A randomized clinical trial of a human acellular dermal matrix demonstrated superior healing rates for chronic diabetic foot ulcers over conventional care and an active acellular dermal matrix comparator

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ABSTRACT

This study compared the efficacy and safety of a human acellular dermal matrix (ADM), D-ADM, with a conventional care arm and an active comparator human ADM arm, GJ-ADM, for the treatment of chronic diabetic foot ulcers. The study design was a prospective, randomized controlled trial that enrolled 168 diabetic foot ulcer subjects in 13 centers across 9 states. Subjects in the ADM arms received one application but could receive one additional application of ADM if deemed necessary. Screen failures and early withdrawals left 53 subjects in the D-ADM arm, 56 in the conventional care arm, and 23 in the GJ-ADM arm (2:2:1 ratio). Subjects were followed through 24 weeks with major endpoints at Weeks 12, 16, and 24. Single application D-ADM subjects showed significantly greater wound closure rates than conventional care at all three endpoints while all applications D-ADM displayed a significantly higher healing rate than conventional care at Week 16 and Week 24. GJ-ADM did not show a significantly greater healing rate over conventional care at any of these time points. A blinded, third party adjudicator analyzed healing at Week 12 and expressed "strong" agreement ($\kappa = 0.837$). Closed ulcers in the single application D-ADM arm remained healed at a significantly greater rate than the conventional care arm at 4 weeks posttermination (100% vs. 86.7%; $p = 0.0435$). There was no significant difference between GJ-ADM and conventional care for healed wounds remaining closed. Single application D-ADM demonstrated significantly greater average percent wound area reduction than conventional care for Weeks 2–24 while single application GJ-ADM showed significantly greater wound area reduction over conventional care for Weeks 4–6, 9, and 11–12. D-ADM demonstrated significantly greater wound healing, larger wound area reduction, and a better capability of keeping healed wounds closed than conventional care in the treatment of chronic DFUs.

Diabetes mellitus is known for its multifaceted complications, including diabetic foot ulcers (DFUs), with approximately 29.1 million people (9.3%) living in America with the disease.[1] It is estimated that the global prevalence of diabetes mellitus will rise to almost 600 million by 2035 and around 80% of these people will live in developing countries.[2,3] Although the frequency and severity of foot problems vary from region to region,[4–6] DFUs are the most prevalent problem, with a yearly incidence of around 2–4% in developed countries and likely even higher in developing countries.[5,7] Among people with diabetes, the prevalence of foot ulcers ranges from 4 to 10% and its lifetime incidence may be as high as 25%.[8] The most important factors underlying the development of foot ulcers are peripheral sensory neuropathy, foot deformities related to motor neuropathy, minor foot trauma, and peripheral artery disease.[7,9–11] Once the skin is ulcerated, it is susceptible to becoming infected, an urgent medical problem. DFUs are difficult to treat, frequently are infected, and are a leading cause of diabetes-related hospital admissions. Compared to healthy individuals, patients with diabetes mellitus hold a 15- to 20-fold increased risk of lower extremity amputations and up to 85% of diabetes amputations are reported to be preceded by a poor healing ulcer.[4,12] Once amputation occurs, 50% of patients will develop an ulcer in the contralateral limb within 5 years.[13]

The 5-year survival rate after one major lower extremity amputation is about 50%.[13]

Foot problems in persons with diabetes not only represent a major personal tragedy, these problems also affect that person's family and place a substantial financial burden on healthcare systems and society in general. An estimated $9–13 billion was spent annually on the treatment of diabetic foot ulcers in the United States, with the average DFU patient incurring additional healthcare costs between $11,710 and $16,883 based on the research of a 2014 study.[14] Furthermore, the cost of treating a single patient with a DFU in the United States averages $31,419 over a one year period, more than twice the expense of treating a diabetic patient without a DFU.[1] Investing in evidence-based, appropriate diabetic foot care guidance is likely among the most cost-effective forms of healthcare expenditure, provided it is goal focused and properly implemented.[6] Further, these aforementioned financial burdens should prompt the treatment community to search for more effective advanced evidence-based treatments that can mitigate these issues via enhanced clinical efficiency and reduced need for multiple treatment applications.

One treatment modality, acellular dermal matrices (ADMs), has shown success in treating DFUs in clinical trials.[15–17] The decellularization process theoretically removes potentially immunogenic material thus creating a scaffold that can be used for tissue engineering through host cellular and vascular in-growth.[18–20] However, not all ADMs perform equivalently, which is likely due to differences in the processing methods. Different ADM products have shown significant differences in early host tissue integration, revascularization, remodeling, and recellularization.[21] The differences in time to complete cellular migration between ADMs may come from a difference in the density of initial migratory inflammatory cells which allow matrix remodeling and subsequent revascularization.[21] Different ADMs have also demonstrated varied levels of residual DNA content.[18] A lower residual DNA content indicates a more thorough decellularization process which enables a different and more desirable host response.[22] Finally, the sterility assurance level (SAL) of an ADM is an important consideration with the constant threat of infection associated with DFUs. However, it seems confusion still exists over the term "aseptic" and "terminal sterilization." Aseptic processing merely means the processing is done under aseptic conditions, and certainly may involve bioburden reduction steps. These allografts may then be either tested for sterility, e.g., following USP <71>,[23] or terminally sterilized to a validated sterility level. If terminally sterilized by a validated method, implants may, e.g., have a Sterility Assurance Level (SAL) of 10^{-3} indicating a 1 in 1,000 probability that a packaged implant contains a viable microorganism, while an SAL of 10^{-6} indicates a 1 out of 1,000,000 chance of the same.[24] The latter, SAL of 10^{-6}, is the same level expected for a sterile-labeled implantable medical device.[25,26]

One particular human ADM, D-ADM, is processed to ensure thorough decellularization, evidenced by $\geq$97% donor DNA removal, terminal sterilization with a SAL of 10^{-6}, and provided fully hydrated and ready-to-use with storage at ambient temperature.[18] It has also shown success in treating DFUs in early case series as well in the interim results of this trial.[16,27–29] Another human ADM, GJ-ADM, has also shown success in healing DFU's in clinical studies.[16,30] However, GJ-ADM undergoes a different processing method than D-ADM, resulting in aseptic, freeze-dried grafts that must undergo 10–40 minutes of rehydration before implantation.[31] The interim study showed preliminary results through 16 weeks follow-up while the trial presented here contains outcomes for the full 24 weeks.

The primary objective of this prospective, randomized trial, the largest human ADM trial to date, was to compare the healing rates of D-ADM for chronic DFUs with that of a conventional care arm. Secondary objectives explored differences in time to wound closure, economic burden, quality of life questionnaires and product utilization between D-ADM, conventional care, and a second active comparator human ADM.

METHODS

Study design

This study was a multicenter, randomized, controlled, open-label trial designed to evaluate the safety and efficacy of D-ADM on the wound healing rate of chronic ulcers of the lower extremities (Clinical trial registration number NCT01970163, http://ClinicalTrials.gov). The trial design, methods, and informed consent were reviewed and approved by a central institutional review board (IRB), Western Institutional Review Board, as well as local IRBs. Although the primary focus of the study was 168 DFU subjects, an exploratory arm was enrolled to assess the efficacy of D-ADM on venous leg ulcers (VLUs). The results of the VLU cohort will be published separately. The numbers reported here are only for the DFU portion of the study. For the DFU arms, subjects from 13 outpatient wound care centers in 9 states were evaluated for entry into the study during a screening period up to 30 days before treatment at baseline. Subjects with a diagnosis of diabetes mellitus on a stable treatment regimen (no changes in treatment for 30 days prior to screening) who presented to the clinic for care of a chronic lower extremity ulcer were invited to participate in the study. After providing voluntary informed consent, the patient demographics (Table 1) were collected and each individual was screened for eligibility based upon the inclusion and exclusion criteria listed in Table 2. Subjects with DFUs who met all inclusion requirements and none of the exclusion requirements were randomized into one of the three treatment arms: D-ADM (DermACELL; LifeNet Health, Virginia Beach, VA), conventional care wound management, or GJ-ADM (GraftJacket; Wright Medical Technology, Memphis, TN) at a ratio of 2:2:1. There are several published reports demonstrating the safety and effectiveness of GJ-ADM. Therefore, this active comparator arm was not powered to detect a difference between the ADMs, it was added only to establish a baseline ADM healing rate for comparison in our site populations.

Concealment of the treatment arm was safeguarded using numbered envelopes that contained the treatment number and arm assigned. All envelopes were prepared by a Contract Research Organization (CRO; Medpace, Cincinnati, OH) and all investigators were blinded to the

Table 1. Demographic variables for the intent to treat population

		D-ADM (*N* = 71)	Conv care (*N* = 69)	GJ-ADM (*N* = 28)
Gender	Male	57 (80.3%)	51 (73.9%)	20 (71.4%)
	Female	14 (19.7%)	18 (26.1%)	8 (28.6%)
BMI	Mean	32.56	32.82	31.41
	Median	31.70	31.50	32.10
	Standard deviation	8.276	6.929	5.082
	Range	19.9–81.6	19.5–50.2	23.4–44.2
Age (years)	Mean	59.1	56.9	58.5
	Median	58.0	56.0	60.5
	Standard deviation	12.76	10.86	9.83
	Range	24–85	33–85	34–80
Circulating Hemoglobin A1c (%)	Mean	8.51	8.38	7.63
	Median	8.40	8.30	7.50
	Standard deviation	1.81	1.87	1.38
	Range	4.8–12.4	5.4–12.3	5.8–11.0
Diabetes type*	Type 1	4 (5.6%)	2 (2.9%)	2 (7.1%)
	Type 2	64 (90.1%)	67 (97.1%)	26 (92.9%)
Smoking Status	Never	38 (53.5%)	40 (58.0%)	21 (75.0%)
	Past	22 (31.0%)	20 (29.0%)	5 (17.9%)
	Current	11 (15.5%)	9 (13.0%)	2 (7.1%)

*May not equal 100% due to prediabetic patients.

Table 2. Screening criteria for exclusion and inclusion.

Inclusion criteria included but was not limited to:

- All enrolled patients must have had the ability to comply with offloading and dressing change requirements.
- The target wound had to have been open and receiving standard of care for 30 days with an area greater than or equal to 1 cm^2 and less than 25 cm^2.
- The patient must have been between 21 and 80 years of age, have a single target DFU with a Wagner Ulcer Classification Grade of 1 or 2, and an absence of infection.
- Patients must have had adequate circulation to the affected area, defined as having at least one of the following criteria within the past 60 days: transcutaneous oxygen measurement at the dorsum of the foot $\geq$ 30 mmHg, ankle-brachial index (ABI) ranging from 0.8–1.2, or at least biphasic Doppler arterial waveforms at the dorsalis pedis and posterior tibial arteries.

Exclusion criteria included but was not limited to:

- Patient had wound treatments involving biomedical or topical growth factors within 30 days prior to screening.
- Patient had circulating hemoglobin A1c exceeding 12% within 90 days of the screening visit, serum creatinine concentrations of 3.0 mg/dL or greater within 30 days prior to screening.
- The presence of peripheral vascular disease, active infection or untreated malignancy, Charcot's disease, or necrosis, purulence, or sinus tracts that could not be removed by debridement.
- Patient underwent a revascularization procedure aimed at increasing blood flow in the target limb, or received a living skin equivalent within 4 weeks before screening.
- Patient had a sensitivity to lincomycin, gentamicin, polymyxin B, vancomycin, polysorbate 20, N-lauroyl sarcosinate, benzonase, or glycerol.

randomization code that matched the treatment numbers. The assignment envelope was opened only after all screening procedures were completed. No subjects withdrew consent between randomization and initiation of care. One subject randomized to conventional care withdrew consent at the Week 3 clinic visit to seek more advanced wound care options. When a subject was scheduled for randomization, the next sequential envelope was opened and provided the clinician with the treatment arm. While it was not possible to blind the treatment arm to the implanting surgeon or subject due to the appearance of the wound after each treatment (i.e., the ADM is seen on the surface of the wound), a clinician blinded to treatment arm was engaged to review wound images for confirmation of healing to limit bias.

The primary endpoint was to compare the proportion of chronic DFUs completely closed at the end of 12 weeks of follow-up. Complete wound closure was defined as 100% reepithelialization without drainage or dressing requirements confirmed at two consecutive study visits 2 weeks apart as encouraged by the Agency for Healthcare Research and Quality (AHRQ) in its 2011 Technology Assessment for Skin Substitutes for Treating Chronic Wounds. Secondary endpoints included comparing the proportion of completely healed wounds, time to complete wound closure, proportion of healed wounds that remained closed posttermination, and percent wound area reduction among all three treatment groups (D-ADM, conventional care, and GJ-ADM) through 24 weeks. Safety was measured by the incidence of treatment emergent severe adverse events (SAEs), changes in vital signs, ABI, and physical examination.

Surgical procedure/treatment phase of the study

At baseline for subjects in all treatment groups, the wound bed was thoroughly debrided with a sharp blade, scissors or Versajet system to remove necrotic tissue and then the wound area was recorded using Aranz Medical's Silhouette Advanced Wound Assessment and Management System (Silhouette System). Each investigator was trained to trace the wound image after image capture via computer. The area traced was calculated by the Silhouette System and used for all change in wound area analyses. Meshed, 4 × 4 cm (thickness range, 0.5–1.0 mm) D-ADM or meshed, 4 × 4 cm (thickness range, 0.38–1.02 mm) GJ-ADM was applied to subjects in the D-ADM and GJ-ADM arms and covered with an appropriate non-adherent dressing. The state of the wound and the appropriate dressing to use was determined by the investigator. A second ADM application was allowed to be administered if determined medically necessary by the investigator, no fewer than 3 weeks but no longer than 12 weeks (Weeks 3–12) after the first application of ADM in subjects with DFUs. For subjects in the conventional care arm, the debrided wound underwent advanced wound care with moist-wound treatment utilizing alginate, foam, or hydrogel dressings after thorough debridement. In all treatment arms, the dressing covered the wound for at least 5 days, but no more than 9 days, (7 days ± 2 days) until the next study visit and dressings were only changed by the study team. The dressings were standardized across all sites in an attempt to reduce treatment variability. The investigators agreed upon a listing of approved dressings from the following categories: alginate, gauze, foam, hydrogel and petroleum impregnated gauze. Debridement was performed at the Baseline treatment visit with a sharp blade, scissors or Versajet system to remove all necrotic tissue. Subsequent debridements were encouraged at the clinic visits to remove any necrotic tissue, if deemed necessary by the investigator. Off-loading using a removable cast walker, diabetic shoe, surgical shoe, walker cast, or a total contact cast was required for all treatment arms unless the investigator deemed it was not appropriate, such as in those cases that the subject was wheelchair bound or the wound was on the dorsal surface of the foot. Although either removable or nonremovable offloading methods were allowed, 95% of all patients used some sort of removable method with 68% of those using removable boots and 16% using surgical shoes. Subsequent study visits occurred every week until complete wound healing or until 24 weeks after treatment initiation. If the wound had achieved healing, posttermination visits were conducted 4, 8, and 12 weeks posttermination to assess if the wound had remained closed (Figure 1).

Assessment methods

Surface area of the wound and depth of the ulcer were measured and recorded at each visit. Measurements of the wound area were taken and recorded. Once wound closure was observed (defined as 100% reepithelialization of the wound without drainage), a second visit occurred 2 weeks after the initial observation to confirm complete wound closure in accordance with the Food and Drug Administration (FDA) guidance on skin substitutes and AHRQ recommendations.[32,33] If complete wound closure was observed during this second visit, this visit was considered the termination visit, and the subject's wound was classified as closed at the first observance of healing. If the wound was not closed at the termination visit, weekly assessments continued until wound closure was confirmed. Follow-up visits occurred at 4, 8, and 12 weeks after final confirmation of complete wound closure. If the wound had not closed at Week 24, the subject's participation in the study was terminated. If the wound had failed to close during the treatment period, the investigator assessed reasons for failed wound closure (e.g., infection, inflammation, arrested healing, allergic reaction, or noncompliance causing detachment of ADM). Assessments were completed to obtain information on wound area, granulation/epithelialization, and safety at each study visit. Adverse events (AEs) were captured from the point of enrollment through study exit. An AE was defined in the study as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the DFU treatment, regardless of whether it was considered to be related to that treatment. Each sign, finding, symptom or disease was collected as a separate AE and followed until resolution or completion of the termination visit.

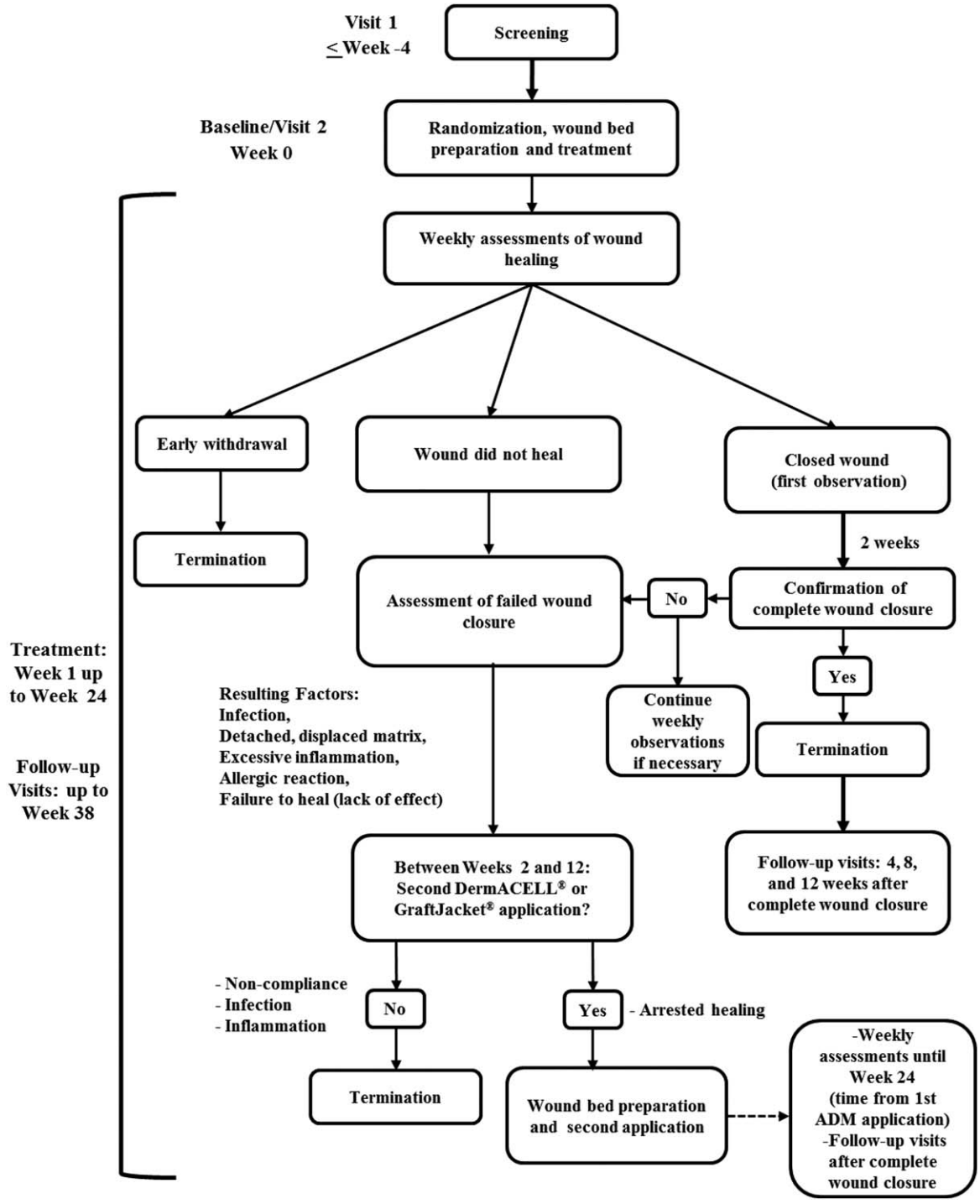

Figure 1. Flowchart depicting treatment processes.

Statistical methods

The percentage of wounds healed was reported for both single-application and all applications for those subjects enrolled in the D-ADM and GJ-ADM arms. A power analysis conducted before study initiation determined 66 patients in each D-ADM and conventional care arm would be needed to be enrolled in each arm to have an 80% chance of obtaining a statistically significant result. Statistical significance was not sought or expected for the GJ-ADM arm so it was not included in the power analysis. The "all applications" of ADM analyses included all

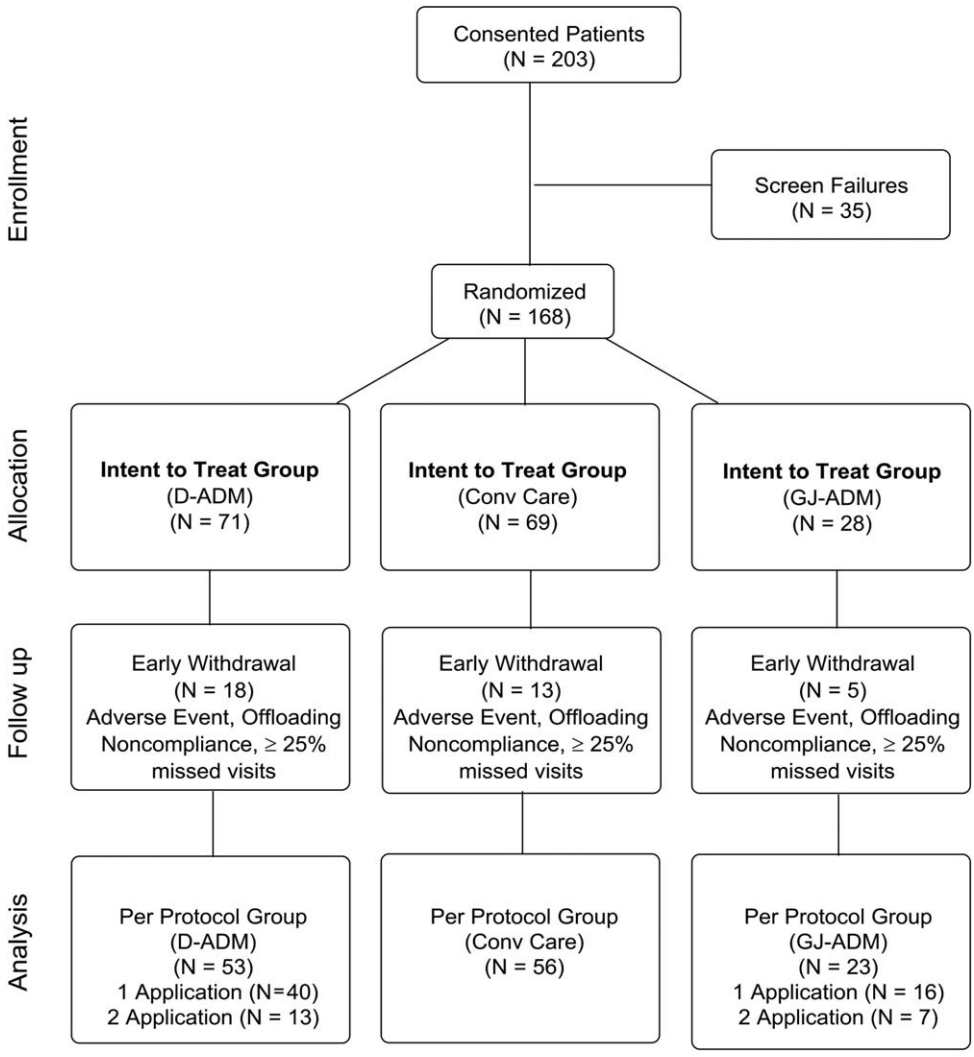

Figure 2. Flowchart showing subject populations throughout study.

subjects who received either one or two applications of the ADMs. Percent wound area reduction (PAR) was calculated using the equation {PAR = ([WM–BM]/BM)*–100} where WM = wound measurement and BM = baseline measurement. Categorical data was analyzed using either Fisher's exact test for pairwise comparisons or ANOVA tests for determining significance among all three treatment arms (e.g., baseline ulcer area). A Kaplan–Meier survivorship analysis was performed for nonhealing probability over time by treatment and the *p*-values were calculated using the Log-rank (Mantel-Cox) test (PRISM, Graphpad, La Jolla, CA). Hazard ratios,[34] used here to indicate the probability of closure at any given point in time for D-ADM versus conventional care treated ulcers, were analyzed using the Mantel-Cox test with 95% confidence intervals. A logistic regression model was used to assess the effect of the following baseline variables on requiring a second application of ADM of either treatment: ulcer duration, ulcer area, subject age, subject BMI, and subject HbA1c. The *p*-values for weekly % wound area and % wound area reduction through 24 weeks was calculated using *t* tests. Interrater reliability for the assessment by the blinded adjudicator was calculated using Cohen's kappa. All *p*-values, for both the categorical and continuous data, were calculated using a two-sided alpha of 0.05. All significance was verified by an independent statistician.

RESULTS

A total of 203 DFU subjects were consented and 35 subjects were removed as screen failures, leaving an intent-to-treat population of 168 subjects. The intent-to-treat population consisted of 71 subjects in the D-ADM arm, 69 subjects in the conventional care arm, and 28 in the GJ-ADM arm. Eighteen subjects in the D-ADM arm, thirteen subjects in the conventional care arm, and five subjects in the GJ-ADM arm withdrew early due to SAEs that impacted the ability to follow the target wound, offloading noncompliance, or $\geq$ 25% missed visits. This resulted in a per protocol population of 53 subjects for D-ADM with 40 of these patients receiving one application, 56 subjects for

Table 3. Ulcer variables at baseline for the intent to treat population.

		D-ADM ($N = 71$)	Conv Care ($N = 69$)	GJ-ADM ($N = 28$)
Ulcer location	Plantar forefoot	44 (62.0%)	46 (66.7%)	18 (64.3%)
	Plantar heel	12 (16.9%)	6 (8.7%)	3 (10.7%)
	Dorsal surface	12 (16.9%)	15 (21.7%)	6 (21.4%)
	Other	3 (4.2%)	2 (2.9%)	1 (3.6%)
Ulcer size at baseline (cm^2)	Mean	3.9	3.6	3.3
	Median	1.90	2.30	2.00
	Standard deviation	4.15	3.61	2.69
	Range	1.0–21.0	1.0–20.0	1.0–11.0
	Statistical difference between 3 arms	$p = 0.6932$		
Ulcer duration at baseline (weeks)	Mean	40.0	36.4	36.8
	Median	20.1	15.3	13.5
	Standard deviation	71.56	38.84	53.60
	Range	6.0–479.0	2.0–167.0	2.0–226.0
	Statistical difference between 3 arms	$p = 0.7656$		
Wagner grade	Grade 1	12 (16.9%)	14 (20.3%)	5 (17.9%)
	Grade 2	59 (83.1%)	55 (79.7%)	23 (82.1%)

conventional care, and 23 subjects for GJ-ADM with 16 of these patients receiving one application (Figure 2).

Baseline ulcer characteristics, including wound size, were similar between the three intent-to-treat arms (Table 3). The mean age for subjects in the D-ADM group was 59.1, 56.9 in the conventional care group, and 58.5 in the GJ-ADM group (Table 1). The subjects were 76.2% male and had a mean BMI of 32.6. The mean circulating HbA1c at screening was 8.51% in the D-ADM arm, 8.38% in the conventional care arm and 7.63% in the GJ-ADM arm. Subjects diagnosed with Type II diabetes comprised 93.5% of the enrolled population, with 90.1% randomized to D-ADM, 97.1% randomized to conventional care and 92.9% randomized to GJ-ADM. The treatment regimens prescribed for diabetes control were evenly distributed across study arms, thereby eliminating the potential confounding effect of insulin levels on cell responses and healing. Close to half of the subjects (41.1%) were current or past smokers with 58.9% having never smoked. Table 3 also depicts ulcer location of subjects included in the intent-to-treat population and stratifies them by plantar forefoot, plantar heel, dorsal surface, and other. The wounds were classified using the Wagner Ulcer Classification Scale. Most of the subjects in the D-ADM (83.1%), conventional care (79.7%), and GJ-ADM (82.1%) groups had ulcers classified as Wagner class 2, which are ulcers that extend into tendon or capsule.

The time course for healing was followed through 24 weeks for all three treatment arms. In the per protocol population (Figure 3), single application D-ADM demonstrated a significantly greater wound healing probability over conventional care through all three endpoints at Week 12 (65.0% vs. 41.1%; HR = 1.969; 95% confidence interval (CI) = 1.1–3.5; $p = 0.0123$), Week 16 (82.5% vs. 48.1%; HR = 2.397; 95% CI = 1.4–4.1; $p = 0.0003$), and Week 24 (89.7% vs. 67.3%; HR = 2.107; 95% CI = 1.3–3.5; $p = 0.0008$). D-ADM subjects that received all applications also showed a significantly greater wound healing probability over conventional care through the endpoints at Week 16 (67.9% vs. 48.1%; HR = 1.716; 95% CI = 1.04–2.831; $p = 0.0283$) and Week 24 (83.7% vs. 67.3%; HR = 1.546; 95% CI = 0.9821–2.435; $p = 0.0489$). The median survival time to healing was 9.0 weeks for the 1 app D-ADM arm versus 16.5 weeks for the conventional care arm ($p = 0.0020$). No significant differences were seen between GJ-ADM and conventional care or between D-ADM and GJ-ADM. In the intent to treat population (Figure 4), single application D-ADM demonstrated significantly greater healing over conventional care through endpoints at Week 16 (66.0% vs. 37.7%; HR = 1.918; 95% CI = 1.139–3.23; $p = 0.0093$) and Week 24 (70.0% vs. 49.3%; HR = 1.589; 95% CI = 0.9824–2.572; $p = 0.0442$). No other statistically significant differences were observed.

Additional analyses were conducted on the per protocol population for secondary objectives. In a week by week analysis (Figure 5), statistical significance was seen for one application D-ADM versus conventional care at weeks 7–24. All applications D-ADM demonstrated a significantly higher healing rate than conventional care at Weeks 15–16 and all applications GJ-ADM at weeks 22–23. GJ-ADM did not show a significantly greater healing rate over conventional care at any time point. Both D-ADM and GJ-ADM treatment arms used an average 1.1 applications of ADM for healed DFUs. For all ulcers, the D-ADM arm received an average 1.2 applications of D-ADM and the GJ-ADM arm received an average 1.3 applications of GJ-ADM. Healed ulcers in the D-ADM arm remained closed at a significantly higher rate than the conventional care arm at 4 weeks posttermination (100% vs. 86.7%; $p = 0.0435$), but the rates evened out at 8 and 12 weeks posttermination (Table 4). There was no significant

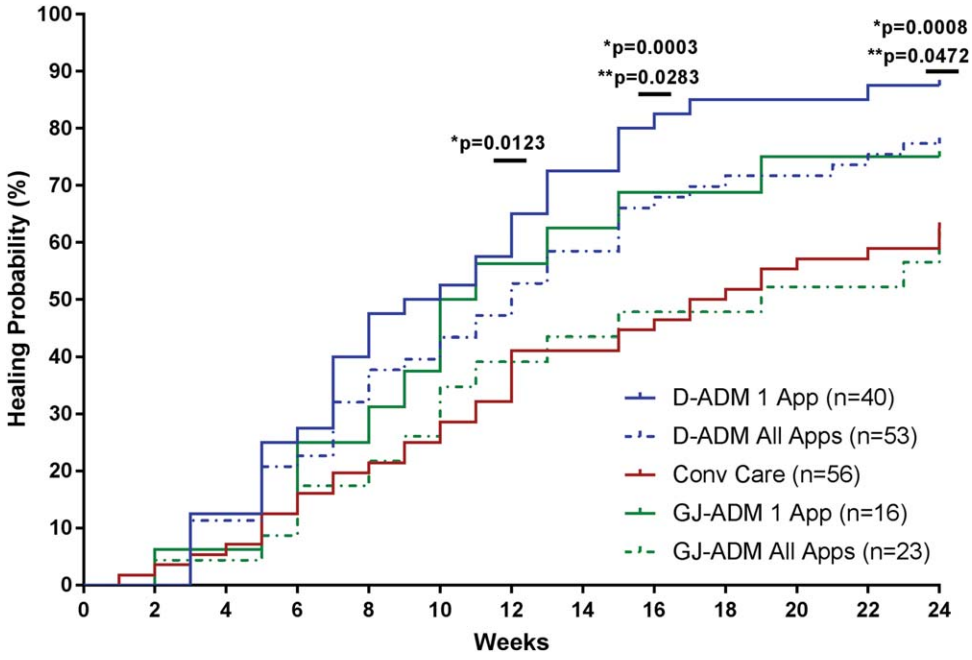

Figure 3. Kaplan-Meier survival curve depicting healing probability through endpoints at Week 12, Week 16, and Week 24 for the per protocol population. *Indicates a statistically significant difference between single application D-ADM and conventional care. **Indicates a statistically significant difference between all applications of D-ADM and conventional care. There were no statistical differences seen between the other arms.

difference between GJ-ADM and conventional care. Ulcers in the single application D-ADM treatment arm demonstrated a significantly greater percentage of wound area reduction than conventional care for Weeks 2–24 while single application GJ-ADM showed significantly greater percent wound area reduction over conventional care for Weeks 4–6, 9, and 11–12 (Figure 6). Also of note, the baseline area of ulcers had a significant effect on requiring a second application of ADM while the other baseline variables had no impact ($p = 0.0181$; Odds Ratio = 1.187; 95% CI = 1.030–1.369) (Figure 7).

The SF-36 v2.0 (Optum, Inc.) was used to capture the subject's perception of general health in eight areas. The mean, overall SF-36 scores at subject termination were 425 for D-ADM, 430 for conventional care, and 404 for GJ-ADM. There were no significant differences noted between treatment arms for the overall total score or in any of the eight areas.

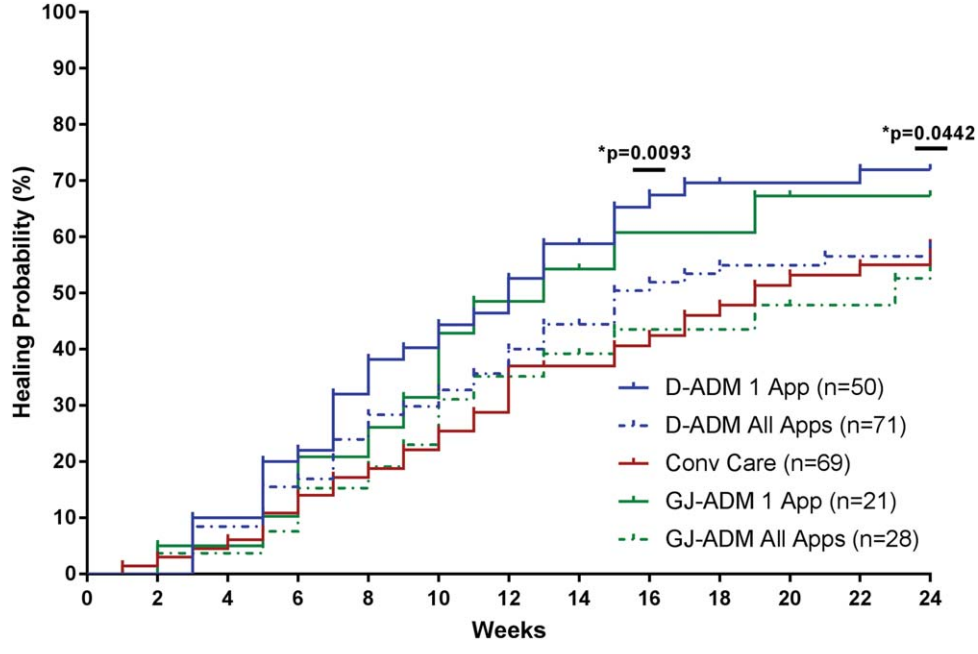

Figure 4. Kaplan-Meier survival curve depicting healing probability through endpoints at Week 12, Week 16, and Week 24 for the intent to treat population. *Indicates a statistically significant difference between single application D-ADM and conventional care. There were no statistical differences seen between the other arms.

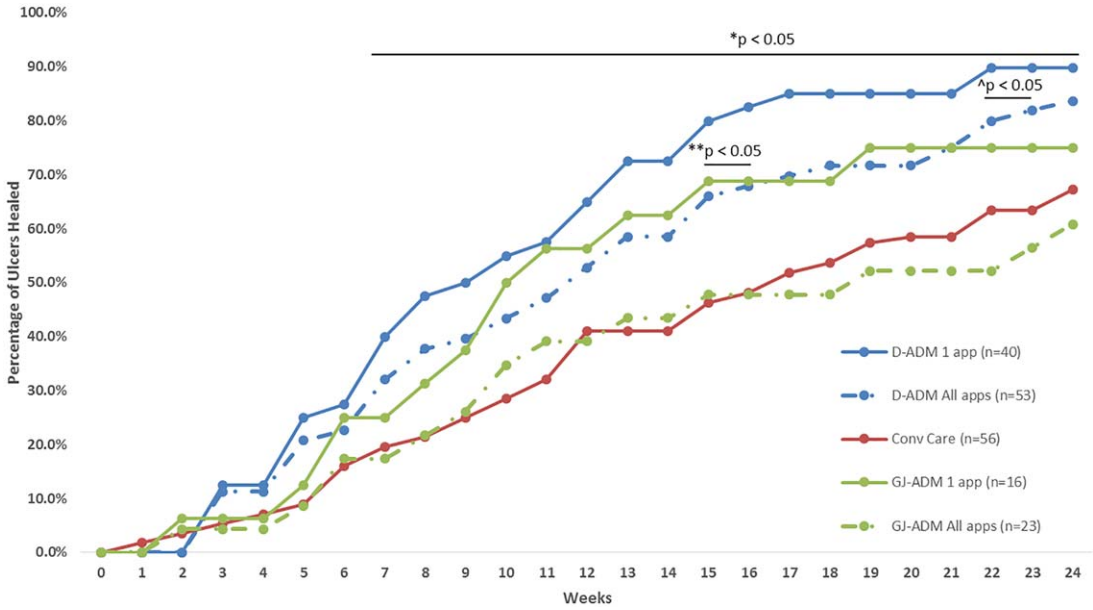

Figure 5. Percentage of healed wounds each week through 24 weeks for the per protocol population. *Statistically significant difference between single application D-ADM and conventional care for weeks 7–24. **Statistically significant difference between combined all applications of D-ADM and conventional care for weeks 15 and 16. ^Statistically significant difference between all applications of D-ADM and all applications of GJ-ADM for weeks 22 and 23.

A total of 167 subjects were followed for safety (Table 5). No AEs or SAEs were deemed related to the procedure or to the study product by the site investigators. One subject was randomized to conventional care but did not have a post baseline assessment so was not included in this analysis. One death was reported in the D-ADM treatment arm after the subject healed and completed a Termination visit, but prior to completion of the Post-Termination follow-up period. This event was determined to not be related to the study procedures or study product. The bulk of adverse events occurred during weeks 13–24, the final 12 weeks of follow-up. Infections were also closely monitored. For those infections deemed severe in intensity by a study physician, osteomyelitis was the most common infection reported in 5.6% of the D-ADM group, 5.9% of the conventional care group and 10.7% of the GJ-ADM group. Because the hospital course for these types of infections can by lengthy, resulting in missed visits and a negative impact on the ability to heal or amputations, those subjects with these severe infections were excluded from the per protocol population. The proportion of overall early withdrawals and the proportion of SAEs were comparable among the three treatment groups based on relative population size ($p \geq 0.05$).

DISCUSSION

This multicenter, randomized, controlled trial demonstrated D-ADM was an effective treatment for completely healing difficult-to-treat DFUs and also for rapidly reducing the wound area of still open ulcers. The reduction in wound area of DFUs is an important outcome as it can be an early

Table 4. Percentage of healed wounds that remained closed at posttermination visits from the Single Application ADMs and Conventional Care Per Protocol Populations

	D-ADM	Conv care	GJ-ADM
4 Week posttermination	100% (29/29)	86.7% (26/30)	88.9% (8/9)
p-value vs. conv care	**$p = 0.0435$**		$p = 0.8629$
8 Week posttermination	88.9% (24/27)	84.4% (27/32)	100.0% (9/9)
p-value vs. conv care	$p = 0.6169$		$p = 0.2113$
12 Week post-termination	92.9% (26/28)	93.8% (30/32)	90.0% (9/10)
p-value vs. conv care	$p = 0.8909$		$p = 0.6913$

*Posttermination data not available for all healed patients at each follow-up time point. Bold value indicates statistical significance $p < 0.05$.

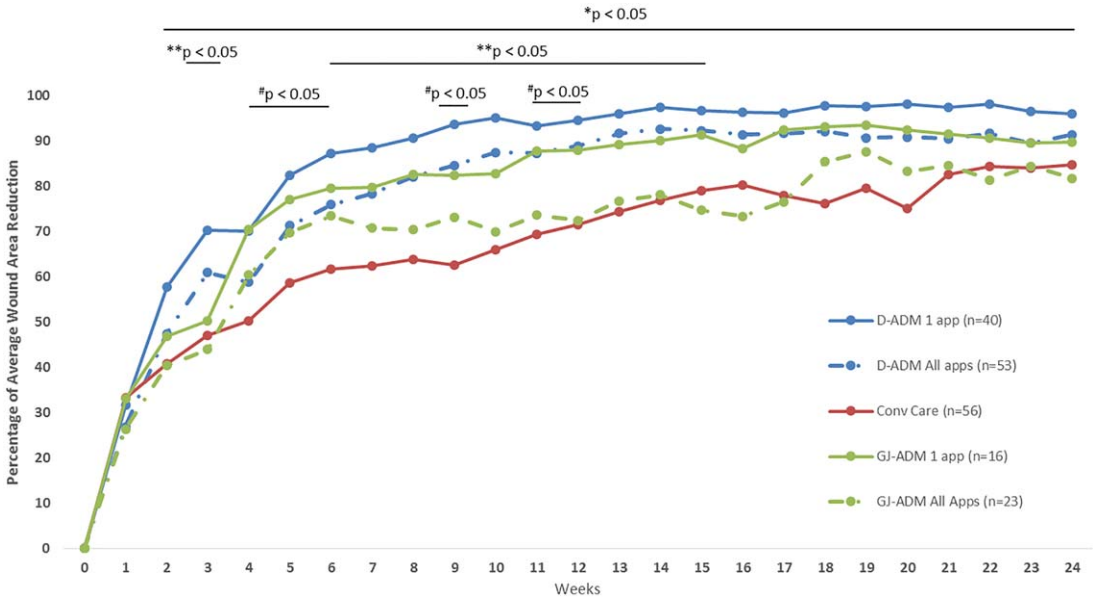

Figure 6. Average percent wound area reduction through 24 weeks for the per protocol population. *Statistically significant difference between single application D-ADM and conventional care for weeks 2–24. **Statistically significant difference between all applications of D-ADM and conventional care for weeks 3 and 6–15. #Statistically significant difference between single application GJ-ADM and conventional care for weeks 4–6, 9, and 11–12.

predictor of later closure, as well as, reduced risk of infection.[35,36] Notably, both D-ADM and GJ-ADM were able to completely close DFUs with an average 1.1 applications, thus establishing these ADMs' ability to heal chronic DFUs of a complex nature usually with a single application. These results indicate that a single application of D-ADM or GJ-ADM is sufficient for closing most DFUs and the results of the single application sub-cohort is the most relevant to "real world" D-ADM and GJ-ADM use. Intent to treat analyses, considered the gold standard in clinical trial reporting,[37] were presented. As some subjects were removed for major protocol violations and missing outcomes, the per protocol analyses were also included to report the healing rate of compliant patients. A per protocol analysis may convey a more accurate treatment efficacy rate[38,39] in a population prone to non-compliance and missing outcomes though this view is contentious and non-compliant patients can also be expected in actual use. Subjects removed from the per protocol population included one subject who was withdrawn after missing 15 visits, another who was withdrawn after Week 7 for lung cancer, and a conventional care subject who withdrew consent at Week 3.

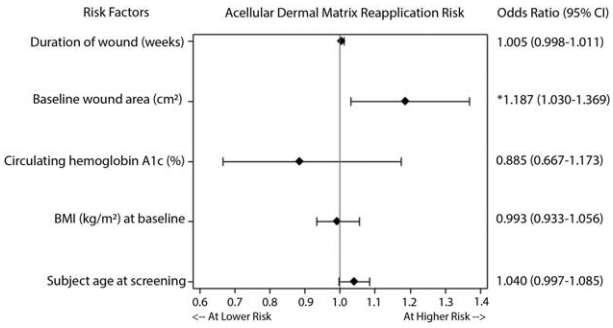

Figure 7. A logistic regression analysis showing baseline factors related to the risk of receiving a second application of acellular dermal matrix.

As the largest human ADM controlled trial to report multiple applications, the study presented here may provide results that could be used as a benchmark for future trials of other ADM products. Other studies report a primary endpoint for wound healing that varies from 12 to 16 weeks follow-up.[16,17] The decision to include detailed results for both 12 and 16 weeks follow-up in the trial presented here allows a more thorough comparison to the literature. The final endpoint of 24 weeks in the current study also provided an extended time for ulcers to heal across the treatment arms, especially those ulcers belonging to subjects who were noncompliant or had comorbidities. For instance, there were two subjects in this study that healed after 16 weeks of treatment but may have healed earlier had there been more than sporadic compliance with off-loading throughout the treatment phase. Furthermore, by analyzing each week through 24 weeks, this study is able to provide an idea of how early significantly improved healing from an ADM treatment can be expected and also, perhaps as importantly, the duration of significantly improved healing rates. This may have substantial implications on clinical care by helping treating clinicians decide how long to persist with a given treatment before moving on to another. A single application of D-ADM applied at baseline resulted in a statistically significant healing rate at 7 weeks that continued

Table 5. Subjects with TEAEs* reported through 24 weeks of treatment (safety population).

	D-ADM ($N = 71$)	Conv care ($N = 68$)	GJ-ADM ($N = 28$)
TEAEs (% of subjects with events)	203 (64.8%)	168 (64.7%)	108 (71.4%)
p-value for all treatment arms†		0.7935	
Related to procedure	2 (2.8%)	4 (4.4%)	0 (0.0%)
Related to study product	0 (0.0%)	0 (0.0%)	0 (0.0%)
Maximum Severity of TEAE			
Mild	137 (23.9%)	117 (29.4%)	66 (32.1%)
Moderate	47 (22.5%)	38 (19.1%)	28 (21.4%)
Severe	19 (18.3%)	13 (16.2%)	14 (17.9%)
Serious TEAEs	33 (28.2%)	22 (27.9%)	26 (28.6%)
p-value for all treatment arms†		0.9980	
Related to procedure	0 (0.0%)	0 (0.0%)	0 (0.0%)
Related to study product	0 (0.0%)	0 (0.0%)	0 (0.0%)

*Treatment emergent adverse events.
†There was not a significant difference in the rates of TEAEs and Serious TEAEs between treatment arms.

exhibiting significant healing over conventional care through the final study endpoint at 24 weeks. This long duration suggests that not only does D-ADM influence healing quickly but also that this effect continues for almost six months past the initial application.

The 24 week time point was also hypothesized to provide those subjects receiving a second application of ADM by 12 weeks another full 12 weeks in which to close before termination from the study. Across the 24 weeks, D-ADM demonstrated a significantly greater percentage of closed wounds and a greater percentage of reduction in wound area than the conventional care arm (Figures 3–6). The percentage of healed wounds that remained closed in the months following termination is an important metric for real world treatments but is not often tracked in the literature. Of note, D-ADM exhibited a significantly greater percentage of healed wounds that remained completely closed one month after termination than healed wounds in the conventional care arm.

Although the number of applications used for treatment has been limited for previous human ADM trials, other skin substitutes have observed as many as 15 applications were needed for wound closure.[17] One recent randomized trial[17] of 307 patients compared the healing rate for DFUs between a bilayer matrix consisting of bovine and synthetic components (Integra Dermal Regeneration Template (DRT)) and a conventional care arm that used moist wound care with daily dressing changes. At 16 weeks follow-up, the investigators found a 51% (79/154) healed rate for the bilayer matrix arm versus 32% (49/153) for the control arm. The 16 week healed rate of 51% with an average of 2 applications and a maximum of 15 applications of bilayer matrix contrasted strongly with D-ADM's 16 week healed rate of 67.9% with an average 1.2 applications and a maximum of 2 applications of ADM.

A wide disparity exists in the efficacy of a product versus the average acquisition cost per patient among different treatments for DFUs. When compared to Integra DRT, GJ-ADM, EpiFix, and Apligraf, D-ADM, as studied here, not only demonstrated the greatest healing rate at 12 weeks, but D-ADM also showed the lowest average product cost per patient (Figure 8). D-ADM (all applications) exhibited an average healing rate of 53% at 12 weeks with an average 1.2 applications and thus an average cost of $1,441 per patient based on product list fee.[40] GJ-ADM (all applications) displayed an average healing rate of 39.1% at 12 weeks with an average 1.3 applications with an average expense of $2,237 per patient based on our cost to purchase. In the previously discussed Integra DRT study, patients showed a 12 week healing rate of 45% with an average of two applications which cost $1,490, assuming that the average graft used was 5 cm $\times$ 5 cm.[40–42] Despite only demonstrating a 12 week healed rate of 28%, EpiFix patients required a mean 3.5 applications at an average cost of $4,463 per patient.[43] Apligraf also showed a higher cost and lower healed ulcer rate with an observed 12 week healed rate of 48% while the average patient needed 2.5 applications at a product cost of $3,238.[43] The data for EpiFix and Apligraf come from a recent retrospective publication by Kirsner et al.[43] which examined the real-world healing rates for these two treatments at 99 wound care centers. The Kirsner et al.[43] study, which used subjects from multiple medical centers across the country in a similar method to our study, may be the most accurate source of real-world healing rates for EpiFix and Apligraf. While another study has reported much higher healing rates for EpiFix and lower rates for Apligraf,[44] there are several concerns about this study including a small sample size, narrow geographical distribution, and that the results can be considered an extreme outlier in the wound healing literature.[43] The striking differences in the cost and efficacy of different products makes comparison important for investigators in light of the ever increasing costs of healthcare combined with the potentially severe consequences for unhealed DFUs.

The results for D-ADM also compared favorably with the 69.6% healed rate reported for GJ-ADM at 12 weeks

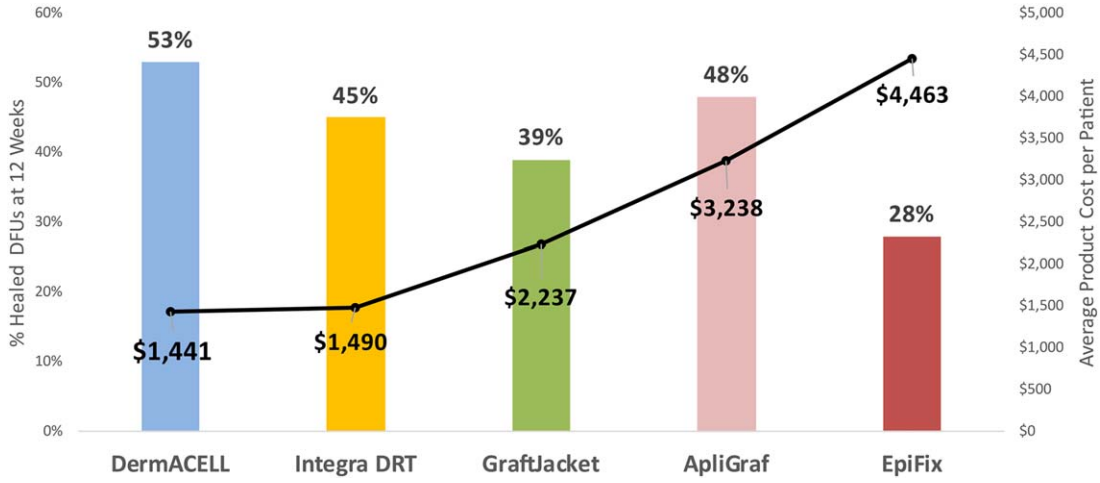

Figure 8. A comparison of recently published 12 week healing rates along with the average cost of product per patient for advanced treatments including DermACELL,[37] Integra DRT*,[38,39] GraftJacket**, Apligraf,[40] and EpiFix.[40] *Assumes the average application provided to each patient was 5 cm × 5 cm. **Expense per patient based on our cost to purchase.

follow-up in a 2009 study by Reyzelman et al.[16] However, it is unclear whether subjects who received multiple applications of GJ-ADM were included in the analysis, leaving uncertain the average number of GJ-ADM applications actually needed for healing and whether the reported healing rate is only for a specific subsection of the patient population. Another concern is that as an older study published in 2009, the healed rate is higher due to a more elastic definition for healed ulcers. The AHRQ has advocated use of the FDA Guidance for Industry: Chronic Cutaneous Ulcer and Burn Wounds[32] which has raised the bar for clinical evidence by requiring confirmation of healing at two consecutive study visits 2 weeks apart. The additional week for confirmation allows the healing outcomes to better reflect real world healing rates where the first indication of healing may not result in termination of care. It is very important to keep in mind when comparing healing rates in the literature that older studies likely use an older definition which allowed an ulcer to be labeled healed at the first instance of closure and some recent studies use a definition of reepithelialization without draining or dressing requirement confirmed at two consecutive visits only 1 week apart.[45] The study presented here is one of the first large DFU trials to use the more rigorous definition of healing. The required extra week for healing can make a substantial difference in the healed rate as well as the mean survival time to healing. For example, 5 D-ADM subjects had an imaged wound area of 0.0 cm^2 at 12 weeks but were not considered healed due to the AHRQ healing requirement and clinician assessment despite having a completely closed wound. It is unknown how high the proportion of other studies' healed wounds contain similar wounds that should not be considered healed under this criteria, but it is worth citing that no wounds considered unhealed in Reyzelman et al. reported an area of 0.0 cm^2 in contrast to the current study. Additionally, at 12 weeks follow-up, there were four D-ADM subjects with a wound area of just 0.1 cm^2. The measuring laser is very accurate and it is possible that these would have been considered healed by an evaluator using their eye, a ruler, and the old definition for healed wounds. In fact, a blinded evaluator determined an additional two D-ADM wounds should have been considered healed that had been designated unhealed, although these were not considered healed in the analysis reported here as the study criteria were followed. This amounts to nine unhealed wounds for D-ADM (17% of the D-ADM arm) that could have arguably been labeled as completely healed in previous studies, but we applied the more conservative definitions for our analysis. The potential impact would only apply to D-ADM as the conventional care arm only had a single patient with a wound area ≤ 0.1 cm^2 at 12 weeks. Finally, as with the novel inclusion of both one and two human ADM applications, the use of the strict AHRQ guidelines for healing may make the results presented here useful as a comparison for future DFU studies that also use the AHRQ guidelines moving forward.

The study reported here was designed to encompass both the strength of a randomized controlled trial along with the inclusion of a large and geographically diverse patient population to determine an accurate healing rate for D-ADM that is representative of real world practice. The study protocol was designed to allow collection of data that was generalizable to the standard wound care population in US wound care centers. One weakness of this design was that the study sites were encouraged to utilize the current standard of care in place at their wound care centers. Although a listing of approved dressings was supplied, there were marked differences in frequency of weekly debridement of wounds and the use of other dressings besides gauze. Conversely, measuring the performance of an ADM directly juxtaposed to a more realistic, diverse conventional product formulary of dressing modalities that are considered of a more advanced nature, only enhances the robustness and practicality of this comparison. As in other investigations, utilizing only gauze or

mitigated dressing options, which may not necessarily be appropriate for certain wound bed characteristics, does not allow the clinician to exercise full practice of wound bed preparation in those comparisons. Therefore, these findings should be more characteristic of real world outcomes in so-called conventional care.

Although this study utilized stringent criteria for evaluation, the lack of information surrounding additional applications of human ADMs in the literature proved challenging for study design. This resulted in the study being erroneously powered using healing rates reported in other human ADM studies that reported only a single application of product with a 12 week follow-up period. Although the single application wound healing rate shown in our study was significantly better than conventional care throughout, the healing rate for all subjects did not become statistically significant until Week 15 even though the percent wound area reduction was statistically significant from Week 6. This study provided a very detailed analysis of healing rates and it should be noted that multiple probability tests were applied to this data set without correcting related probabilities. While some may consider this a source of probability bias, more recent views[46–48] have found this acceptable. Elucidating the effect of a second application on the overall wound environment and its ability to heal was not considered during protocol development. Furthermore, since additional applications of ADMs were allowed at investigator discretion and a few of these wounds healed quickly thereafter, more second applications may have occurred than were necessary. Criteria for the timing of second applications for ADMs are not standardized and are an area of consideration for additional research. The results of the logistic regression analysis (Figure 7) indicated that baseline wound area size should be a focal point of further research. Additionally, although wound depth was collected at each visit, this data was not used as the Silhouette System had difficultly reliably determining depth. It appeared that the depth measurements may have changed depending on the angle or distance of the camera. However, investigators used a ruler to measure wound depth to determine if a subject passed the inclusion criteria so there was no concern about the accuracy of the screening process. It should be noted that in contrast to the unreliable depth measurements, the Silhouette system was extremely accurate in measuring the wound area. Furthermore, the outlined area image was double-checked for every subject at each visit to ensure the wound area was accurately measured.

Another weakness of this study was that the investigators were not blinded to the treatment type when assessing wound closure. However, this was mitigated by the use of the Aranz laser system which eliminated the bias in measuring wound area reduction. Additionally, a blinded, third-party adjudicator assessed healed wounds and those close to healing by 12 weeks follow-up. The adjudicator expressed "strong" agreement ($\kappa = 0.837$)[49] with investigator designations and found an additional 2 healed wounds for D-ADM, 1 healed wound for GJ-ADM, and no change for conventional care subjects. These additional healed wounds were conservatively not included in the data analysis but are evidence that there was no investigator bias in favor of D-ADM specifically or ADMs in general. Another disadvantage mentioned previously is the possibility of an artificially lowered healing rate for as many as 9 D-ADM wounds due to the stricter definition of healing applied in this study. The different definitions in healing should be taken into account when comparing this study with older literature, but this study may provide a benchmark for healed rates as more published studies transition to the new AHRQ guidelines for determining the healed status of wounds.

A thorough review of the safety profiles of available skin substitutes demonstrated a plethora of adverse event definitions and collection guidance. One of the strengths of this study was the strict adverse event definition used for safety data collection. An adverse event was required to be recorded when any change in a subject's health status was noted, regardless of severity or causality, in an attempt to draw an accurate safety profile for treatment with human ADMs in the challenging diabetic with chronic wounds of the lower extremity. This safety profile as well as the length of time over which AEs are collected should be considered when comparing the products studied here to others with less formal control over collection of changes in health status. The thorough safety profile presented here may serve as a benchmark for future DFU studies which undertake an inclusive approach to safety data collection.

The greater healed ulcer rates and positive safety profile for D-ADM may come from the processing method which removes $\geq 97\%$ of donor DNA from the graft while maintaining the biomechanical and biochemical properties.[18,50] Further, published references have been made to D-ADM demonstrating more rapid host cellular infiltration and revascularization as compared to other similar dermal substitutes.[21,51] The processing method also allows D-ADM to be stored fully hydrated at ambient temperature and terminally sterilized to the same 10^{-6} SAL as expected for implantable medical devices.[18,25] Furthermore, as noted in the introduction, an estimated 80% of patients suffering from diabetes will live in developing countries by 2035[2] and the availability of advanced treatments with a reasonable cost are essential for this population. In accordance with LifeNet Health's mission as a non-profit organization, efforts are currently underway to make D-ADM available in low resource countries including India, Bolivia, Korea, South Africa, Pakistan, Paraguay, Philippines, Myanmar, Indonesia, Cambodia, and Guatemala.

In conclusion, the results of this multicenter, randomized, controlled trial demonstrate that D-ADM, a thoroughly decellularized, ready to use, sterile allograft, can successfully heal and rapidly reduce the wound area of difficult to heal DFUs with one or two applications and at significantly higher rates than conventional care treatment with minimal complications.

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Conflict of Interest: Dr. Cazzell, Dr. Vayser, Dr. Pham, Dr. Walters, and Dr. Reyzelman were among the clinical

trial investigators for this study sponsored by LifeNet Health and do not report any other conflicts of interest. Mr. Samsell, Ms. Dorsch, and Dr. Moore are employees of LifeNet Health. Efforts to mitigate potential conflicts of interest included only permitting investigators to determine the healed status of wounds, submitting those designations to a third–party adjudicator blinded to treatment type, using an impartial laser system to determine ulcer areas, and contracting with an independent contract research organization to verify the significance of all statistical analyses.

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