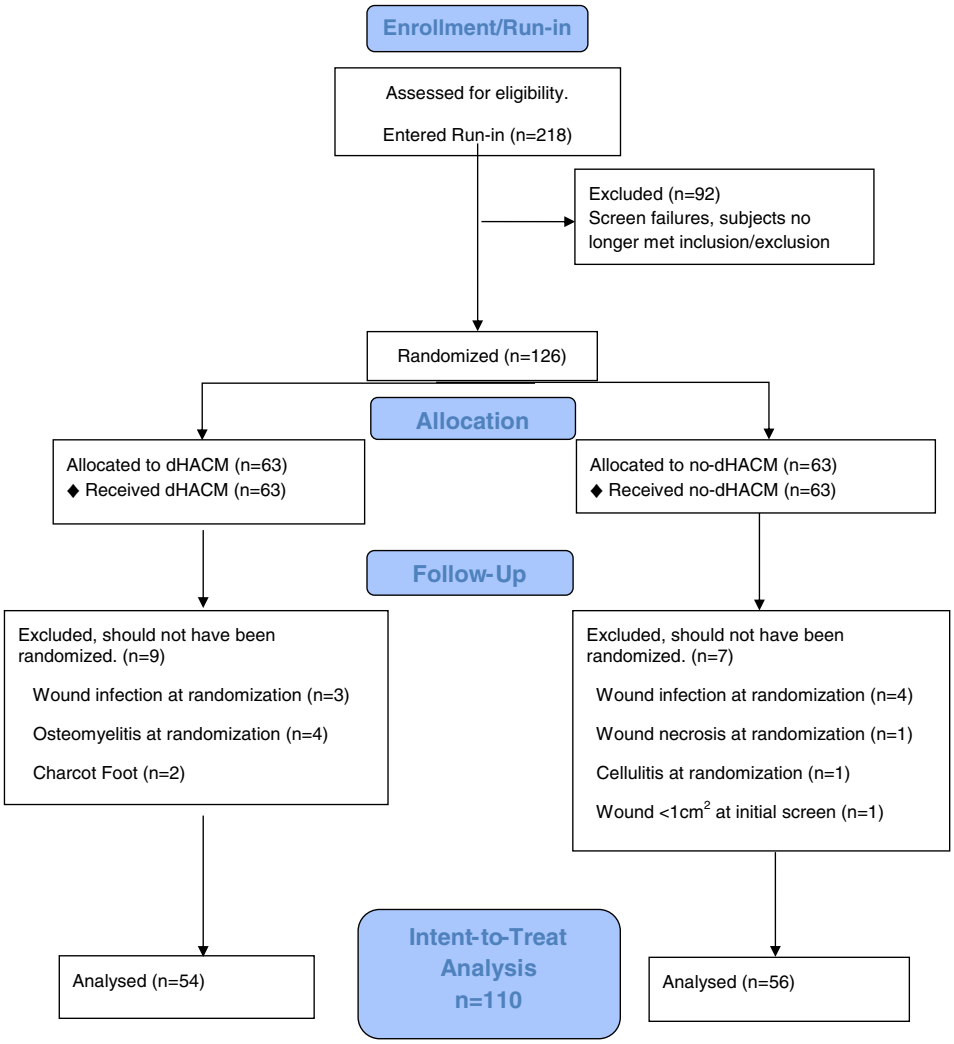

FIGURE 1 Consort flow diagram

TABLE 2 Clinical characteristics at study enrolment

	Overall ($N = 110$)	dHACM ($n = 54$)	No-dHACM ($n = 56$)
Mean age, years (SD)	57.2 (10.5)	57.4 (10.6)	57.1 (10.5)
	56.9 (34, 83)	55.9 (34, 80)	57.2 (34, 83)
Age ≥ 65 years (*n*, %)	25 (23%)	12 (22%)	13 (23%)
Male gender (*n*, %)	80 (73%)	40 (74%)	40 (71%)
Race (*n*, %)			
Caucasian	92 (84%)	46 (87%)	46 (82%)
African American	14 (13%)	6 (11%)	8 (14%)
Hispanic ethnicity (*n*, %)	42 (38%)	22 (41%)	20 (36%)
BMI (SD)	35.2 (8.7)	35.8 (8.9)	34.6 (8.5)
	33.5 (19.1, 70.1)	34.1 (19.1, 64.0)	32.8 (23.6, 70.1)
Obese BMI ≥ 30 (*n*, %)	74 (67%)	39 (72%)	35 (63%)
A1c %	8.3 (1.7)	7.8 (1.4)	8.8 (1.8)
	8.0 (5.1, 12.4)	7.7 (5.1, 11.9)	8.6 (5.9, 12.4)
Smoker (*n*, %)	39 (37%)	22 (41%)	17 (32%)
Alcohol use (*n*, %)	42 (40%)	21 (40%)	21 (40%)
History of recurring ulcers (*n*, %)	21 (20%)	12 (23%)	9 (18%)
History of cardiovascular abnormalities (*n*, %)	47 (44%)	23 (43%)	24 (45%)
Prior amputation (*n*, %)	27 (25%)	11 (20%)	16 (29%)

Abbreviation: BMI, body mass index. Data presented as mean (SD), median (minimum, maximum), or number (percent) as indicated.

TABLE 3 Characteristics of study ulcer at baseline

	dHACM ($n = 54$)	No-dHACM ($n = 56$)	*P*-value
Ulcer position (n, %)			
Plantar	25 (48%)	37 (74%)	0.0190
Dorsal	17 (33%)	8 (16%)	
Ulcer location (n, %)			
Toe	7 (14%)	4 (8%)	0.9095
Forefoot	27 (53%)	30 (60%)	
Midfoot	8 (16%)	8 (16%)	
Hindfoot	8 (16%)	7 (14%)	
Ulcer size (cm^2, SD)	3.2 (2.8)	3.9 (3.8)	0.3327
	2.2 (1.0, 11.7)	2.4 (0.7, 16.8)	
Ulcer duration, weeks (SD)	20.8 (18.5)	21.4 (15.8)	0.8747
	12.0 (4, 96)	15.0 (4, 56)	

Data presented as mean (SD), median (minimum, maximum), or number (percent) as indicated.

been randomised to receive continuation of standard care without dHACM (no-dHACM group). Of these 110 subjects, 98 completed the study per protocol (47/54 in the dHACM group and 51/56 in the no-dHACM group).

Patient demographics and pertinent medical history are presented in Table 2. Underscoring the risk factors for poor healing in the study population, 73% of the study population was male, 67% were obese, 37% were smokers, 40% consumed alcohol, 33% had an A1c ≥ 9%, and 25% had a prior amputation. Characteristics of the study ulcers are presented for each study group in Table 3. Overall, 61% of study ulcers were plantar, with a median duration of 14 (range 4, 96) weeks at study enrolment. Plantar ulcers were less prevalent in the dHACM group than in the no-dHACM group.

3.1 | Study outcomes

In the ITT analysis, at the end of the 12-week treatment phase, 70% (38/54) of dHACM-treated ulcers had completely healed, a significantly greater number compared with 50% (28/56) of healed ulcers in the no-dHACM group ($P = 0.0338$). For those subjects completing the study per protocol, healing rates at 12 weeks were 81% (38/47) for those in the dHACM group and 55% (28/51) for those not receiving dHACM ($P = 0.0093$). Through the evaluation of Silhouette wound images captured after debridement during the treatment phase of the study, adjudicators determined that, overall, 95 of 110 (86%) study ulcers had been adequately debrided. Inadequate debridement occurred in 9 of 54 (17%) and 6 of 50 (11%) of dHACM and no-dHACM treated wounds, respectively. Of the 38 healed wounds treated with dHACM during the 12-week treatment phase, 36 of 38 (95%) remained closed at the week 16 follow up, while 24 of the 28 wounds healed without dHACM (86%) had remained closed.

The median number of grafts applied per healed wound was 5 (range 1–12). The median cost per dHACM healed ulcer was $2252.33 (range $306.95–12, 394.02). The dHACM allograft is available in multiple sizes to reduce waste through the ability of applying a wound size-appropriate graft. Of the 227 allografts applied, 190 (83%) were 14 mm or 18 mm grafts.

3.2 | Cox regression modelling

Cox regression modelling, a multivariate assessment controlling all factors and measuring the impact of each specific variable in the model, was performed to examine patient demographics, medical history, ulcer characteristics, and clinical treatment influencing ulcer healing within the 12-week treatment period. Covariates were entered as a block into the Cox regression model: dHACM treatment group, patient age ≥ 65 years, presence of obesity, A1c ≥ 9%, smoking, alcohol use, Caucasian race, Hispanic ethnicity, male gender, history of cardiovascular abnormality, history of prior recurrent ulcers, history of amputation, ulcer duration ≥14 weeks, baseline wound size ≥2.2 cm^2, lack of adequate debridement of ulcer during the study period, wound location, and wound position. Table 4 shows the corresponding hazard ratios (HRs) for the covariates in the initial model. Refinement of the model was then performed through elimination of stepwise covariates with descending *P*-values. The final model includes only those covariates found to be significantly associated with complete ulcer healing. The definitive Cox regression results and corresponding HRs are presented in Table 5. In the population

TABLE 4 Corresponding hazard ratios (HRs) for the covariates in the initial Cox regression model

Variables	*P*-value	Hazard ratio	95% CI for HR	
			Lower	Upper
Treatment: dHACM	0.080	1.69	0.94	3.06
Age: ≥65	0.899	0.95	0.45	2.04
Gender: Male	0.354	1.45	0.66	3.17
Race: Caucasian	0.021	2.98	1.18	7.49
Ethnicity: Hispanic	0.207	1.52	0.79	2.92
BMI ≥ 30	0.418	0.76	0.39	1.49
A1c ≥ 9%	0.345	0.72	0.36	1.43
Abnormal cardiovascular history	0.235	1.46	0.78	2.73
History of amputations	0.414	0.74	0.36	1.52
Diabetes (type 2)	0.598	1.55	0.31	7.82
Smoker: Yes	0.411	1.33	0.68	2.62
Drinker: Yes	0.332	1.47	0.68	3.20
History of recurring ulcers: Yes	0.117	0.46	0.17	1.22
Target ulcer history ≥ 14 weeks	0.467	0.81	0.46	1.42
Baseline ulcer size ≥ 2.2 cm^2	0.011	0.44	0.24	0.83
Inadequate debridement	0.139	0.47	0.17	1.28
Ulcer position: Plantar	0.185	0.62	0.31	1.25
Ulcer location: Toe	0.023	3.85	1.21	12.25
Ulcer location: Forefoot	0.069	2.28	0.94	5.53
Ulcer location: Midfoot	0.117	2.30	0.81	6.52

TABLE 5 Refined Cox regression results after eliminating stepwise covariates with descending *P*-values

Variables	*P*-value	Hazard ratio	95% CI for HR	
			Lower	Upper
Treatment: dHACM	0.003	2.15	1.30	3.57
Race: Caucasian	0.008	3.01	1.33	6.80
History of recurring DFU: Yes	0.029	0.42	0.20	0.92
Baseline ulcer size ≥ 2.2 cm^2	0.003	0.44	0.26	0.75
Inadequate debridement	0.022	0.36	0.15	0.86
Ulcer location: Toe	0.013	3.29	1.29	8.38
Ulcer location: Forefoot	0.043	2.12	1.02	4.39
Ulcer location: Midfoot	0.095	2.20	0.87	5.55

studied, factors associated with complete ulcer healing included Caucasian race, toe and forefoot ulcer location, and treatment with dHACM. Factors with a negative influence on healing during the 12-week study period included larger ulcer size, history of recurring ulcers, and inadequate wound debridement. Subjects identified as having inadequate debridement were 64% less likely to heal within 12 weeks when controlling for other significant factors in the model.

3.3 | Kaplan–Meier plot of time to heal

A Kaplan–Meier plot of time to heal within 12 weeks by study group demonstrated a superior wound-healing trajectory for dHACM-treated ulcers compared with lower extremity ulcers not receiving dHACM and being treated with standard care alone. The log-rank test of equality of the healing function over the two study groups produced a χ^2 test statistic of 5.5294, with a $P = 0.0187$ (Figure 2).

3.4 | Adverse events

All 126 subjects randomised were followed for evaluation of safety. An adverse event was defined as any untoward medical event, including any unfavourable and unintended sign, symptom, or disease occurring during study enrolment, but which may not necessarily have a causal relationship with study treatment. All adverse events, even those not related to the study ulcer or study procedures, were captured and reviewed. All adverse events were reviewed by site investigators and a Clinical Events Committee including the study sponsor to determine if the event was product or study related. Overall, there were 230 adverse events recorded. The most common adverse event was development of an additional ulcer ($n = 34$). A total of 112 adverse events occurred in subjects receiving dHACM and 118 in subjects not receiving dHACM. Fifty-three adverse events (23%) were ulcer-related. In the dHACM-treated subjects, 30 adverse events were ulcer-related. In subjects not receiving dHACM, 23 events were ulcer-related. Of the 53 ulcer-related events, 30 were infectious events. There were 11 target ulcer infections (6 dHACM-treated and 5 no-dHACM), 15 cases of cellulitis (7 dHACM-treated and 8 no-dHACM), and 4 cases of osteomyelitis (3 dHACM-treated and 1 no-dHACM). There were three events classified as possibly being product related. These included one case of wound maceration and two positive wound cultures (1 Providencia Stuartii, 1 Pseudomonas Aeruginosa).

4 | DISCUSSION

In the present study, we have shown that diabetic lower extremity ulcers treated with dHACM had significantly

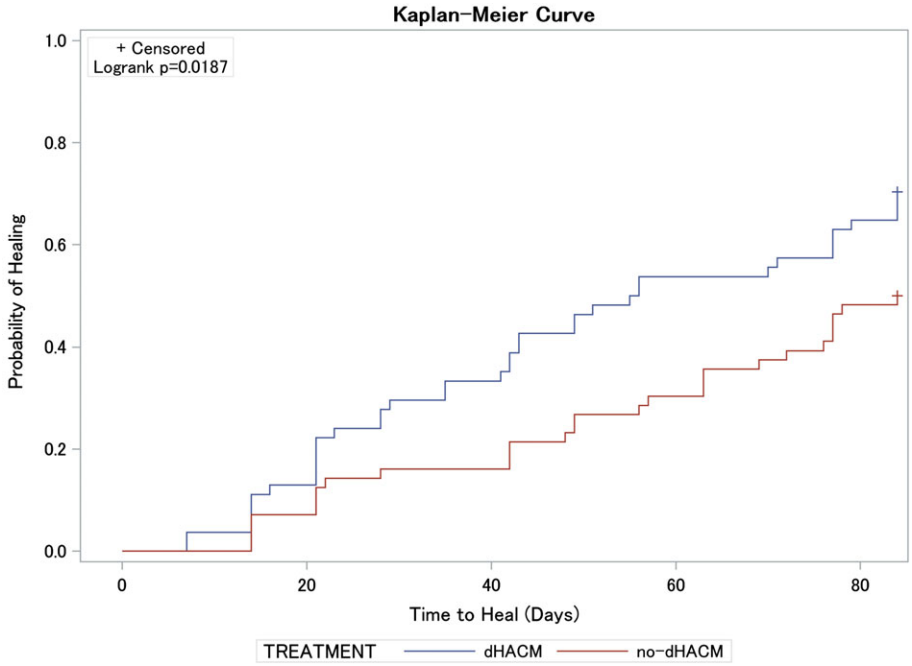

FIGURE 2 A Kaplan–Meier plot of time to heal within 12 weeks by study group

greater rates of complete healing and healed more rapidly than wounds treated with standard care alone. These results confirm the findings of previous studies examining the efficacy of dHACM for the treatment of diabetic lower extremity ulcers.[9–11]

A chronic wound develops when the normal healing process of haemostasis, inflammation, fibroplasia, epithelialisation, and maturation is disrupted or stalled. Underlying disorders, such as peripheral artery disease, diabetes, venous insufficiency, nutritional deficiencies, and other disease states and comorbidities, as well as patient habits such as smoking, alcohol and/or drug abuse, obesity, poor hygienic practices, and non-compliance with clinical recommendations, all contribute to non-healing and the development of a chronic wound.[12] Appreciation of these factors is necessary when a clinician is determining a treatment plan and/or evaluating the results of a therapeutic intervention. Clinicians must also acknowledge that their own preferences, skills, and level of expertise play a role in ulcer healing. To promote healing, a healthy ulcer environment must be created by removal of necrotic tissue, management of bioburden, and maintenance of an appropriate moisture balance. Methods and thoroughness of debridement as well as chosen topical products, advanced treatments, and dressings all influence healing outcomes.

The requirement for successful healing is characterised by a tissue microenvironment with high levels of bioactive proteins, such as growth factors and other soluble mediators of cell signalling, functional fibroblasts, keratinocytes, and vascular endothelial cells, as well as controlled levels of proteases and bacteria.[13] Persistent inflammation, cell senescence, growth factor deficiencies, high levels of bioburden, elevated concentrations of destructive proteases, and stem cell deficiencies are characteristics inherent of chronic wounds.[13] Advanced wound care products, such as dHACM, can ameliorate these destructive properties that are often present in chronic wounds; provide an opportunity for more rapid and complete healing, which reduces the risk for significant morbidity associated with infection; and may very well reduce health care costs.

ELISA assays performed on samples of dHACM have shown quantifiable levels of vascular endothelial growth factor (VEGF), platelet-derived growth factors AA and BB (PDGF-AA and PDGF- BB), transforming growth factors alpha and beta (TGFα and TGFβ1), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and granulocyte colony-stimulating factor (GCSF).[14] Interleukins 4, 6, 8, and 10; Tissue Inhibitors of Metalloproteinases (TIMPs) 1, 2, and 4; and signalling molecules, including 14 cytokines and 10 chemokines known to regulate inflammation and 12 cytokines known to regulate wound-healing processes, have also been identified in dHACM.[15]

Quantifiable levels of the angiogenic cytokines angiogenin and angiopoietin-2 (ANG-2) have also been measured in dHACM.[16] The dHACM allograft has been shown to promote amplification of angiogenic cues by inducing endothelial cell proliferation and migration and by upregulating the production of endogenous angiogenic growth factors by endothelial cells. Laboratory studies have shown that subcutaneous dHACM implants displayed a steady increase in microvessels over a period of 4 weeks, indicative of a dynamic intra-implant neovascular process.[16] SDF-1, a factor that has been shown to recruit stem cells, is known to be deficient in diabetic wounds,[17] yet in vitro and in vivo studies have confirmed that the dHACM allograft can stimulate the migration of mesenchymal stem cells to a wound, as well as bone marrow-derived haematopoietic stem cells.[18]

An initial feasibility study of dHACM as a treatment for lower extremity ulcers in 25 patients with diabetes conducted in 2012 and 2013 at a single centre was able to demonstrate reasonable safety and effectiveness of the allograft.[9] Results showed a large effect size compared with standard care, with healing rates of 77% versus 0% at week 4 and 92% versus 8% at week 6 for dHACM ($n = 13$) and standard care ($n = 12$), respectively.[9] In another randomised controlled trial, dHACM was compared with BSS and standard care without dHACM.[11] The study was conducted in 2014 at four study sites, three being in Virginia and one in Oklahoma, with efficacy at 12 weeks reported.[11] In that comparative effectiveness study, healing rates of 73% with BSS ($n = 33$), 97% with dHACM ($n = 32$), and 51% with standard care ($n = 35$) were reported at week 12, adjusted $P = 0.00019$.[11] The results of these prior studies can be considered extreme outliers when compared with results of other advanced products in the wound-healing literature, and these early studies have been criticised for having a small sample size, a narrow geographical distribution, few study sites, and homogeneity of patients and providers. However, it is also apparent that these four study sites in the comparative study were very clinically effective and consistent, as demonstrated by the high levels of complete healing in the standard care group, as well as patient groups receiving advanced treatments.

The current study was conducted at 14 wound centres in seven states. Even when non-compliant and withdrawn subjects were included in the final analysis, ulcers treated with dHACM continued to show a statistically significant higher healing rate of 70% at week 12, compared with 50% for ulcers not treated with the allograft ($P = 0.0338$). In subjects completing the study per protocol, rates of complete healing of 81% and 55% were observed for the dHACM and standard care groups, respectively. While there appears to be a reduction in the overall rates of healing in the current study compared with earlier reports, this is not at all surprising given the clinical and methodological heterogeneity between the previous and current study sites, clinician practice patterns across a wider geographical distribution, and a more complex patient population. A larger sample size; diversity

in demographic and clinical characteristics in study patients across the multiple study sites, including high numbers of large ulcers, males, smokers, drinkers, and those with prior amputations; and obesity and poor glucose control, as well as observed dissimilarities in study protocols, debridement techniques, racial distribution, and patient and provider compliance, are believed to be contributing factors to variances observed in healing rates between the previous and current studies.

It is an expected phenomenon in clinical research that, as additional studies are completed and as more wounds are treated in broader, less homogenous patient populations and clinical settings, there will be some regression to the mean for treatment outcomes.[19,20] Irrespective of the initial treatment effect size, it is more important to observe if the overall significance remains when an intervention is implemented in a broader patient population cared for by a differing array of clinical specialists with varying skill levels in a wide range of clinical settings. Another important observation is if a significant difference in treatment effect persists in both ITT and per protocol analysis of data. The results of the present study continue to support that dHACM is an efficacious treatment for chronic lower extremity ulcers.

Frequent debridement has been reported as improving wound healing and has been called a vital adjunct in the care of patients with DFUs.[21,22] Unfortunately, it is difficult to uniformly categorise the quality of debridement across study sites given that many wound care specialists have typically never received hands-on standardised clinical training in surgical wound debridement. A unique feature of the current study is the evaluation of the thoroughness of debridement and its influence on healing outcomes. At study completion, adjudicators evaluated all wound images blinded to study group, study site, and primary investigator. Adjudicators made a determination if adequate debridement had been performed throughout the study period for each subject. Adequate debridement was defined as occurring when post-debridement images revealed exposure of healthy tissue in the ulcer with no significant eschar, callous, necrotic tissue, or foreign material present in or around the wound. Site investigators were not aware that the thoroughness of debridement would be evaluated. Results of the Cox regression analysis support our assumption that variation in the thoroughness of debridement played an important role in ultimate healing outcomes. After controlling for treatment, ulcer size and position, wounds receiving inadequate debridement were 64% less likely to heal than those adequately debrided. Sharp debridement facilitates growth factor delivery by restoring the expression of growth factor receptors that are not properly expressed at the non-healing edge of chronic ulcers, thus making insensitive cells more responsive to exogenous growth factor therapy.[23] We believe that meticulous debridement is essential in order to observe the greatest healing benefits when implanting an allograft such as dHACM into a chronic wound.

While the best approach in managing patients with diabetes at high risk for lower extremity ulcers is to promote prevention through extensive patient education, early assessment, and aggressive treatment of a developing wound is often necessary. The goal of treating a lower extremity ulcer is rapid and complete healing to avoid a cascade of events, which could lead to the need for amputation. When developing an individual treatment plan based on wound characteristics, efficacy and cost of advanced wound dressings are important considerations for patients, clinicians, and payors. A meta-analysis published in 2017 reported that data strongly favour the use of amniotic membrane to improve wound healing with potentially significant cost savings.[24] Repeatedly, dHACM has been shown to be an efficacious treatment for chronic lower extremity ulcers. In the present study, the median graft cost for an dHACM-healed ulcer was $2,252.33, which is substantially lower than the median cost burden of a minor amputation, which was reported to be $37,598 and $53,779 for a major amputation in 2010.[25] Multiple graft sizes that allow smaller, less expensive grafts to be used as a wound decreases in size reduces waste and promotes cost-effectiveness of dHACM.

There are strengths and weaknesses to every study. An overwhelming strength of the present study lies in its randomised, multicentre design, which is considered the gold standard, providing Level 1 evidence of treatment efficacy. Objective measurement of wound area was standardised across all study sites through the use of the Silhouette camera. To eliminate potential bias, final healing status was adjudicated by a group of three physicians who examined all wound images. Adjudicators were blinded to study site and patient group assignment. To further reduce bias and overestimation of treatment effect, data were analysed in a modified ITT fashion, only excluding those subjects who did not receive assigned treatment after randomisation or who were found to not meet study inclusion criteria. Patients were included in their original assigned group even when they were not treated per protocol, withdrew from study participation, were non-compliant, lost to follow up, or experienced adverse events or severe adverse events where continuation in the study was not feasible. While ITT analysis is recognised as a method to reduce potential bias, it must also be recognised that inclusion of non-compliant subjects, study dropouts, and those with protocol and treatment deviations results in a diluted and conservative estimate of treatment effect and may be prone to Type II error.[26]

Use of Cox regression analysis allowed for the control of multiple factors that can influence healing and differences in clinical characteristics between the study groups. It is well recognised by clinicians that a myriad of patient-specific factors, including medical history, demographics, diet, habits such as smoking and drinking, compliance with medical

advice, and size and location of ulcer, influence treatment success. The ability to control for these factors when analysing study data allows for a more robust and appropriate interpretation of the results. Indeed, while there was a greater preponderance of plantar ulcers in the no-dHACM group, plantar ulcer location was not found to be a significant factor influencing healing when controlling for multiple clinical factors in the Cox model.

An unfortunate weakness in any study of advanced wound care products compared with a "standard care group" is that the level of treatment provided as "standard care" is specified by study protocol and is generally of higher quality and more consistent than what may be provided outside a clinical trial setting, which may reduce the true effect size between treatment and control study arms. In a meta-analysis from 1999, standard "good" wound care consisted of wet-to-dry dressings and resulted in a healing rate of approximately 24% after 12 weeks.[27] In contemporary practice, a wider variety of more advanced dressings is used, and current "standard care" for treatment of lower extremity diabetic ulcers frequently consists of alginate dressings. In the present study, alginate dressings, absorbent non-adhesive hydropolymer secondary dressings, and gauze were used in lieu of basic moist-to-dry dressings, which we believed increased the rates of healing in the standard care group and reduced the treatment effect size. A 12-week healing rate of 50% with alginate dressings, double the rate expected with simple wet-to-dry dressings, speaks to the overall influence of advanced dressings on rates of wound healing. Achieving 70% healing within 12 weeks provides further evidence of the efficacy of dHACM compared with other advanced treatments.

In a perfect world, all wounds could be adequately offloaded at all times, and patients would be 100% compliant with the use of the prescribed offloading device. As in most other wound treatment studies, the inability to truly monitor offloading compliance is a study weakness. In the present study, clinicians were allowed to use their judgement regarding the appropriate offloading device to prescribe, including the use of full-contact casting and when offloading was no longer needed once healing occurred. Given the variety of offloading devices used, we are unable to determine if the type of offloading device influenced our results. As no patients' wounds were offloaded with full-contact casting, we do not know if this would have improved treatment results in either study group. Variations in clinical recommendations for continued offloading of healed wounds was not specified per study protocol, and it is unknown how this influenced the observed rates of wound recurrence.

5 | CONCLUSION

In conclusion, the results of the current multicentre clinical trial support and confirm that dHACM is an efficacious treatment for lower extremity diabetic ulcers. In a heterogeneous patient population across the United States, healing rates with the use of dHACM were superior to those achieved with standard dressings alone, even when ITT data analysis, including non-compliant subjects, was conducted. Reported healing rates within 12 weeks of 70% (ITT analysis) and 81% (per protocol analysis) remain superior to healing rates reported in comparable prospective studies of other advanced wound care products. The results of this 110 patient, multicentre, randomised controlled study provide additional Level I evidence regarding the efficacy of dHACM and are useful to clinicians who are determining which advanced wound care product to choose when caring for their patients and for health care policymakers in both the United States and globally who are challenged to evaluate the benefits of available advanced wound care products compared with costs. The most expensive intervention is the one that does not work effectively. Benefiting patients through the appropriate utilisation of strong evidence-based products such as dHACM is likely among the most cost-effective forms of health care expenditure.

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Conflict of Interest

Dr. Tettelbach, Dr. Cazzell, Dr. Reyzelman, Dr. Sigal, Dr. Caporusso, Dr. Agnew, and members of the EpiFix study group were among the clinical trial investigators and adjudicators for this study sponsored by MiMedx and received research funding. None of the authors had a financial interest in any of the products mentioned in this manuscript during the course of the study. Although Dr. Tettelbach did not have any financial interest, or any other conflicts of interest, during the course of study, he discloses that he is now an employee of MiMedx Group, Inc., the study sponsor.

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ENDNOTES

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