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ORIGINAL ARTICLE

An aseptically processed, acellular, reticular, allogenic human dermis improves healing in diabetic foot ulcers: A prospective, randomised, controlled, multicentre follow-up trial

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Aseptically processed human reticular acellular dermal matrix (HR-ADM) has been previously shown to improve wound closure in 40 diabetic patients with non-healing foot ulcers. The study was extended to 40 additional patients (80 in total) to validate and extend the original findings. The entire cohort of 80 patients underwent appropriate offloading and standard of care (SOC) during a 2-week screening period and, after meeting eligibility criteria, were randomised to receive weekly applications of HR-ADM plus SOC or SOC alone for up to 12 weeks. The primary outcome was the proportion of wounds closed at 6 weeks. Sixty-eight percent (27/40) in the HR-ADM group were completely healed at 6 weeks compared with 15% (6/40) in the SOC group. The proportions of wounds healed at 12 weeks were 80% (34/40) and 30% (12/40), respectively. The mean time to heal within 12 weeks was 38 days for the HR-ADM group and 72 days for the SOC group. There was no incidence of increased adverse or serious adverse events between groups or any graft-related adverse events. The mean and median HR-ADM product costs at 12 weeks were $1200 and $680, respectively. HR-ADM is clinically superior to SOC, is cost effective relative to other comparable treatment modalities, and is an efficacious treatment for chronic non-healing diabetic foot ulcers.

KEYWORDS
diabetic foot ulcers, human acellular dermal tissue, randomised controlled trial, standard of care

1 | INTRODUCTION

Diabetes is 1 of the more serious chronic medical conditions worldwide, with 6.3% of people with diabetes globally having a diabetic foot ulcer (DFU) and over twice that (13%) in North Americans with diabetes.[1] DFUs are a serious diabetes complication that can result in lower extremity amputation with high mortality rates.[2] Such grave outcomes can be lessened with expeditious wound closure, but many DFUs do not heal despite standard of care (SOC) and subsequently become chronic in nature.[3] Allograft tissues have been used for many years to treat non-healing DFUs. One allograft, human acellular dermal matrix, is rich in peptides and growth factors associated with ulcer healing and facilitates cellular activation in the wound bed, mediates the inflammatory response, and enhances tissue repair.[4–7]

Until recent years, human acellular dermal matrices have been prepared from the more superficial layers of the

donated human dermal tissue. The superficial layers are characterised by a heterogeneous network structure that varies in density from 1 side to the other, impacting both cellular infiltration and the remodelling process.[6,8–10] In contrast, when the dermal matrix is prepared from the deeper reticular layer, it has an elastic and porous structure comprised of multiple structural elements, including elastin, collagens, and reticular fibres,[5,6,11,12] that promote graft integration, cellular infiltration, tissue remodelling, and potentially address scar formation.[5,6,12]

The allogeneic graft studied in this clinical trial is composed of the reticular dermal layer and is prepared using aseptic techniques and mild processing to maintain the native structural integrity and matrix proteins of the tissue while minimising immunogenicity.[4–6,13] Histological analyses confirmed that this aseptically processed human reticular acellular dermal matrix (HR-ADM) retains the homogenous, porous structure and key ECM components, including retention of collagen type I, III, IV, and VI and elastin, that are naturally present in the human reticular dermis[6] (Figure 1).

Recently, we reported the use of HR-ADM in a study enrolling 40 patients.[5] In that study, we found that at 6 weeks, 65% of patients were healed using the construct vs 5% of patients with the SOC. At 12 weeks, 80% ulcers healed with HR-ADM vs 20% with SOC. Although this result was statistically significant, the investigators sought additional data from a larger population to validate and extend the initial findings. Here, we report the results from the entire 80-patient cohort.

Key Messages

- this multicentre, randomised, controlled clinical follow-up study demonstrated the clinical effectiveness of an aseptically processed human reticular acellular dermal matrix (HR-ADM) in improving wound outcomes when applied to non-healing diabetic foot ulcers (DFUs) compared with standard of care (SOC)
- the proportion of wounds healed at 6 and 12 weeks was significantly higher for the HR-ADM group (68% and 80%, respectively) compared with the SOC group (15% and 30%, respectively)
- the mean time to heal within 12 weeks was significantly shorter for the HR-ADM group at 38 days compared with 72 days in the SOC group
- the mean cost to heal in the HR-ADM group was $800 and $1200 at 6 and 12 weeks, respectively

2 | METHODS

This randomised clinical trial (RCT) was conducted at 5 outpatient wound care centres across the United States, in which HR-ADM plus SOC vs SOC alone was assessed in a total of 80 patients with diabetes. The preliminary results of the interim analysis of the first 40 patients have been reported, with the final cohort of 80 being evaluated for the complete trial.[5] Each patient had at least 1 chronic neuropathic DFU that failed to heal following a minimum 4 weeks of documented SOC. The Western Institutional Review Board reviewed and approved the study protocol and subject consent form (#20142081). The trial was pre-registered in ClinicalTrials.gov (NCT02331147). The study adhered to the Declaration of Helsinki, Good Clinical Practice, and HIPAA patient confidentiality requirements. All subjects provided their written consent prior to enrolment.

2.1 | Patient recruitment and randomisation

The complete inclusion and exclusion criteria used by site investigators to screen patients for study eligibility are listed in Table 1. Patients were required to have a DFU present for a minimum of 4 weeks and demonstrate adequate renal function and adequate perfusion to the affected extremity (Table 1). Prior to randomisation, patients who met the inclusion criteria were first treated only with SOC for a 2-week screening period, during which time they were evaluated on site weekly for ulcer assessment/measurements and sharp debridement. During the first screening visit, patients underwent a comprehensive physical examination and had their medical history documented. If multiple ulcers were present, the largest ulcer was selected as the study ulcer (referenced within this manuscript as "index-ulcer"). The index ulcer was assessed for infection using the Woo and Sibbald guidelines.[14] Ulcers were then cleaned and surgically debrided using a 15 blade or curette. Next, each ulcer was digitally photographed, and the area was measured using acetate tracing.[5] Ulcers within 3 cm of another ulcer were excluded. A sterile, ophthalmological probe was used to perform a probe-to-bone test on the index ulcer. Any ulcers with bone involvement were excluded. Serum creatinine and glycosylated haemoglobin (HbA1c) was documented. Vascular assessments using dorsal transcutaneous, ankle brachial index, or Doppler arterial waveforms tests were performed on the affected extremity.

During the 2-week screening period, collagen-alginate dressings, gauze, soft roll, and a compressive dressing were applied to the ulcer. Offloading was performed using a removable cast walker (Royce Medical, Inc., Camarillo, California) or similar generic device. In the cases where a patient could not be fitted with a removable device, a total contact cast was used. In addition, if the investigator observed patient non-adherence to offloading, the patient was fitted with an instant total contact cast, which requires the addition of a fibreglass layer on top of the diabetic cast walker to prevent removal or non-compliance. Patients were provided with dressing supplies to change their dressings

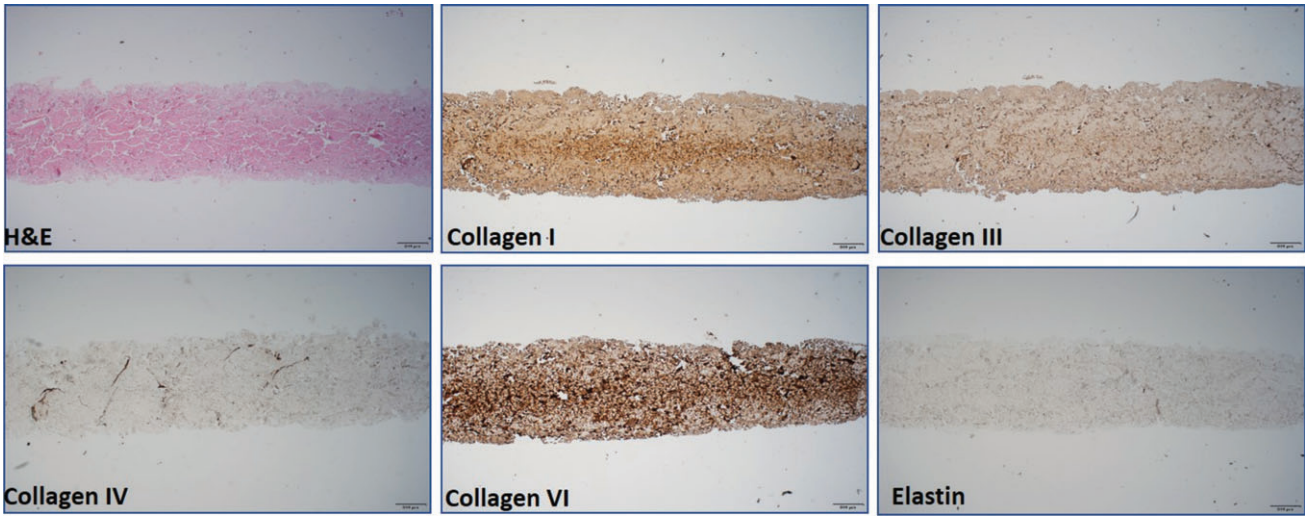

FIGURE 1 Immunohistochemical staining of aseptically processed, pre-hydrated human, reticular acellular dermis for matrix proteins revealed retention of collagen type I, III, IV and VI, and elastin (magnification ×2)

daily. At 2 weeks, patients with index ulcers that had not healed more than 20% were randomised 1:1 to receive HR-ADM plus SOC or SOC alone.

A paper block system was used for patient randomisation.[5] Sealed envelopes were distributed to each study site, where investigators were blinded to the randomisation and allocation processes.

2.1.1 | HR-ADM allograft

Study investigators evaluated AlloPatch Pliable (MTF, Musculoskeletal Transplant Foundation, Edison, New Jersey), a reticular layer preparation of human dermal tissue that is aseptically processed to preserve the biological properties and structure of the native tissue.[5] The HR-ADM was provided in sizes as small as 1.5 cm × 1.5 cm to optimise donor tissue use during this study. Prior to application, the dermal tissue was rinsed with saline, trimmed to fit the ulcer using sterile scissors, and fenestrated to prevent the formation of a haematoma or seroma.

2.1.2 | Procedures

Patients received weekly examinations and treatments for up to 12 weeks or until the index ulcer completely healed. Per protocol, a patient was withdrawn from the study if an adverse event (AE) occurred, or if the ulcer failed to decrease in size by 50% in 6 weeks. Vital signs were taken,

TABLE 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria

• Aged 18 years or older
• Type 1 or type 2 diabetes mellitus[a]
• Non-infected wound, diabetic in origin, larger than 1 cm^2, and located on the foot (beginning below the malleoli of the ankle)
• Wound present for a minimum of 4 weeks duration, with documented failure of prior treatment to heal the wound
• Additional wounds may be present but not within 3 cm of the index wound
• HbA1c <12% (prior to randomisation)
• Adequate circulation to the affected extremity, as demonstrated by 1 of the following within the past 60 days:
  ○ Dorsum TCOM ≥30 mm Hg
  ○ ABI with results of ≥0.7 and ≤1.2
  ○ Triphasic or biphasic Doppler arterial waveforms at the ankle of affected leg
• Serum creatinine less than 3.0 mg/dL
• Patient is willing to provide informed consent and is willing to participate in all procedures and follow-up evaluations necessary to complete the study
	• Patients previously randomised into this study or presently participating in another clinical trial
	• Wound probing to bone (UT Grade IIIA-D)
	• Index wound larger than 25 cm^2
	• Active infection at index wound site
	• Wound treated with a biomedical or topical growth factor within the previous 30 days
	• HbA1c >12% within previous 90 days
	• Serum creatinine level ≥3.0 mg/dL
	• Patients with a known history of poor compliance with medical treatments
	• Patients with ongoing radiation therapy or chemotherapy
	• Patients with known or suspected local skin malignancy to the index wound
	• Patients with uncontrolled autoimmune connective tissues diseases
	• Non-revascularisable surgical sites
	• Any pathology that would limit the blood supply and compromise healing
	• Patients who are pregnant or breastfeeding
	• Patients who are taking immune system modulators that could affect graft incorporation
	• Patients taking a Cox-2 inhibitor
	• Patients whose wounds heal >20% during the screening period

Abbreviations: ABI, ankle brachial index; TCOM, transcutaneous oxygen test; UT, University of Texas.

[a] American Diabetes Association diagnostic criteria used.

and an Accu-Chek test was used to measure blood glucose levels at each visit. Patients with inadequate diabetes management were referred to their primary care physician or endocrinologist for treatment and were allowed to continue in the clinical trial while their blood sugar was optimised.

At each visit, the index ulcer was cleansed with sterile normal saline solution, photographed, and appropriately debrided before surface area and depth measurement.[5,15] A wound culture was taken with both anaerobic and aerobic swabs if infection was suspected. Systemic antibiotics were administered until the infection was clinically resolved. Patients were withdrawn from the study if the infection worsened in severity such that it interrupted HR-ADM treatment or interfered with study visits.

Treatment in the SOC group consisted of daily dressing changes with a collagen alginate (Fibracol, Systagenix, Gargrave, Yorkshire, UK), followed by a 3-layer padded generic dressing of gauze, soft roll, and a compressive wrap, which were documented at each weekly study visit.

Patients allocated to the treatment group received weekly applications of HR-ADM during the study period. Following immersion in sterile saline for 5 to 10 seconds, the graft was pie-crusted with a 15-scalpel blade, not greater than ×1.5 to ×1.0, and cut to size using sterile scissors and applied to the entire ulcer surface ensuring maximum surface contact.[5] A non-adherent dressing (Adaptic Touch, Systagenix) was applied over the graft, followed by a moisture-retentive dressing (hydrogel bolster) and a padded 3-layer dressing (Dynaflex, Systagenix or equivalent) until complete closure (100% reepithelialisation) had occurred.

As in the screening period, all patients in both groups were offloaded using a removable cast walker (Royce Medical, Inc., Camarillo, California), total contact cast, or similar generic device. Percentage area reduction (PAR) was calculated for the index ulcer at 6 weeks after randomisation using the following formula: $\mathrm{PAR} = ([A_I - A_{6W}]/A_I)100$, where A_I is the area of the index ulcer at randomisation, and A_{6W} is the area at 6 weeks. Patients whose ulcer had a poor wound-healing trajectory at 6 weeks (PAR $\leq$ 50%) were withdrawn from the study.

2.1.3 | Validation of healing

Complete ulcer healing was based on the site investigator's assessment, as evidenced by complete (100%) reepithelialisation without drainage and need for dressing. A follow-up validation visit was conducted 1 week after ulcer closure was first observed to confirm durability of ulcer closure.

The principal investigator reviewed ulcer photographs and confirmed healing status. An independent panel of wound care experts, who were blinded to the patient allocation process and the principal investigator's assessment, reviewed all study-related decisions made by the site investigators and confirmed healing status. The validation team included a general surgeon, 2 plastic surgeons, a vascular surgeon, a podiatrist, and an internal medicine specialist.

2.1.4 | Study outcomes

The primary endpoint of this study was the difference between the 2 groups in the proportion (%) of ulcers healed at 6 weeks. Secondary endpoints were: differences in proportion of ulcers healed at 12 weeks, time to heal between study groups at 6 and 12 weeks, the number of grafts used, product wastage, and the cost to closure of the product for the HR-ADM group. Product wastage was measured as a percentage by subtracting the ulcer area at each visit from the total area of the full HR-ADM product available during the same visit and dividing the result by the total product area. The sum of the costs of each applied HR-ADM from all visits was used to calculate the total product cost for each ulcer per patient.

2.1.5 | Sample size calculations and statistical analysis

The sample size of 40 in each group was enough to detect a difference of 0.3 between the group proportions with 80% power. The proportion in the HR-ADM group was assumed to be 0.3 under the null hypothesis and 0.6 under the alternative hypothesis. The proportion in the SOC group was 0.3. The test statistic used was the 2-sided Z test with pooled variance, with significance level targeted at .05. The significance level actually achieved by this design was .048.

Statistical analysis was performed using PASW 19 (IBM, Chicago, Illinois). All analyses used an intent-to-treat (ITT) approach. The ITT population comprised all patients who were randomised and received at least 1 treatment. The last observation carried forward principle was used for missing data. Continuous variables were summarised as means and SDs, unless the Shapiro-Wilk test determined that the data distributions were non-normal, for which medians were also reported. Proportions or percentages were used for categorical variables. Parametric and non-parametric tests were used as appropriate.

Statistical testing between study groups at baseline was undertaken for all 80 subjects. In addition, analysis was performed for the first cohort of 40 subjects, and a separate analysis was conducted for the second cohort of 40 subjects. For normal continuous variables, means between groups were analysed by the t test or the Kolmogorov-Smirnov test, when data distributions were non-normal; categorical data were analysed by the χ^2 or Fisher exact test when cell values $\leq$5 were encountered.

The χ^2 or Fisher's exact tests were also performed to test for statistical differences among the percentage healed between the 2 study groups. The time to heal within 6 and 12 weeks was compared between the study groups using a Kaplan-Meier analysis with 95% confidence intervals (CIs). Time to heal was also analysed using Cox regression adjusted for covariates known to influence ulcer healing, such as smoking and obesity. Using stepwise regression, all

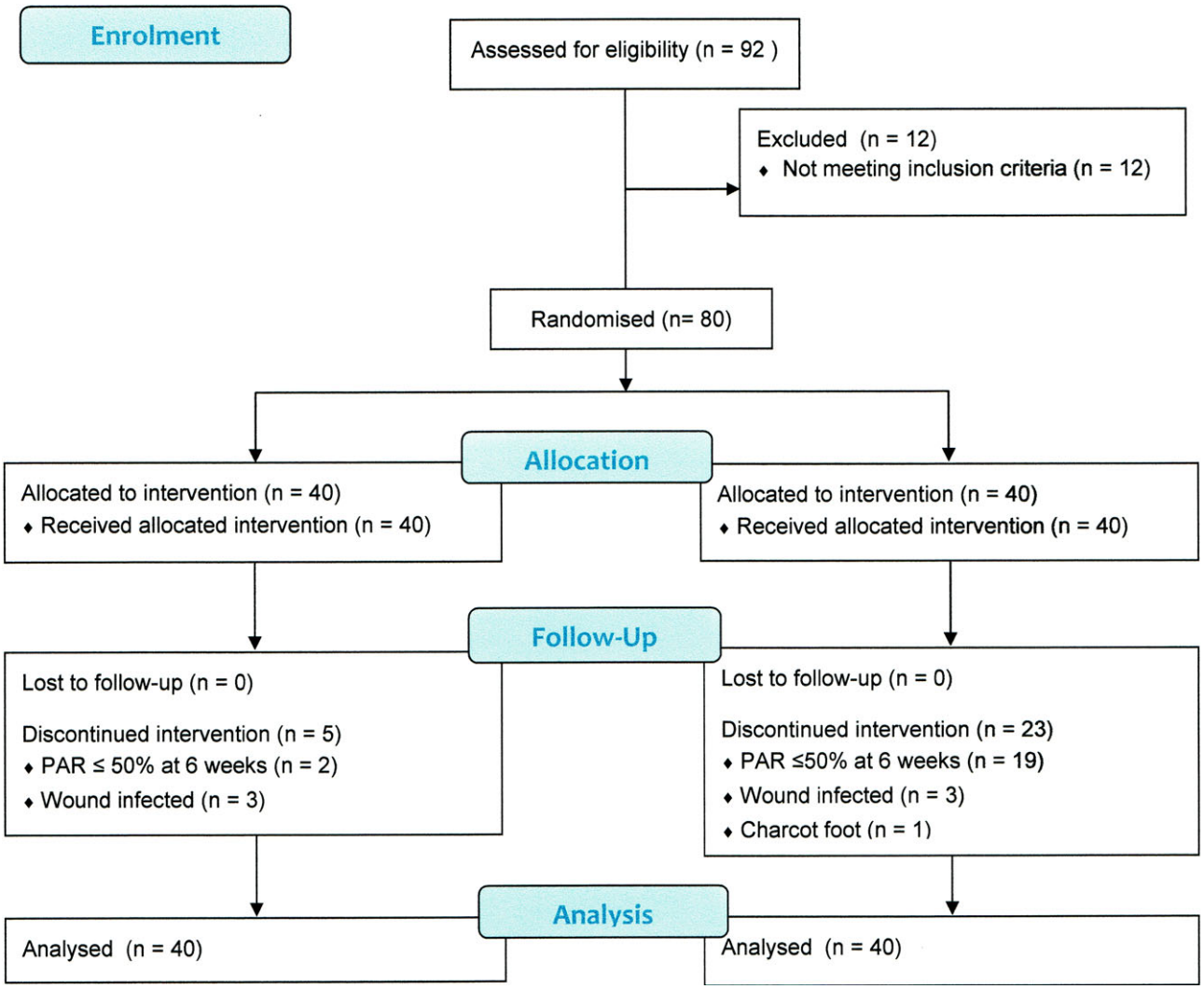

FIGURE 2 Participant flow chart

covariates were entered in 1 block, and non-significant covariates were eliminated stepwise 1 at a time from the initial model based on descending *P* values. Proportional hazard assumptions for each covariate in the final model were verified by examining the slope of the Schoenfeld residuals and adding additional time-dependent covariates if slopes were found to be non-linear. The PAR was analysed using a Mann-Whitney test. All *P* values were adjusted for the family-wise error rate using the Hochberg step-up procedure, except for group baseline values and Kaplan-Meier values at 12 weeks. Adjusted 2-sided *P* values <.05 were considered significant.

3 | RESULTS

This study took place from December 16, 2014 to March 29, 2017. Following consent to participate in the study, 92 patients were screened. Of these, 80 were eligible to participate and 12 were ineligible based on inclusion and exclusion criteria. Eligible subjects were randomised to HR-ADM plus SOC ($n = 40$) or SOC alone ($n = 40$) (Figure 2). All subjects received their assigned intervention and were included in the ITT analysis. At 6 weeks, a significantly higher number, 68% (27/40), of the HR-ADM-treated ulcers had healed compared with 15% (6/40) of the ulcers treated with SOC alone ($P = 2.7 \times 10^{-6}$) (Table 4).

In the first 40 subjects enrolled, the initial ulcer size was larger, 4.7 cm^2 for HR-ADM vs 2.7 cm^2 for the SOC group. In the second 40 subjects enrolled, there were significantly more smokers in the HR-ADM group (7 vs 1, $P = .044$) (Table 2), and mean age was significantly higher for the SOC group compared with the HR-ADM group (67 years vs 55 years, $P = .008$). In addition, serum creatinine levels were higher in the SOC group compared with the HR-ADM group (1.3 mg/dL vs 0.9 mg/dL, $P = .008$). However, pooled patient and ulcer-related variables for all 80 subjects were similar at enrolment (Table 3), with the exception of serum creatinine levels, which were marginally higher in the SOC group (1.2 mg/dL; SD: 0.45) compared with the HR-ADM group (0.97; SD: 0.40), $P = .04$.

TABLE 2 Wound- and patient-related variables between study groups at baseline for first 40 subjects enrolled and the second 40 subjects enrolled

	First 40 subjects[5]			Second 40 subjects		
Variable	**HR-ADM**	**SOC**	***P*-value**	**HR-ADM**	**SOC**	***P*-value**
Age (y)	62 (11)	57 (11)	.21	55 (13)	67 (14)	**.008**
Race						
White	20 (100)	19 (95)		16 (80)	19 (95)	
African American	0 (0)	1 (5)	1.0	4 (20)	1 (5)	.34
Gender						
Male	16 (80)	12 (60)	.	12 (60)	12 (60)	1.0
Female	4 (20)	8 (40)	30	8 (40)	8 (40)	
BMI	34 (8.7)	32 (6.9)	.53	35 (7.0)	35 (10)	0.92
Smoker	4 (20)	6 (30)	.72	7 (35)	1 (5)	**.044**
Drinks alcohol	5 (25)	4 (20)	1.0	2 (10)	5 (25)	.41
HbA1c	7.9 (1.6)	7.8 (1.8)	.87	7.7 (1.5)	7.3 (0.95)	.29
Creatinine	1.1 (0.38)	1.1 (0.35)	.94	0.9 (0.40)	1.3 (0.53)	**.008**
Wound area (cm^2)	4.7 (5.3)	2.7 (2.3)	.14	1.7 (0.61)	2.6 (2.7)	.15
Wound location						
Toe	6 (30)	7 (35)		5 (25)	6 (30)	
Forefoot	5 (25)	7 (35)		13 (65)	6 (30)	0.10
Midfoot	7 (35)	2 (10)	.28	1 (5)	4 (20)	
Heel/ankle/hindfoot	2 (10)	4 (20)		1 (5)	4 (20)	

Abbreviations: BMI, body mass index; HR-ADM, human reticular acellular dermis matrix; SOC, standard of care. Continuous variables are reported as means and SDs and categorical variables as number (*n*) and percentage (%). Statistically significant differences between groups are in bold.

The difference in mean PAR at 6 weeks between study groups was statistically significant ($P = 2.7 \times 10^{-6}$)—HR-ADM: 62% (SD: 160) vs SOC: 50% (SD: 41). Mean time to heal at the 6-week time point was 27 days (95% CI: 23-32 days) for the HR-ADM group and 41 days (95% CI: 39-42 days) for the SOC group ($P = 9.9 \times 10^{-7}$) (Table 4). Two patients from the HR-ADM group (5%) and 19 patients from the SOC group (48%) were withdrawn from the study at 6 weeks per protocol because their ulcers did not decrease in area by at least 50%.

At 12 weeks, a significantly higher number, 80% (32/40), of the HR-ADM-treated ulcers had healed compared with 30% (12/40) of the ulcers treated with SOC alone ($P = 8.4 \times 10^{-6}$) (Figure 3, Table 4). From week 6 to week 12, the median PAR remained consistent at 100% for the HR-ADM group, whereas it continued to slightly fluctuate in a decreasing trend for the SOC group. At 12 weeks, mean PARs were similar to 6 weeks—HR-ADM: mean: 64% (SD: 160); SOC: mean: 52% (SD: 43) ($P = 1.0 \times 10^{-5}$). Mean time to heal within 12 weeks was 38 days (95% CI: 29-47 days) for the HR-ADM group and 72 days (95% CI: 66-78 days) for the SOC group ($P = 3.9 \times 10^{-7}$) (Table 4, Figure 4). After adjusting for patient age and ulcer area at randomisation, the hazard ratio (HR) for HR-ADM compared with SOC was 8.0 (95% CI: 3.8-16.8, $P = 3.7 \times 10^{-7}$) (Table 5, Figure 5).

The mean number of HR-ADM grafts applied per ulcer to achieve closure by 6 weeks was 3.4 [SD: 2.1; median: 3; interquartile range (IQR): 5] and at 12 weeks was 4.7 [SD: 3.4; median: 3; IQR: 4]. Mean product cost to heal a closed ulcer ($n = 27$) at 6 weeks was \$800 (SD: \$687; median: \$675; IQR: \$850). The corresponding cost at 12 weeks was \$1200 (SD: \$1209; median: \$675; IQR: \$994; $n = 32$). The mean wastage at 12 weeks was 57% (SD: 11; $n = 32$).

TABLE 3 Wound- and patient-related variables between study groups at baseline

Variable	**HR-ADM, $n = 40$**	**SOC, $n = 40$**	***P*-value**
Age (y)	59 (12)	62 (13)	.20
Race			
White	36 (90)	48 (95)	.68
African American	4 (10)	2 (5)	
Gender			
Male	28 (70)	24 (60)	.35
Female	12 (30)	16 (40)	
BMI	35 (7.9)	34 (8.8)	.62
Smoker	11 (28)	7 (18)	.28
Drinks alcohol	7 (18)	9 (23)	.58
HbA1c	7.8 (1.5)	7.6 (1.4)	.45
Creatinine	0.97 (0.40)	1.17 (0.45)	**.04**
Wound area (cm^2)	3.2 (4.0)	2.7 (2.4)	.26
Wound location			
Toe	11 (28)	13 (33)	
Forefoot	18 (45)	13 (32)	.32
Midfoot	8 (20)	6 (15)	
Heel/ankle/hindfoot	3 (7)	8 (20)	

Abbreviations: BMI, body mass index; HR-ADM, human reticular acellular dermis matrix; NS, not statistically significant; SOC, standard of care. Continuous variables are reported as means and SDs and categorical variables as number (*n*) and percentage (%). Statistically significant differences between groups are in bold.

TABLE 4 Healing analysis based on χ^2 or Fisher's exact tests (percentage healed) and Kaplan-Meier with log-rank test (time to heal)

	Healed at 6 weeks		Healed at 12 weeks		Mean time to heal (6 weeks)			Mean time to heal (12 weeks)		
Study group	***N* (%)**	***P*-value**	***N* (%)**	***P*-value**	**Days**	**95% CI**	***P*-value**	**Days**	**95% CI**	***P*-value**
HR-ADM, $n = 40$	27 (68)	2.7×10^{-6}	32 (80)	8.4×10^{-6}	27	23-32	9.9×10^{-7}	38	29-47	3.9×10^{-7}
SOC, $n = 40$	6 (15)		12 (30)		41	39-42		72	66-79	

Abbreviations: CI, confidence interval; HR-ADM, human reticular acellular dermis matrix; SOC, standard of care.

Sixteen AEs occurred during this trial, 9 of which were serious adverse events (SAEs). None of the AEs were related to study treatment. There were 8 AEs in the HR-ADM group, 3 of which were diabetic foot infections that required hospitalisation and subsequent IV antibiotic therapy and were classified as SAEs. Three subjects were withdrawn from the study due to infection. There were 8 AEs observed in the SOC group, 6 of which were SAEs. Five SAEs resulted from infection that led to hospitalisation and subsequent IV antibiotic therapy, with 3 of these subjects withdrawn from the study. The other SAE was related to an acute Charcot foot, and the subject was also withdrawn from the study.

4 | DISCUSSION

Controlled trials in wound care are often small and statistically underpowered with regard to primary and secondary endpoints.[16,17] In addition, the heterogeneity of treatment effects and population heterogeneity leading to different ulcer-healing capabilities at baseline may still occur.[18,19] Consequently, while the primary results of the initial 40-patient study[5] were promising and appropriately designed with adequate statistical power, we chose to continue and expand the trial to 80 patients. This was deemed advantageous to further validate the preferential healing with HR-ADM and to include a cohort size comparable to other peer-reviewed published studies of human dermal matrices.[20]

This continuation study of 80 patients with HR-ADM vs SOC corroborates results from the previously published initial 40-patient study and confirms that HR-ADM provides a viable treatment modality for DFUs when used in conjunction with SOC.[5]

In this trial, the addition of HR-ADM to SOC was clearly shown to improve the wound-healing trajectory, leading to a 2-fold improvement in the speed of healing of diabetic foot ulcers when compared with use of SOC treatment alone.

Randomised controlled trials, by their nature, study a defined population of patients that may not be generalizable to a more heterogeneous "real-world" population, a valid criticism of RCTs. However, we point out that, in this study, nearly half of patients (47.5%) in the SOC group in our trial were exited at 6 weeks because their ulcers did not adhere to satisfactory wound-healing trajectories for this population, compared with only 2 patients (5%) in the HR-ADM group.[21–24] In addition, the population of smokers was statistically significantly higher in the second 40-patient cohort in the HR-ADM group, which further supports the effectiveness of this technology to promote healing, even in the presence of this significant comorbid factor.

The mechanisms underlying superior healing with HR-ADM have been studied in vitro. HR-ADM is aseptically processed and provided sterile to a 10^{-6} sterility assurance level (SAL) without any terminal sterilisation and provides an open, uniform, 3-dimensional framework with the retention of endogenous extracellular matrix (ECM) proteins for

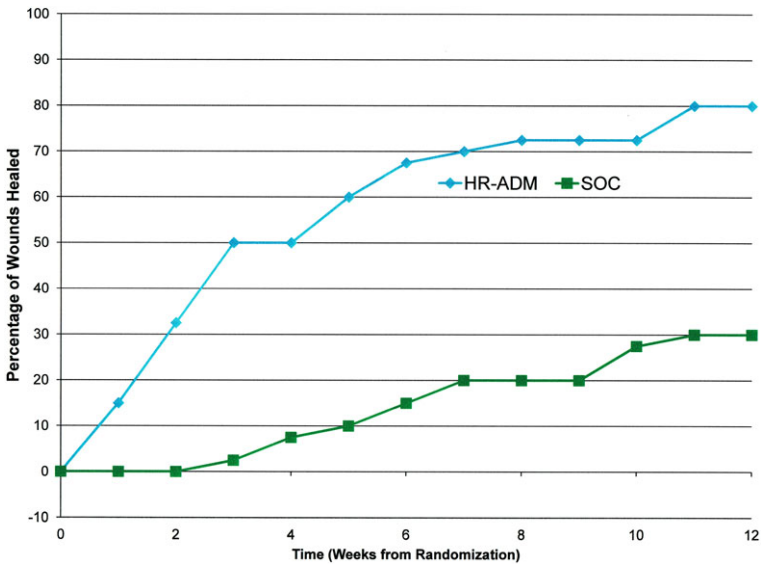

FIGURE 3 Percentage of wounds closed by week by treatment group. HR-ADM, human reticular acellular dermis matrix; SOC, standard of care

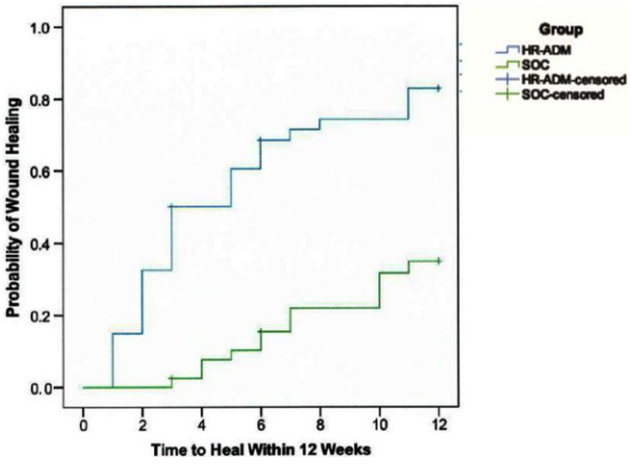

FIGURE 4 Kaplan-Meier time-to-heal plot within 12 weeks. HR-ADM, human reticular acellular dermis matrix; SOC, standard of care

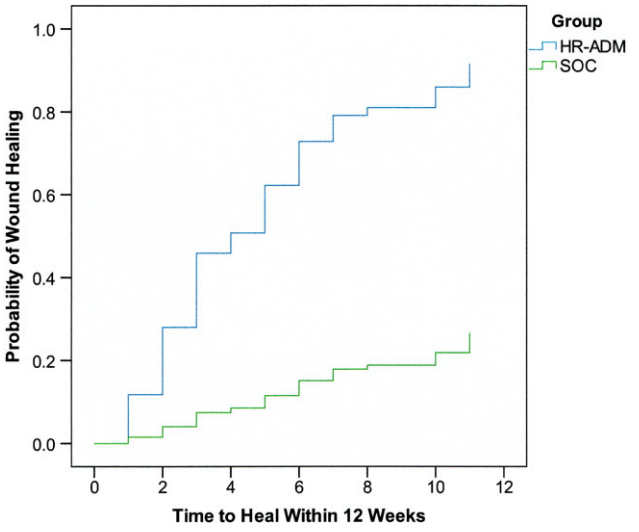

FIGURE 5 Cox regression model of time to heal within 12 weeks after controlling for patient age and initial wound area. HR-ADM, human reticular acellular dermis matrix; SOC, standard of care

cell attachment and remodelling activities.[6] The HR-ADM's open architecture and ECM proteins encourage human dermal fibroblast and human endothelial cell proliferation and infiltration, which culminate in the secretion of an abundance of new matrix proteins supporting granulation activities and the formation of tubular networks providing evidence of robust angiogenesis.[6] These synergistic cell interactions contribute to restoring the ulcer microenvironment and modulating cellular activities.[25]

Another unique advantage of HR-ADM is that it comes from human donors with large dermal sheets procured from the back and the legs. The larger dermal grafts are first prepared for use in burn and abdominal wall repairs and breast reconstruction following mastectomy. Once these larger grafts are prepared, smaller wound tissue sizes can be harvested from the remaining tissue, which increases the overall utility of the donor gift and allows a single donation to benefit even more potential recipients.

This study demonstrated that the use of the HR-ADM results in far less product wastage (57%) compared with previously reports of bioengineered cellular tissue use, where product wastage was reported to be more than 97%.[26] In addition, the efficiency of HR-ADM use is comparable with other recently published studies using size-specific allografts where wastage was reported to be 56%.[26]

TABLE 5 Time to heal based on Cox regression within 12 weeks

				95% CI for HR	
	B	*P*-value	Hazard ratio	Lower	Upper
Patient age (y)[a]	0.048	.001	1.1	1.0	1.1
Initial wound area (cm^2)					
2.0-3.99	−0.89	.019	0.41	0.20	0.87
≥4.0	−1.62	.001	0.20	0.08	0.51
HR-ADM[b]	2.07	3.7×10^{-7}	8.0	3.8	16.8

Abbreviations: CI, confidence interval; HR-ADM, human reticular acellular dermis matrix.

[a] Values for each increase in 1 year of age.

[b] Category reference: standard of care.

In terms of published cost to closure, HR-ADM use resulted in $1200 mean cost to closure at 12 weeks relative to previously published randomised controlled trials of bioengineered cellular tissue products with a mean cost to closure that is nearly ×7.5 greater.[26]

The strengths of this trial included a 2-week run-in period prior to randomisation and strict adherence to the CONSORT guidelines for conducting and reporting RCTs,[27] with allocation concealment. The sample size of 80 patients provides further statistical strength to the study, addresses the potential heterogeneity of treatment effect and population heterogeneity leading to different disease risks at baseline that may occur, and provides comparable sample sizes to studies published with other human dermal matrices with similar-sized cohorts.[18–20]

Limitation of this study include the fact that it was an open study that did not blind the patient or the investigator to the intervention allocated because blinding was not feasible (although reviewers were blinded to the type of treatment in their evaluation of wound closure). It was also limited in ulcer size and depth, in that there was no tendon, capsule, muscle, or bone exposure, which is frequently seen in complex ulcers presenting to the wound clinic. Following the positive wound outcomes demonstrated in the patient population treated with HR-ADM in this study, future trials may assess the use of HR-ADM on deeper wounds and more medically complex patient populations as frequently seen in the "real-world" population.[5]

5 | CONCLUSION

Consistent with the previous 40-patient study, we demonstrated, with a larger 80-patient, cohort that HR-ADM plus SOC was more effective than SOC alone in the healing of

chronic DFUs. Because of the variety of sizes available, HR-ADM was shown to be an efficient tissue form in terms of both a reduction in cost of treatment and tissue wastage perspective. The issues of clinical efficacy and reduced cost and wastage are meaningful in the context of a wound care environment where economics and effectiveness are key drivers in selection of grafts.

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