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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

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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

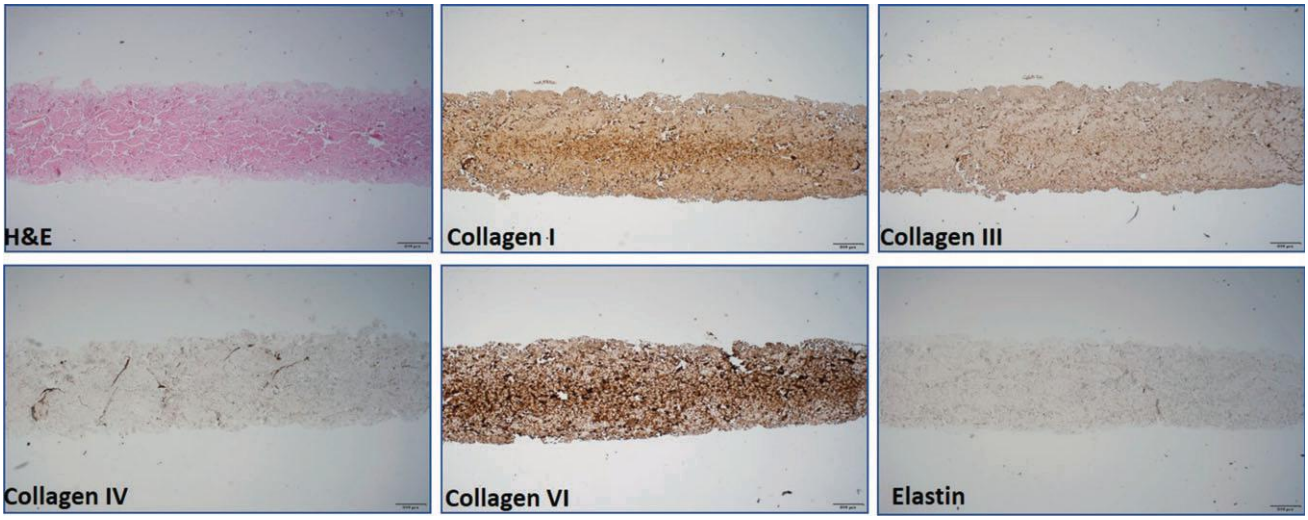

FIGURE 1 Immunohistochemical staining of aseptically processed, pre-hydrated human, reticular acellular dermis for matrix proteins revealed retention of collagen type I, III, IV and VI, and elastin (magnification ×2)

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

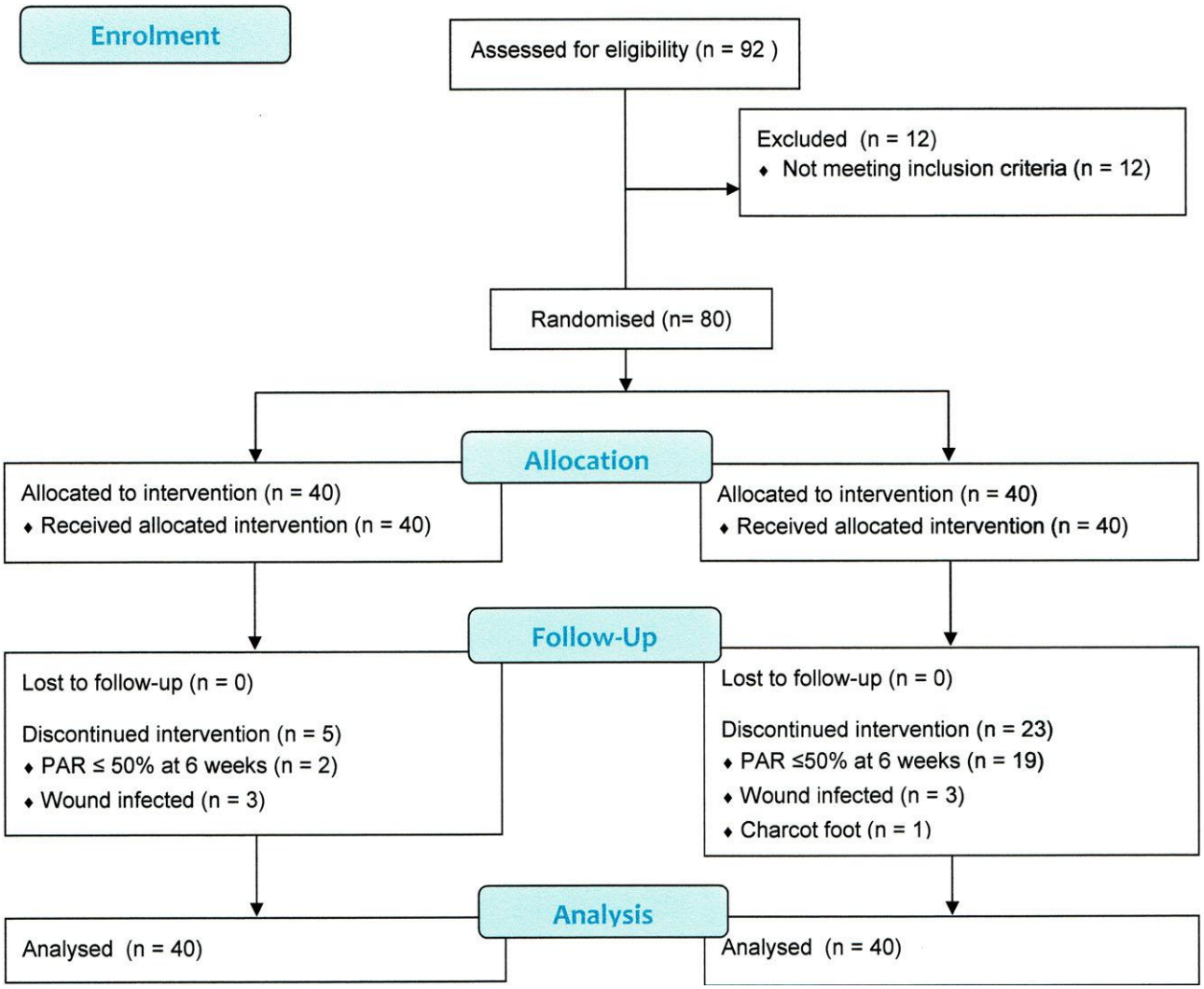

FIGURE 2 Participant flow chart

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

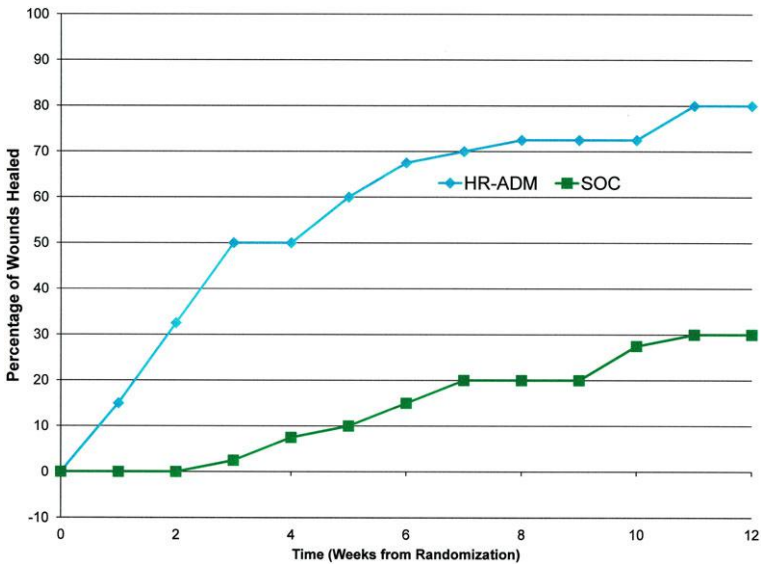

FIGURE 3 Percentage of wounds closed by week by treatment group. HR-ADM, human reticular acellular dermis matrix; SOC, standard of care

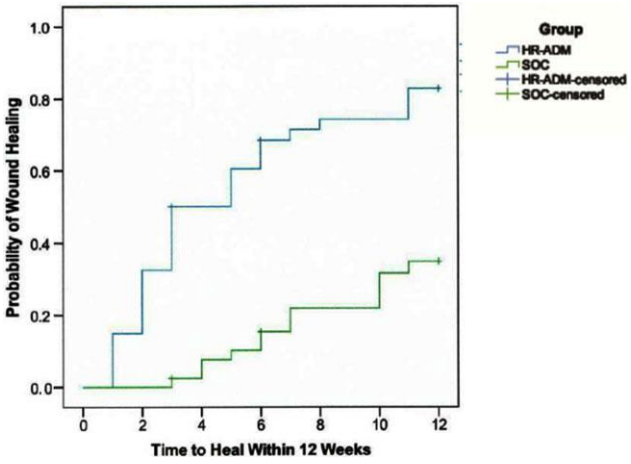

FIGURE 4 Kaplan-Meier time-to-heal plot within 12 weeks. HR-ADM, human reticular acellular dermis matrix; SOC, standard of care

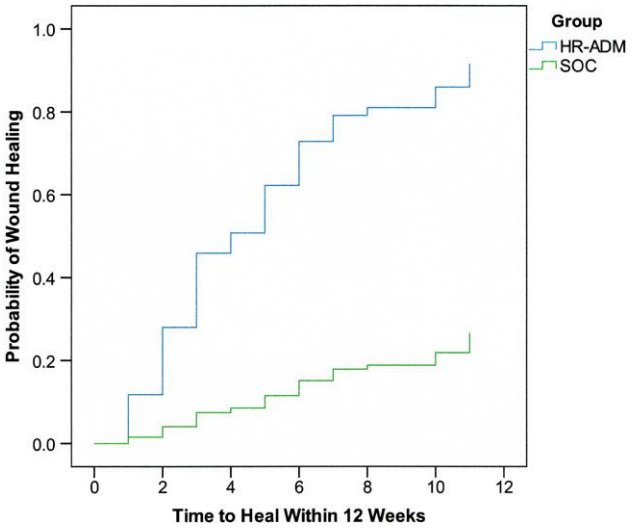

FIGURE 5 Cox regression model of time to heal within 12 weeks after controlling for patient age and initial wound area. HR-ADM, human reticular acellular dermis matrix; SOC, standard of care

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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ORIGINAL ARTICLE

A prospective, randomised, controlled, multicentre clinical trial examining healing rates, safety and cost to closure of an acellular reticular allogenic human dermis versus standard of care in the treatment of chronic diabetic foot ulcers

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Key words
Diabetic foot ulcers; Human acellular dermal tissue; Randomised controlled trial; Standard of care

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Abstract

Acellular dermal matrices can successfully heal wounds. This study's goal was to compare clinical outcomes of a novel, open-structure human reticular acellular dermis matrix (HR-ADM) to facilitate wound closure in non-healing diabetic foot ulcers (DFUs) versus DFUs treated with standard of care (SOC). Following a 2-week screening period in which DFUs were treated with offloading and moist wound care, patients were randomised to either SOC alone or HR-ADM plus SOC applied weekly for up to 12 weeks. At 6 weeks, the primary outcome time, 65% of the HR-ADM-treated DFUs healed (13/20) compared with 5% (1/20) of DFUs that received SOC alone. At 12 weeks, the proportions of DFUs healed were 80% and 20%, respectively. Mean time to heal within 12 weeks was 40 days for the HR-ADM group compared with 77 days for the SOC group. There was no incidence of increased adverse or serious adverse events between groups or any adverse events related to the graft. Mean and median graft costs to closure per healed wound in the HR-ADM group were $1475 and $963, respectively. Weekly application of HR-ADM is an effective intervention for promoting closure of non-healing DFUs.

Introduction

Diabetes and its complications pose a major health system challenge. In 2011–2012, the estimated unadjusted prevalence of diabetes in the United States using National Health and Nutrition Examination Survey (NHANES) data from the CDC was 14·3%, with nearly a third of adults undiagnosed (1).

Key Messages

- this 12-week, multicentre, randomised, controlled, clinical study demonstrated that diabetic foot ulcers (DFUs) treated with human reticular acellular dermis

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matrix (HR-ADM) healed more rapidly compared with DFUs treated with standard of care (SOC)
- complete wound healing of DFUs at 6 weeks using HR-ADM plus SOC was significantly higher compared with SOC alone (65% versus 5%)
- proportion of completely healed wounds at 12 weeks was also significantly higher for the HR-ADM plus SOC group compare to the SOC group (80% versus 20%); time to heal within 6 and 12 weeks was also significantly faster for the HR-ADM plus SOC group compare to the SOC group

However, the age-standardised prevalence was 12% between 2008 and 2012, suggesting that incidence might be peaking (2). Serious complications of diabetes include diabetic foot ulcers (DFUs) and associated lower extremity amputations (LEAs). The annual incidence of DFUs and LEAs calculated from the Medicare population is approximately 6% and 0·4%, respectively (3). The mortality rate for Medicare beneficiaries having a DFU was 10·7% in 2008, with rates doubling following a LEA (3).

Non-healed DFUs are prone to infection, increasing the risk for tissue necrosis and osteomyelitis. Moreover, rapid DFU healing is highly desirable to avoid LEAs and other complications. A meta-analysis of patients studied in controlled trials demonstrated, on average, healing rates of 31% at 20 weeks with standard of care (SOC) (4). The clinical practice guideline of assessing if the surface area of a DFU has been reduced by 50% or more within 4 weeks is critical when treating DFUs (5–9). Using this guideline, when SOC fails to heal the indolent DFU, advanced wound therapies can offer a better alternative for such wounds with complex pathologies.

Biological scaffolds for wound healing typically consist of an extracellular matrix (ECM) that provides both structural support for cells and signalling cues to modulate beneficial cellular responses (10). Among the biological options, human dermis provides an anatomic architecture that can provide matrix proteins physiologically inherent for wound healing. The human dermis is comprised of two distinct layers: the papillary or superficial layer and the reticular dermal layer (Figure 1). The reticular layer is rich in collagens, elastin and reticular fibres woven throughout (Figure 2), and these matrix proteins provide strength and elasticity (11). This type of reticular network is known to promote regeneration versus repair and scar formation (12–15). Evidence that the two layers behave differently started with a single case study many decades ago in a badly burned patient who received deceased donor skin allografts. When the allografts were abraded to remove the epidermal layer, subsequent application of cultured epidermal autografts resulted in complete skin reconstitution (16). Further work in burn patients has confirmed the success of the basic technique in which a de-antigenised dermal matrix is implanted first followed by a split-thickness skin graft (17).

In general, full-thickness skin grafts contract less and provide better cosmetic results than split-thickness skin grafts. In healthy human volunteers, a mean depth greater than 0·57 mm was found to cause a scar – about a third of normal hip skin thickness (14). This is approximately the depth at which the junction lies between the reticular and papillary dermis, although in the elderly, the junction would be closer to the skin surface because the papillary dermis is considerably thinner.

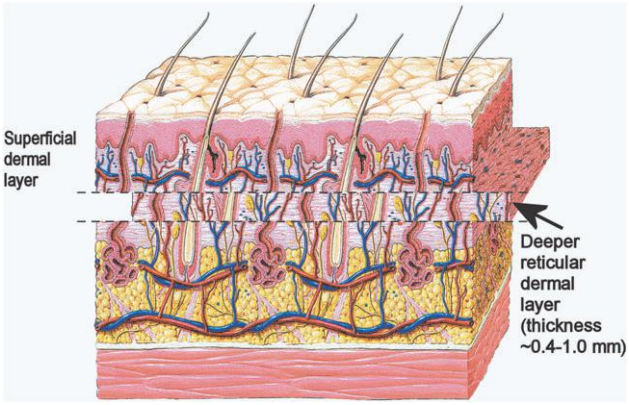

Figure 1 Comparison of superficial and deep reticular cut dermal graft layers.

Considering these attributes of reticular dermis, this study set out to demonstrate that human reticular acellular dermal matrix (HR-ADM) provides a scaffold that improves wound healing time compared with the SOC.

The primary objective of this study was to compare complete wound healing of DFUs at 6 weeks using HR-ADM plus SOC compared with SOC alone. Secondary objectives included the proportion of completely healed wounds at 12 weeks, time to heal within 6 and 12 weeks, the incidence of adverse and serious adverse events, the product cost of therapy from start of study to closure and graft wastage.

Methods

This multicentre, randomised controlled study screened patients with diabetes who had at least one non-healing neuropathic foot ulcer, which failed a minimum of 4 weeks of documented conservative care, for a period of 2 weeks prior to study enrolment. Patients needed to have adequate renal function as assessed by a blood draw of serum creatinine with a value less than 3·0 mg/dl and adequate circulation to the affected extremity, as demonstrated by one of the following within the past 60 days: transcutaneous oxygen test (TCOM) with results ≥30 mmHg, ankle brachia index (ABIs) with results of ≥0·7 and ≤1·2 or Doppler arterial waveforms, which were triphasic or biphasic at the ankle of affected leg. Eligible patients meeting inclusion and exclusion criteria were randomised 1:1 to HR-ADM plus SOC or SOC alone. This study was conducted at five outpatient wound care centres in Virginia and Ohio. The study protocol and subject consent form were reviewed and approved by an institutional review board on November 13, 2014 (#20142081), and written consent was obtained from all participants prior to any study-related procedure. The trial was pre-registered in ClinicalTrials.gov (NCT02331147) and conducted in compliance with applicable regulatory requirements in accordance with the provisions of the Declaration of Helsinki and in adherence to Good Clinical

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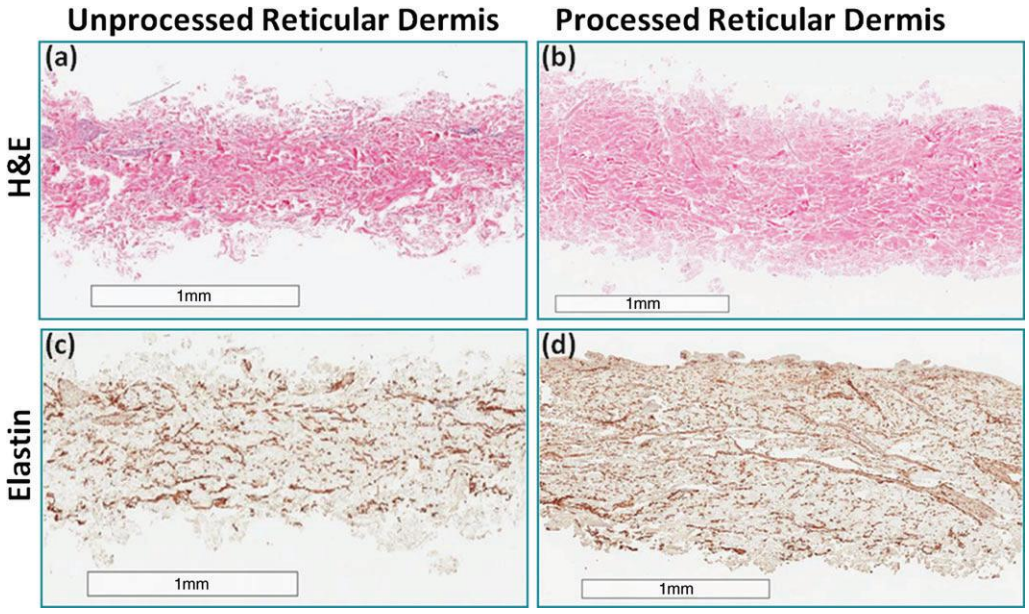

Figure 2 Aseptic processing preserves inherent architecture of the tissue and the key matrix protein, elastin, similar to unprocessed reticular dermis (magnification 2X). Key: (a) unprocessed reticular dermis with H&E stain; (b) processed reticular dermis with H&E stain; (c) unprocessed reticular dermis showing elastin; (d) processed reticular dermis showing elastin.

Practice. Confidentiality was maintained with all patient records in accordance with HIPAA. The trial was conducted between 16 December 2014 and 25 November 2015 with 40 patients enrolled in the study, and they were followed-up to study withdrawal or study completion.

Patient screening, eligibility and randomisation

Patients with type 1 or 2 diabetes who had a foot ulcer of at least 4 weeks duration were screened for study eligibility based on inclusion and exclusion criteria (Table 1). Eligible patients who consented received a full physical examination on the first screening visit, and their medical history was documented. Each study wound was examined for infection per the guidelines of Woo and Sibbald (18) and cleaned and debrided. Digital photographs were taken at a distance of 30 cm and included a graded centimetre ruler in which markings were directly adjacent to the ulcer, a legible label and entire wound clearly visible within the photographic field. Wound surface area was measured by a ruler from an acetate tracing according to length, width and depth. The largest wound was selected if multiple wounds in a single patient were present. Any wound that was within 3 cm of another wound was excluded from the study. The index (study) wound was evaluated using a probe-to-bone test with a sterile, ophthalmological probe. Patients with bone involvement were excluded. Blood was drawn for serum creatinine and glycosylated haemoglobin (HbA1c) analysis, and a vascular assessment was performed on the extremity in which the wound was located using dorsal TCOM, ABI or Doppler arterial waveform tests.

All eligible participants meeting inclusion and exclusion criteria were treated with SOC alone for a 2-week screening period prior to randomisation. Surgical debridement as part of SOC was accomplished using a 15-blade or curette to remove all necrotic tissue, and wounds were offloaded using a total contact cast, removable cast walker (Royce Medical, Inc., Camarillo, CA, USA) or similar generic device. If patient non-compliance with offloading was subsequently discovered by the investigator, the device was converted into an instant total contact cast. Wounds were dressed with collagen-alginate and gauze, and dressing supplies were provided for patients to perform daily dressing changes. Patients were evaluated weekly in the clinic during the screening period for wound assessment, sharp debridement and wound measurements. Patients whose index wound had not healed greater than 20% at 2 weeks were then randomised to the HR-ADM plus SOC or SOC alone groups.

Randomisation used a paper block system. Sheets of paper in blocks of ten with five sheets having an assignment of SOC and the other five having the assignment of HR-ADM plus SOC were placed in a blank envelope that was sealed. The envelopes were shuffled and then labelled 1 through 10. This process was observed by the principal investigator and study staff, with the process being repeated four times and distributed to the individual sites. The site investigators did not have knowledge of the process used to create assignments, and randomisation of patients proceeded individually at their first post-screening treatment.

HR-ADM allograft

The HR-ADM studied was AlloPatch® Pliable™ (Musculoskeletal Transplant Foundation, Edison, NJ, USA), a preparation of a reticular cut of human dermis aseptically processed to preserve the native tissue and retain the standard amount of collagens and elastins normally present. (Figures 1 and 2). It requires no rehydration or refrigeration prior to use and can be

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Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Male or female aged 18 or older • Type 1 or 2 diabetes mellitus (ADA diagnostic criteria) • Signed informed consent • Patient's wound diabetic in origin and larger than 1 cm^2. • Wound present for a minimum of 4 weeks duration, with documented failure of prior treatment to heal the wound • Wound has no signs of infection • Wound present anatomically on the foot as defined by beginning below the malleoli of the ankle • Additional wounds may be present but not within 3 cm of the study wound • Serum creatinine less then 3·0 mg/dl • HbA1c less than 12% taken prior to randomisation • Patient has adequate circulation to the affected extremity, as demonstrated by one of the following within the past 60 days: • Dorsum transcutaneous oxygen test ≥30 mmHg • ABI with results of ≥0·7 and ≤1·2 • Doppler arterial waveforms, which are triphasic or biphasic at the ankle of affected leg • Patient is of legal consenting age • Patient is willing to provide informed consent and is willing to participate in all procedures and follow-up evaluations necessary to complete the study	• Wound probing to bone (UT Grade IIIA-D) • Index wound greater than 25 cm^2 • HbA1c greater than 12% within previous 90 days • Serum creatinine level 3·0 mg/dL or greater • Patients with a known history of poor compliance with medical treatments • Patients previously randomised into this study or presently participating in another clinical trial • Patients currently receiving 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 • Active infection at index wound site • Any pathology that would limit the blood supply and compromise healing • Patients who have received a biomedical or topical growth factor for their wound within the previous 30 days • Patients who are pregnant or breast feeding • Patients who are taking medications that are considered immune system modulators that could affect graft incorporation • Patients taking a Cox-2 inhibitor • Patients with wounds healing greater than 20% during the screening period

ABI, ankle brachia index.

stored at ambient temperature. This dermis differs from many of the other human dermal matrices available that are derived from a more superficial cut of the dermis, which contains both papillary and reticular portions of the dermis. The HR-ADM provided in this trial came in size-specific grafts as small as 1·5 cm × 1·5 cm to minimise wastage and was trimmed using sterile scissors to fit the wound with a saline lavage prior to application.

Treatments

Patients were examined and treated weekly during the study period until the index wound closed, for up to 12 treatment weeks or if the patient did not achieve greater than 50% closure at 6 weeks, they were withdrawn from the study at that time. At each visit, vital signs were taken and blood glucose levels measured using an Accu-Chek test. Patients determined to be in poor metabolic control of their diabetes at any visit were referred to their primary care physician or endocrinologist to ensure proper diabetes management during the study. No patients were withdrawn from the study because of inadequate diabetes management.

The index wound was cleansed with sterile normal saline solution and appropriately debrided at each visit. The post-debridement surface area was then calculated from the acetate sheet tracing (19) and the wound depth measured. The wound was photographed at each step for documentation.

The patient's wound was assessed for infection at each weekly follow-up visit. If infection was suspected, a wound culture was obtained with both anaerobic and aerobic swabs of the suspected infected area, and appropriate systemic antibiotic treatment was administered until the infection was clinically resolved. If the wound infection was sufficiently severe to preclude application of the HR-ADM in the treatment group or interfered with scheduled visits, the patient was removed from the trial.

For patients in the SOC group, daily dressing changes with a collagen-alginate (Fibracol, Systagenix, Gargrave, Yorkshire, UK) were performed and documented at each of the weekly visits.

For patients assigned to the HR-ADM group, the graft was removed from its primary package and rinsed by complete submergence in sterile saline for 5–10 seconds prior to application. Prior to placement over the wound, a sketch of the ulcer was made on the graft with a sterile marker, and an additional photograph was taken to document size and portion of graft not being used (waste). The graft was then cut to size with a sterile scissors, and a 15-scapel blade was used to pie-crust the graft by placing small full thickness cuts into the tissue, to prevent fluid from collecting underneath the graft, if needed. The graft was then placed over the wound site dermal side down, and care was taken to ensure that the graft was consistently covering and adhering to the entire wound surface. The graft was covered with non-adherent dressing (Adaptic Touch, Systagenix, Gargrave, Yorkshire, UK) followed by a moisture-retentive dressing (hydrogel bolster) and a padded 3-layer dressing (Dynaflex, Systagenix or equivalent) until complete epithelialisation had occurred. Application of HR-ADM was continued weekly during the study period. Clinical assessment was performed according to protocol. Six weeks after randomisation, the percentage area reduction (PAR) was calculated for the index wound: $PAR = ((A_I - A_{6W})/A_I) \times 100$, where A_I is the area of the index wound at randomisation, and A_{6W} is the area at 6 weeks. Patients were

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then evaluated and allowed to continue or were removed from the study if the DFU failed to reduce in area by 50% or more. For patients continuing in the study, weekly assessment was performed until the wound closed or until study completion.

Validation of healing

Wounds were defined as healed if there was complete (100%) re-epithelialisation without drainage and without a need for dressing, as determined by the site investigator. In order to confirm durability of wound closure, a follow-up visit was conducted 1 week after 100% re-epithelialisation occurred. Following study exit in all cases, per the protocol, the patients were given diabetic shoes with insoles to help facilitate the best possible preventative care of their diabetic pedal pathology.

The primary investigator was responsible for reviewing photographs and approving protocol pathway decisions regarding wound closure or individual patient continuation in the study. Validation of healing was conducted by an independent panel of physicians specialising in wound care, including a vascular surgeon, two plastic surgeons, a general surgeon and a scientific expert in angiogenesis. These adjudicators, blinded to patient study group assignments, reviewed decisions being made by site investigators regarding patient enrolment, healing and continuation within the protocol.

Study outcomes

The primary endpoint of the study was to compare the proportion of wounds healed at 6 weeks between the two treatment groups. Secondary endpoints included comparison between treatment groups of the proportion of wounds healed at 12 weeks, time to heal within 6 and 12 weeks, numbers of grafts used, graft wastage and graft cost to closure. Waste was determined as a percentage by subtracting the wound area at each visit from the total area of the HR-ADM removed from the package during the same visit and dividing the result by the total HR-ADM product area. Graft costs for each wound were calculated by summing the costs of the applied HR-ADM products from all visits.

Sample size calculations and statistical analysis

Sample sizes of 20 in each group achieved 82% power to detect a difference between the group proportions of 0·45. The proportion in group one (the treatment group) was assumed to be 0·3 under the null hypothesis and 0·75 under the alternative hypothesis. The proportion in group two (the control group) was 0·3. The test statistic used was the two-sided Z test with pooled variance. The significance level of the test was targeted at 0·05, and the significance level actually achieved by this design was 0·053.

An intent-to-treat (ITT) approach was used for all analyses. All patients who were randomised and received at least one treatment were incorporated into the analyses. For missing observations, the last observation carried forward (LOCF) principle was used. Study variables were summarised as means and standard deviations (SDs) for continuous variables unless the data were non-normal, as determined by the Shapiro–Wilk test. In such cases, medians were also reported. Results for categorical variables were presented as proportions or percentages. Parametric and non-parametric tests were used as appropriate. Statistical testing between groups at baseline was not undertaken per CONSORT guidelines (20). For categorical variables, chi square or Fisher exact tests were performed to test for statistical differences. A Kaplan–Meier analysis was conducted to compare time to heal within 6 or 12 weeks for the two treatment groups. A Cox regression was carried out to analyse time to heal within 6 weeks, adjusting for all available covariates known to influence wound healing, such as smoking and obesity. Using stepwise regression, all covariates in one block were entered, and non-significant covariates were eliminated stepwise from the initial model. 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 these were found to be significant. To adjust for the family-wise error rate (FWER), *P* values were reported using the Hochberg step-up procedure. Adjusted two-sided *P* values $<0{\cdot}05$ were considered significant. PASW 19 (IBM, Chicago, IL) was used to perform the statistical testing.

Results

A total of 45 subjects were screened, with 40 meeting the screening criteria followed by randomisation to HR-ADM plus SOC ($n = 20$) or SOC alone ($n = 20$) (Figure 3). Patient and wound characteristics were similar at enrolment, with the exception of mean wound area, which was larger in the HR-ADM group ($4{\cdot}7\ cm^2$) compared with the SOC group ($2{\cdot}7\ cm^2$) (Table 2).

At 6 weeks, 65% (13/20) of the HR-ADM-treated wounds had healed compared with 5% (1/20) of the SOC alone ($P = 0{\cdot}00028$) (Figure 4). The percentage of wound area reduction between the groups changed substantially over time (Figure 5), with a mean time to heal within 6 weeks of 28 days (95% confidence interval (CI): 22–35 days) for the HR-ADM group compared with 41 days (95% CI: 40–43 days) for the SOC group. After adjusting for area of wound at randomisation, the hazard ratio (HR) for HR-ADM compared with SOC was 168 (95% CI: 10–2704), $P = 0{\cdot}00036$ (Table 3). Ten patients from the SOC group (50%) and one patient from the HR-ADM group (5%) exited from the study at 6 weeks per protocol because their wounds failed to reduce in area by at least 50%.

At 12 weeks, 80% (16/20) of the HR-ADM-treated wounds had healed compared with 20% (4/20) of the wounds that received SOC alone ($P = 0{\cdot}00036$) (Figure 6). Mean time to heal within 12 weeks was 40 days (95% CI: 27–52 days) for the HR-ADM group compared with 77 days (95% CI: 70–84 days) for the SOC group ($P = 0{\cdot}00014$).

The mean number of HR-ADM grafts used to achieve closure per wound was 4·7 (SD = 3·3). The mean and median graft costs to heal (healed wounds only) were \$1475 (SD: \$1528; $n = 16$) and \$963, respectively. The mean percentage of wastage (healed wounds only) was 51·7% (SD: 10·7; $n = 16$).

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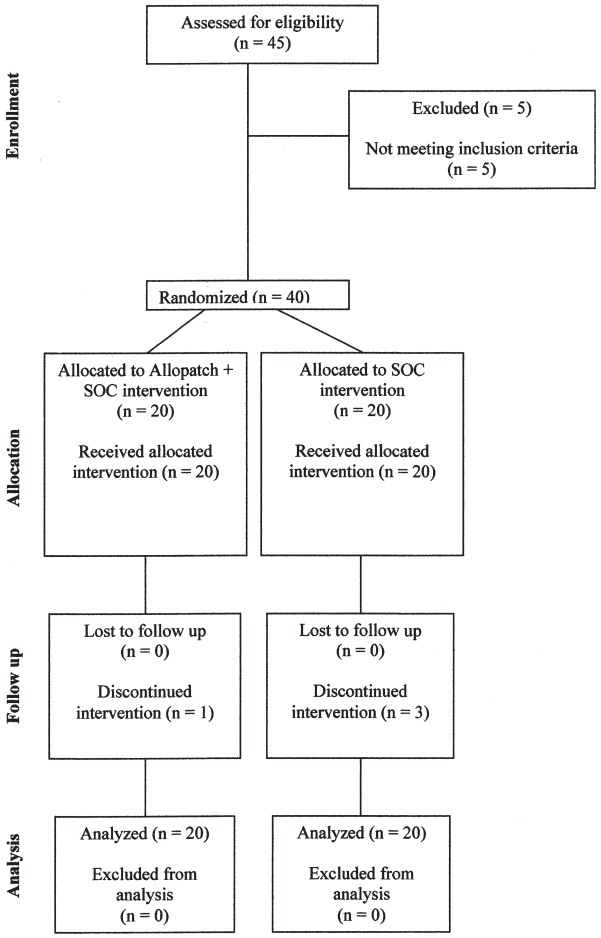

Figure 3 Flow chart of trial participants.

A total of seven adverse events were documented during this trial. Four adverse events were observed in the HR-ADM group, of which two met the criteria for serious adverse events (SAEs). Three adverse events were observed in the SOC group, of which two were SAEs. In the HR-ADM group, all four adverse events were related to diabetic foot infections that occurred during treatment, with two of the infections leading to hospital admission and subsequent IV antibiotic therapy. One subject was removed from the study because of infection. In the SOC group, two of the adverse events were related to diabetic foot infections, one of which required hospital admission and IV antibiotic therapy. The third adverse event in the SOC group was related to an acute Charcot foot. All three of these subjects were removed from the study. None of the adverse events were related to study treatment.

Discussion

Rapid and cost-effective healing of DFUs remains a challenging problem in the care of patients with diabetes. A number of advanced wound care technologies have been demonstrated to accelerate wound healing, including cultured skin equivalents, human allogeneic placental membranes, bioengineered materials and human allogeneic dermal grafts (21–26). In this prospective, randomised, controlled multicentre study, HR-ADM proved to be superior to SOC in promoting DFU closure. This novel graft is an ADM aseptically processed and derived from the reticular layer of the skin. The reticular layer of skin has a more consistent, open architecture than the superficial layer, yet contains key matrix proteins (collagens and elastin) similar to unprocessed tissue (Figure 2). These properties have been shown to facilitate critical cellular responses such as cell attachment and migration (27,28).

This study focused on a comparison of wound healing at 6 weeks using HR-ADM plus SOC versus SOC alone. Secondary objectives included comparing healing at 12 weeks, time to heal

Table 2 Wound and patient variables between groups at baseline. Continuous variables are reported as means and standard deviations (SDs) and categorical variables as number (*n*) and percentage (%)

Variable	HR-ADM	SOC
Age (years)	61·5 (10·85)	57·1 (10·65)
Race		
Caucasian	20 (100)	19 (95)
African American	0 (0)	1 (5)
Gender		
Male	16 (80)	12 (60)
Female	4 (20)	8 (40)
BMI	33·9 (8·72)	32·3 (6·90)
Smoker	4 (20)	6 (30)
Drinks alcohol	5 (25)	4 (20)
HbA1c (%)	7·9 (1·56)	7·8 (1·77)
Creatinine (mg/dl)	1·1 (0·38)	1·1 (0·35)
Wound area (cm^2)	4·7 (5·24)	2·7 (2·26)
Wound location		
Toe	6 (30)	7 (35)
Forefoot	5 (25)	7 (35)
Midfoot	7 (35)	2 (10)
Heel/ankle/hindfoot	2 (10)	4 (20)

HR-ADM, human reticular acellular dermis matrix; SOC, standard of care.

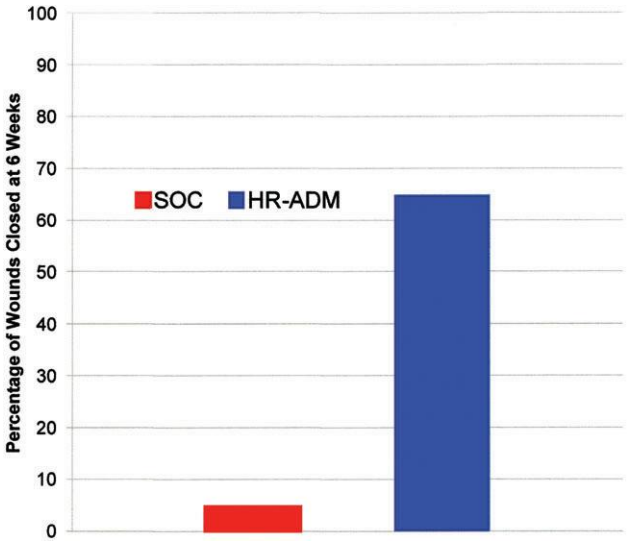

Figure 4 Bar graph showing complete wound healing at 6 weeks between the HR-ADM and SOC group versus the SOC group.

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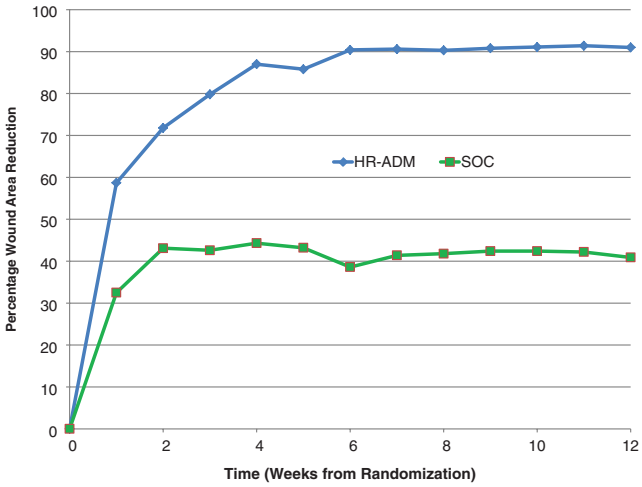

Figure 5 Percentage of wound area reduction by week and treatment group.

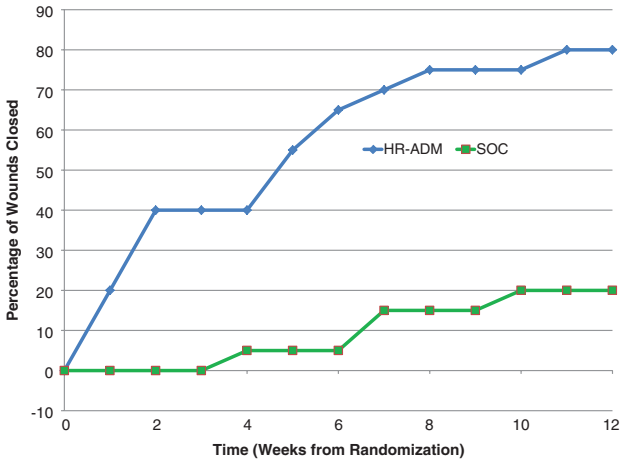

Figure 6 Percentage of wounds closed by week and treatment group.

Table 3 Cox regression, time to heal within 6 weeks. Change in chi square from the null model to the final model was 32·87

			95% CI	
Covariate	*P**	HR	Lower	Upper
HR-ADM†	0·0003	168	10	2704
Area (cm^2)‡				
2–3·99	0·014	0·061	0·007	0·57
≥4	0·002	0·027	0·003	0·27

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

*Not adjusted for study multiplicity of testing; references.

†Standard of care group.

‡<2 cm^2.

at 6 and 12 weeks, graft count, wastage and assessment of product cost to closure. By 6 weeks, 65% of the DFUs had healed in the HR-ADM group compared with 5% in the SOC group ($P = 0{\cdot}00028$), This statistically significant differential was maintained at the completion of the study (12 weeks), where 80% wound healing was achieved with HR-ADMs compared with 20% for SOC alone ($P = 0{\cdot}00036$). At the 6-week time point, DFUs in the HR-ADMs healed over 30% faster compared with DFUs treated with SOC only. Baseline parameters were similar in both groups with exception of average wound area, with patients in the HR-ADM group having larger wounds. It would be expected that smaller wounds would heal faster; however, the Kaplan–Meier survival analysis showed an even greater advantage in healing time for the HR-ADM-treated wounds within 12 weeks. The Cox regression (6 weeks) showed that larger areas significantly reduced the probability of wound healing, but after the adjustment for area, the effective size of the HR-ADM group was still more than ten times that of SOC alone ($P = 0{\cdot}0003$) (Table 3). These results support the effectiveness of HR-ADMs in problematic DFUs when combined with SOC.

Previous studies have demonstrated that acellular dermal grafts are efficacious in wound healing (24,29). Historically, human ADMs have been derived from the papillary or superficial skin layer. A controlled pilot trial (30) studied wound healing in subjects ($N = 40$) with full-thickness lower extremity DFUs of at least 6 weeks non-healing duration. The wounds were treated with either SOC alone or ADM plus SOC. Data on complete wound closure were not published from this 4-week study, but there were statistically significant differences between the ADM group and SOC with regard to wound area and depth reduction. Key CONSORT criteria and patient variables were not discussed, and the statistical analysis did not take multiplicity of testing into account. Brigido (31) published a single-centre randomised controlled trial in which subjects with Wagner 2 lower extremity DFUs ($N = 28$) were randomised to sharp debridement or sharp debridement plus treatment with ADM. In the ADM group, 86% (12/14) wounds healed by 16 weeks compared with 29% (4/14) in the debridement alone group ($P = 0{\cdot}006$). A caveat was that neither trial had a screening period, which is the standard practice with most RCTs. A larger multicentre randomised controlled trial ($N = 86$) was reported by Reyzelman et al. (24) in which patients with UT grade 1 or 2 DFUs were randomised to SOC or SOC plus ADM application. At 12 weeks, 70% of the DFUs had healed in the ADM group versus 46% in the SOC group ($P = 0{\cdot}029$). There was also a statistically significant difference between groups in time to heal within 12 weeks ($P = 0{\cdot}023$) after adjusting for initial wound area (HR for ADM = 2·0).

The availability in the current study of size-specific grafts (e.g.1·5 cm × 1·5 cm) allowed for less product wastage and a lower overall cost to closure. Similar to other studies using size-specific grafts, our percentage waste (51·7% versus 55·8%) as well as the mean graft cost per patient ($1475 versus $1669) was comparable (25). However, product wastage in the current study is far less than previously reported bioengineered alternative tissue products, which are reported at greater than 90% (26).

Strengths of our study include comprehensive SOC, satisfactory allocation concealment, an ITT analysis, adequate statistical power based on sample size and appropriate adjustment for multiple statistical testing and reporting according to CONSORT guidelines. Limitations of this investigation include lack

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of blinding from the patient's and investigator's perspective, an absence of exact tissue-level exposure measurement and reporting for each wound (e.g. Wagner grading), although each wound was evaluated to ensure that no wound reached greater then Wagner 2. There is also extensive right censoring for analyses at 12 weeks because of the decision to exit patients from the study whose wounds did not reduce in area by at least 50% after 6 weeks of either treatment regimen (4,25). This was carried out to ensure safety and the most compassionate care possible for all enrolled patients. In the SOC group, half of the wounds did not achieve greater than 50% closure by 6 weeks, which is consistent with previous studies (7) after adjusting for the 2-week longer time period in this study. However, even with adjustment for patients exiting at 6 weeks, both the 6- and 12-week data demonstrate statistically significant superiority in closure with the HR-ADM over SOC.

Although wounds with depths reaching muscle, tendon and bone were excluded from this trial, as were patients with uncontrolled diabetes, peripheral vascular and renal diseases, such patient populations may also benefit from HR-ADMs based on its ability to speed closure. Further studies will help establish the value of HR-ADM in these higher-risk and more medically complex populations.

In summary, this randomised controlled trial of HR-ADM showed clinical superiority over SOC at 6 weeks and 12 weeks in non-healing DFUs. With the availability of wound size-specific grafts, this therapeutic modality may be a cost-effective solution for DFUs.

Acknowledgements

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Human Reticular Acellular Dermal Matrix in the Healing of Chronic Diabetic Foot Ulcerations that Failed Standard Conservative Treatment: A Retrospective Crossover Study

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Abstract: *Background*. Acellular matrices have been successfully used to heal indolent diabetic foot ulcers (DFUs). These tissues include allogenic dermis as well as xenograft dermis, pericardium, and small intestine submucosa. While all of these tissues show promise for healing DFUs, dermal-derived matrices have shown considerable potential. *Materials and Methods*. The authors retrospectively reviewed healing in patients with DFUs that failed the standard of care (SOC) treatment from a previous prospective randomized, controlled trial (RCT). That trial compared the efficacy of human reticular acellular dermal matrices (HR-ADMs) with the SOC. Of the 16 out of 20 patients who did not heal in the SOC group, 12 were eligible for crossover treatment with the HR-ADM. The authors studied the rate of complete healing in that specific cohort after 12 weeks of crossover treatment. *Results*. Of the 12 patients who were eligible for the HR-ADM, 10 (83%) achieved complete wound healing, with a mean healing time of 21 days to closure. The corresponding wound area reduction was from 1.7 cm^2 to 0.6 cm^2. The mean product cost to closure was $800/patient. *Conclusion*. This study further demonstrates the effectiveness of the HR-ADM in facilitating the closure of nonhealing DFUs refractory to SOC.

Key words: diabetic foot ulcers, dermal matrices, tissue repair, chronic ulcers, wound healing

Wounds Epub 2016 November 21

Diabetic foot ulcers (DFUs) are common complications, with nearly a quarter of people with diabetes experiencing at least 1 DFU in their lifetime.[1] The standard of care (SOC) for treating DFUs is offloading the wound, aggressive debridement, reduction of the wound bioburden, revascularization where indicated, and maintenance of moist wound-healing conditions.[2-6] Typical dressing options include collagen alginates, wound care gels, and antibacterial creams.

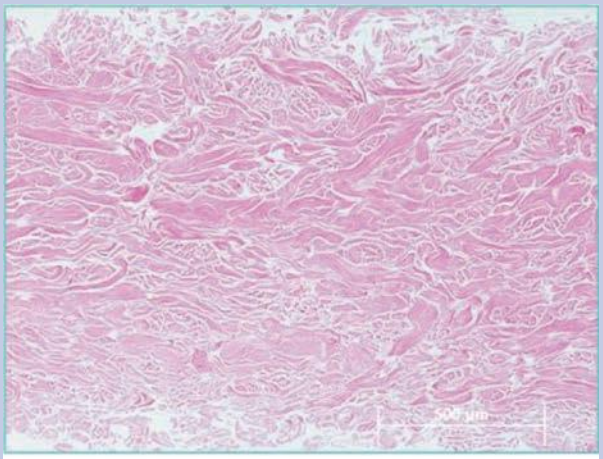

Figure 1. Hematoxylin and eosin stain showcasing the human reticular acellular dermal matrix (HR-ADM) is uniform throughout (symmetrical nature) and similar on both sides of the graft.

Despite excellent wound care, DFUs often take months to close, with many failing to do so. Prolonged wound healing leads to higher rates of infection and lower extremity amputation. Consequently, many interventions have been devised to accelerate wound closure, although only a few have undergone rigorous clinical trials. The literature demonstrates certain growth factors, as well as tissue-cultured skin substitutes, can improve healing of DFUs.[7] In addition, xenograft tissue matrices that have been studied, including dermis, small intestine submucosa, and pericardium, show promise in healing indolent wounds. The authors hypothesized human allogenic dermis, being the most analogous to a patient's tissue, would provide a suitable wound matrix to facilitate healing of chronic DFUs.

A randomized, controlled trial (RCT) was conducted to examine healing of indolent DFUs using a weekly application of aseptically processed human reticular acellular dermal matrix (HR-ADM; AlloPatch Pliable, Musculoskeletal Transplant Foundation [MTF], Edison, NJ) in conjunction with SOC compared to SOC alone. The results demonstrated that 16 of 20 (80%) patients healed with a weekly application of the HR-ADM, compared to 4 out of 20 (20%) patients who healed in the SOC arm after 12 weeks.[8]

After study exit, patients randomized to SOC were immediately offered the option to cross over and receive the HR-ADM for up to 12 weeks. Twelve of the 16 total patients who did not improve with SOC returned for application of the HR-ADM while continuing SOC. The primary objective of this retrospective study was to evaluate the proportion of ulcers that went on to complete closure with a weekly crossover application of the HR-ADM over a period of up to 12 weeks. Secondary objectives included evaluating the healing trajectory and product cost to closure.

Materials and Methods

Study population. Patients were deemed eligible for the study if they did not improve in the SOC arm of the HR-ADM RCT[8] (Western Institutional Review Board, November 13, 2014, #20142081). This included patients who had completed 12 weeks of SOC but whose ulcers did not heal and those who had exited the trial at 6 weeks because their DFUs failed to reduce in area by at least 50%, which was a criterion to ensure safety and the most compassionate care possible for all enrolled patients.[9-12]

Of the 16 patients who failed in the SOC arm, 12 returned to the clinic to receive a weekly application of the donated HR-ADM graft. The other 4 patients did not elect to receive the HR-ADM or were unable to receive it due to prior adverse events or serious adverse events that prevented grafting.

The study was approved by the Western Institutional Review Board, June 16, 2016 (#20161368) and allowed the investigators to review medical records of the entire original cohort from the time of study exit to current treatment. The study met applicable regulatory requirements in accordance with the provisions of the Declaration of Helsinki and in adherence to Good Clinical Practice. The first patient received a donated HR-ADM graft on February 10, 2015, and the last patient on February 12, 2016.

Treatments. At each clinic visit, the study ulcer was examined for presence of infection according to the guidelines of Woo and Sibbald.[13] If the examination suggested infection, a wound culture was taken with anaerobic and aerobic swabs of the suspected infected area, and appropriate systemic antibiotic treatment was administered until the infection clinically resolved. The wound was cleansed with sterile, normal saline solution and debrided as deemed necessary using a number 15 blade or curette to remove necrotic tissue. Hemostasis was obtained, digital photography of the wound was performed, and surface area was documented via acetate sheet tracing.[14]

Table 1. Wound and patient variables at study entry (N = 12) compared to the original standard-of-care (SOC) arm of the randomized, controlled trial (RCT; N = 20)

Variable	Failed SOC arm receiving HR-ADM (current study)	Original SOC arm (prior RCT)
Age	53 (10)	57 (10)
Race		
Caucasian	12 (100)	19 (95)
African American	0 (0)	1 (5)
Gender		
Male	8 (67)	12 (60)
Female	4 (33)	8 (40)
BMI	34 (8)	32 (7)
Smoker	4 (33)	6 (30)
Drinks alcohol	4 (33)	4 (20)
HbA1c (%)	7.1 (0.9)	7.8 (2)
Creatinine (mg/dL)	1.1 (0.4)	1.1 (0.4)
Wound area (cm^2)	1.7 (1.7)	2.7 (2.3)
PAR at end of RCT[a]	25.3 (30.37)	—
Wound location		
Toe	5 (42)	7 (35)
Forefoot	5 (42)	7 (35)
Midfoot	1 (8)	2 (10)
Heel/ankle/hindfoot	1 (8)	4 (20)

Continuous variables are reported as means and standard deviations, and categorical variables are reported as number and percentage.
[a]The percentage area reduction (PAR) at the end of 12 weeks in the original RCT. HR-ADM: human reticular acellular dermal matrix; BMI: body mass index; HbA1c: glycated hemoglobin; PAR: percentage area reduction

The HR-ADM is an aseptically processed graft prepared from the deep layer of human dermis (Figure 1). It differs from most other human dermal matrices because it only contains the deeper reticular portion of the dermis without the papillary layer.

The tissue is obtained through a donation program coordinated by the largest tissue bank in the United States, MTF. The majority of donors were otherwise healthy and relatively young people who died in accidents or from sudden illness such as heart attack or stroke. Donors are thoroughly screened and tested before donation. The screening includes comprehensive medical and social histories including high-risk behaviors for transmissible diseases. Extensive testing and serology is also performed. In addition, exclusion criteria consist of potential donors with histories of conditions that may affect the quality and long-term performance of the tissue.

The tissue is aseptically processed by this organization without terminal sterilization. Human reticular acellular dermal matrix is available commercially through the tissue bank for use in offices, wound centers, and hospitals. The grafts are available in size-specific pieces to minimize cost and waste.

In preparation for treatment, each graft was trimmed to fit the wound if needed and pie-crusted or meshed to no greater than 1.5x to 1.0 with a number 15 blade. The graft was completely submerged in sterile saline for 5 to 10 seconds and then applied, with care taken to ensure complete adherence in the wound bed and coverage of the entire wound surface. A nonadherent dressing (ADAPTIC TOUCH, Acelity, San Antonio, TX) was used to cover the wound, followed by a moisture-retentive dressing (hydrogel bolster) and a padded 3-layer dressing (DYNA-FLEX, Acelity, San Antonio, TX) until complete epithelialization occurred. Wounds were off-loaded using a

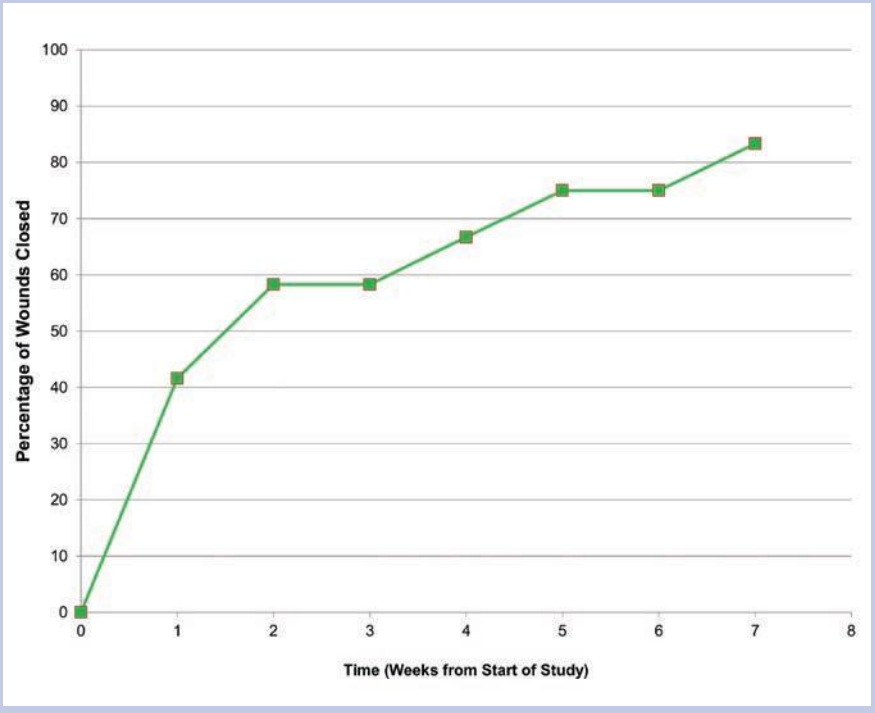

Figure 2. Plot of percentage of wounds healed week by week during the study. After week 7, the percentage remained constant through week 12.

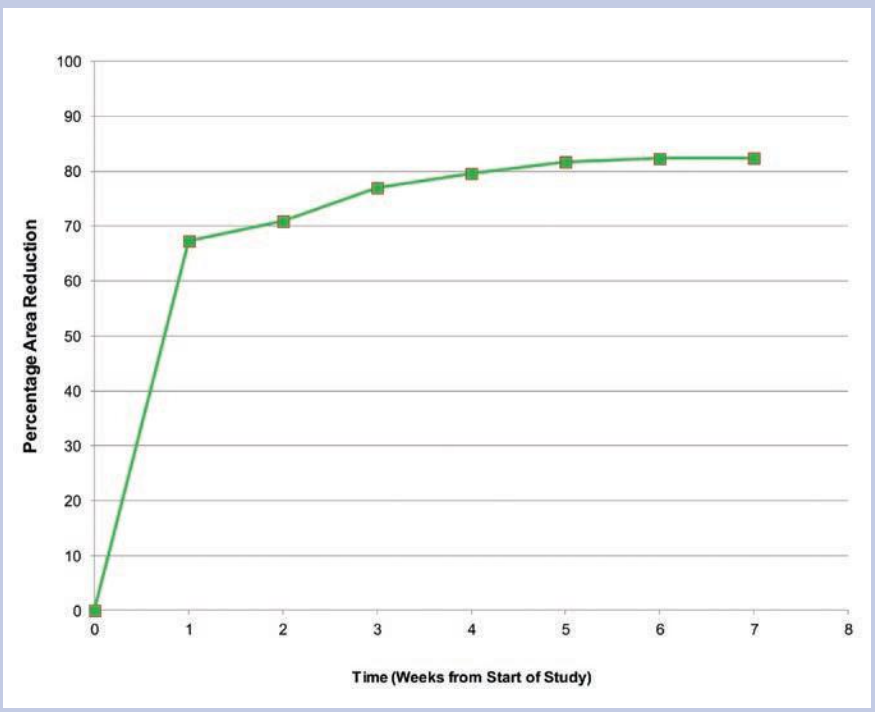

Figure 3. Trajectory of wound healing: plot of percentage area reduction week by week. After week 7, the percentage remained constant through week 12.

total contact cast, removable cast walker (Royce Medical, Camarillo, CA), or similar generic device. Patients were examined weekly with continued weekly application of the HR-ADM for up to 12 weeks or until the wound healed.

Study outcomes/Statistics. The primary endpoint of the study was the proportion of wounds completely healed at 12 weeks. Secondary endpoints looked at the difference in wound area, in which paired values were used for each wound (baseline and end-of-study values) using the Wilcoxon signed-rank test; time-to-heal within 12 weeks was calculated using the Kaplan-Meier approach; the percentage area reduction (PAR) was calculated as $PAR = ((A_I - A_{12W})/A_I)*100$, where A_I was the area of the wound at study entry and A_{12W} was the area at 12 weeks; and mean product cost to wound closure, which was calculated by adding the costs of the applied HR-ADM. An intent-to-treat approach was used for all analyses. For missing observations, last observation carried forward was used. Study variables were summarized as means and standard deviations (SDs) for continuous variables unless the data were not normal. In such cases, medians were also reported. Results for categorical variables were presented as proportions or percentages. Statistical analysis was performed using PASW 19 software (IBM, Chicago, IL).

Results

At entry to this retrospective study, patient characteristics (N = 12) were similar to the overall patient characteristics in the SOC arm of the RCT, with a mean

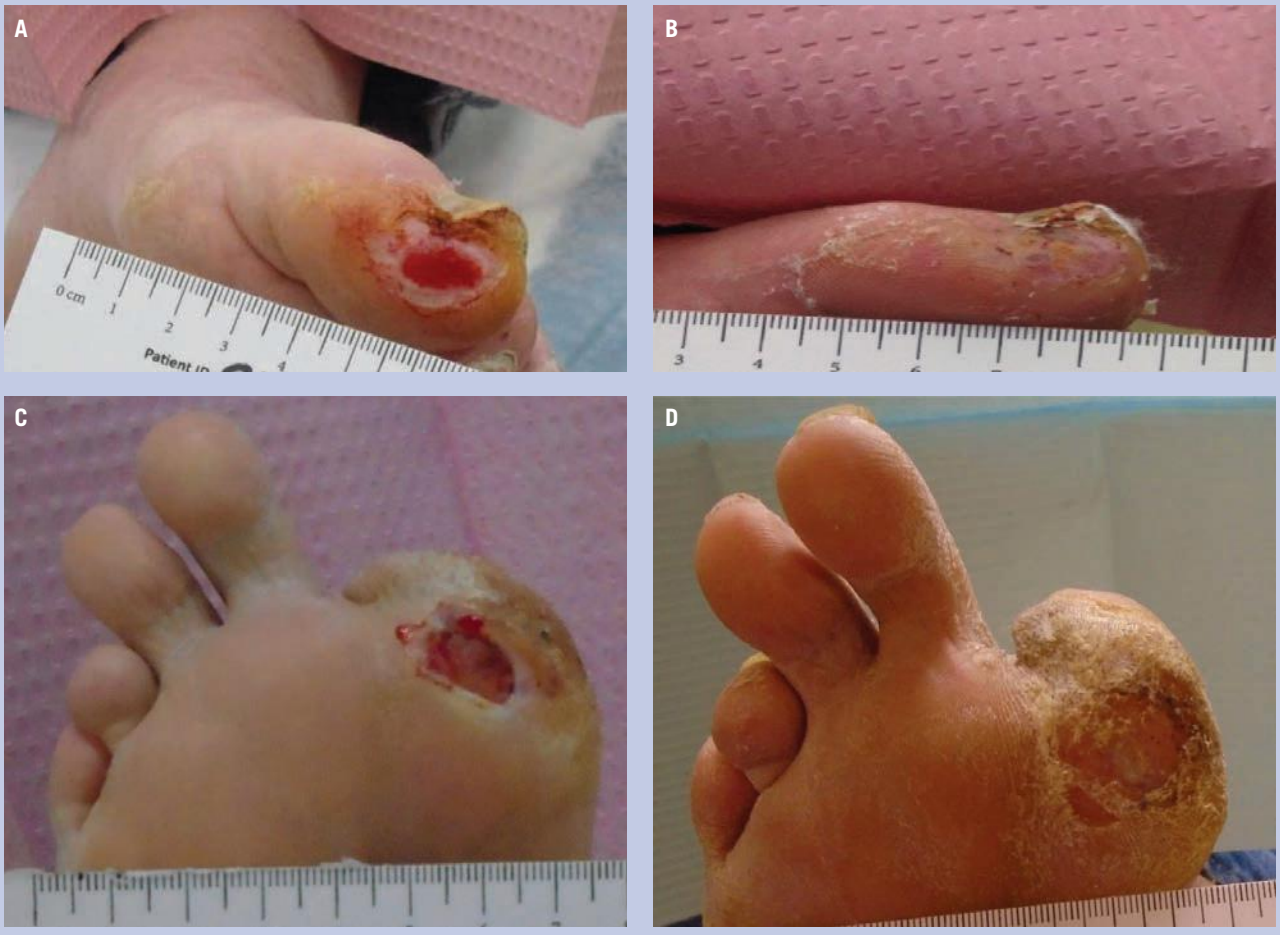

Figure 4. Two patients who achieved healing with human reticular acellular dermal matrix (HR-ADM) graft. (A) Patient 1 exit from standard of care (SOC) after failed treatment; (B) and completely healed with one application of the HR-ADM graft. (C) Patient 2 exit from SOC after failure of treatment; and (D) completely healed after 4 applications of the HR-ADM graft.

age of 53 years (Table 1). Wounds were smaller in area (mean 1.7 cm^2) at the beginning of this study compared to their original mean size at the beginning of the RCT (2.7 cm^2), with a 25% mean reduction in area over the course of the RCT.

Following crossover, 10 of the 12 (83%) wounds achieved complete wound closure (Figure 2). The mean area of the DFUs reduced from 1.7 cm^2 to 0.6 cm^2 (P = 0.006) at 7 weeks for the entire cohort (Figure 3), with a mean time to healing of 21.3 days (95% confidence interval, 11–31). Of the 10 patients who succeeded in healing, the healing was that of durable skin with complete epithialization (Figure 4). Of the 2 patients whose wounds failed to heal during the 12-week period, 1 patient did not return after the initial visit and application of the HR-ADM and the other withdrew from the study after 5 weeks because the wound was not reducing in area. At the end of the study period, the mean PAR was 82% (SD = 41) (Figure 3). The mean cost of the graft used to closure was $800 (SD = $790). No patient experienced adverse events or serious adverse events during treatment. All patients were provided with diabetic shoes and insoles donated by the tissue bank. When examining the results of the original RCT and the crossover cohort, the authors found that of the 32 patients who received the graft in total, 26 had healed completely for a combined healing rate of 81.3%.

Discussion

This is a retrospective study of patients who, following the conclusion of the original RCT, had wounds that did not heal with SOC alone and who subsequently received HR-ADM as a crossover treatment in addition to continued SOC. Twelve out of 16 patients in the cohort

participated. The results showed the use of HR-ADM led to complete healing of 83% of the wounds, with a mean healing time of 21 days (Figure 2). Although this group of patients (N = 12) is smaller than the original group of 20, the percentages of healed wounds were similar (83% versus 80% in the RCT), which further supports the conclusions from the original RCT. The trajectory of closure was also similar to that demonstrated in the RCT (Figure 3). The combined healing rate for both studies was 81%.

In regard to product cost to closure, even though the HR-ADM was donated for this study, the value of the graft used was $800. This amount takes into account the overall smaller wound size (1.7 cm^2) at the start of the crossover study compared to the original RCT (2.7 cm^2 average wound size in the SOC cohort). The cost to closure for patients in the HR-ADM group of the original 12-week RCT[8] was $1475 with an average wound size of 4.7 cm^2. In a recently published 12-week trial[15] comparing a dehydrated human amnion/chorion membrane (dHACM; EpiFix, MiMedx, Marietta, GA), a bioengineered skin substitute (Apligraf, Organogenesis, Canton, MA), and SOC, the baseline wound areas of the first 2 groups were 2.6 cm^2 and 2.7 cm^2, respectively, with corresponding mean costs to closure of $2798 and $8828, respectively. While it is difficult to compare cost to closure from different studies without also looking at initial wound size and other variables, it is clear the cost of the bioengineered skin substitute was far greater than the other options due to the fact that the tissue is only available in a single, large size. In contrast, HR-ADM and dHACM are available in multiple sizes, which minimizes waste.

Because HR-ADM grafts are available in size-specific packages, with the smallest graft measuring 1.5 cm x 1.5 cm, the majority of wounds healed with the smallest appropriate graft size. This reduced overall cost to closure compared with products available only in a single, large size. Cost is an important factor when clinicians choose among a myriad of advanced wound treatments. This study further supports HR-ADM graft use as not only a clinically effective but also a cost-effective intervention.

The HR-ADM used in this study originates from the deeper reticular layer of human dermis, which is known to be rich in collagens, elastin, and other extracellular matrix (ECM) components.[16] The dermis provides an open, uniform structure for cellular ingrowth and, during aseptic processing, specifically retains key ECM components such as collagens and elastin.[8] Furthermore, reticular dermis has a basket-weave structure, similar to fetal tissue, which may facilitate regeneration rather than scar development.[17,18] By providing a wound with this open, uniform, organized framework, HR-ADM may stimulate the type of healing observed in this study.

In addition, HR-ADM has not undergone the terminal sterilization typically used in the majority of allografts available. Terminal sterilization can damage the basement membrane and elastin collagen fibers and subsequently affect the quality of the graft structure and integrity.[19] Aseptically processed HR-ADMs retain ECM components that play important roles in supporting cell migration, cell infiltration, and cell attachment.[20-22]

Study limitations include a small sample size and the fact that patients did not require follow-up since they were regular patients in the wound clinic; therefore, they were under no obligation to return and receive the complimentary graft.

Conclusion

The crossover study showed a high healing rate (83%) among patients who received the HR-ADM with SOC who had failed to heal with SOC alone in the RCT. The mechanism by which the reticular dermis stimulates healing has yet to be fully investigated. However, with the results showing such marked success of the HR-ADM application, this novel approach may provide a cost-effective technology to treat patients with difficult-to-heal DFUs.

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A Novel Reticular Dermal Graft Leverages Architectural and Biological Properties to Support Wound Repair

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Background: Acellular dermal matrices (ADMs) are frequently used in reconstructive surgery and as scaffolds to treat chronic wounds. The 3-dimensional architecture and extracellular matrix provide structural and signaling cues for repair and remodeling. However, most ADMs are not uniformly porous, which can lead to heterogeneous host engraftment. In this study, we hypothesized that a novel human reticular ADM (HR-ADM; AlloPatch Pliable, Musculoskeletal Transplant Foundation, Edison, N.J.) when aseptically processed would have a more open uniform structure with retention of biological components known to facilitate wound healing.

Methods: The reticular and papillary layers were compared through histology and scanning electron microscopy. Biomechanical properties were assessed through tensile testing. The impact of aseptic processing was evaluated by comparing unprocessed with processed reticular grafts. In vitro cell culture on fibroblasts and endothelial cells were performed to showcase functional cell activities on HR-ADMs.

Results: Aseptically processed HR-ADMs have an open, interconnected uniform scaffold with preserved collagens, elastin, glycosaminoglycans, and hyaluronic acid. HR-ADMs had significantly lower ultimate tensile strength and Young's modulus versus the papillary layer, with a higher percentage elongation at break, providing graft flexibility. These preserved biological components facilitated fibroblast and endothelial cell attachment, cell infiltration, and new matrix synthesis (collagen IV, fibronectin, von Willebrand factor), which support granulation and angiogenic activities.

Conclusions: The novel HR-ADMs provide an open, interconnected scaffold with native dermal mechanical and biological properties. Furthermore, aseptic processing retains key extracellular matrix elements in an organized framework and supports functional activities of fibroblasts and endothelial cells. *(Plast Reconstr Surg Glob Open 2016;4:e1065; doi: 10.1097/GOX.0000000000001065; Published online 4 October 2016.)*

Acellular dermal matrices (ADMs) are commonly used in wound healing and tissue repair to facilitate wound closure and regenerative remodeling.[1–3] The extracellular matrix (ECM), a major component of ADMs, provides structure, cell-signaling cues, and mechanical support to facilitate the healing process.[4–8] Key dermal ECM components include collagens, elastin, glycosaminoglycans (GAGs), and hyaluronic acid (HA).[4,9–11] The ECM

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can sequester and control the bioavailability of growth factors that modulate cellular responses by serving as a growth factor reservoir.[4,5,12] Apart from providing biological cues, the ECM imparts mechanical properties in the form of structural, tensile, and compressive support.[13,14] Its architecture influences material stiffness, which regulates cell behavior by affecting cytoskeletal reorganization and cell signaling,[15,16] whereas an open microstructure can facilitate host cell infiltration.[17] These native dermal properties can guide cell behavior and tissue remodeling in a wound care setting.

Exogenous scaffolds replace or replicate native ECM by restoring structural and functional requirements.[1,12,18] They also provide a barrier to protect wounds from infection and desiccation. Scaffold origins can be cellular or acellular and originate from biological, synthetic, or composite materials.[1,19] Although synthetic scaffolds are reproducible and uniform, they lack the biological advantages of native dermal matrices.[5,19] ADMs can be processed to preserve the dermal structure and leverage the dermal biology to reduce scarring and improve tissue regeneration.[2,20–22]

The structure of human dermis can be divided into 2 layers: papillary or superficial and reticular.[1] The fibrils present in the papillary dermis are smaller compared with the reticular dermis. When the papillary dermis is injured (superficial cut or burn), it can often regenerate without a scar. The reticular dermis is the deeper and thicker region composed of dense collagen fibers, elastin, and woven reticular fibers. These characteristics provide this region with strength, extensibility, and elasticity.[23] In a deep wound, this framework is missing, which can lead to scarring. By using an organized structure, this can coordinate new tissue repair and potentially address scarring.

ADM processing aims to remove cellular material to reduce immunogenicity and decontaminates or sterilizes the graft to limit disease transmission.[2,24] If not designed appropriately, however, the processing can negatively impact the endogenous matrix proteins and natural architecture that can hamper host cell integration and result in encapsulation and foreign body response.[25,26] Aseptic tissue processing utilizes gentle decontamination steps to ensure tissue safety, while preserving the matrix configuration.

In this study, we investigate the hypothesis that aseptically processed reticular dermal grafts provide a scaffold possessing biological and mechanical properties that can support wound healing. This unique deeper cut reticular dermis retains architectural elements (open structure), mechanical properties (elasticity, organized collagen and elastin), and key matrix proteins to support physiological cellular responses during regenerative remodeling.

MATERIALS AND METHODS

Tissue Procurement

Human dermal tissue was screened and recovered following industry standard guidelines. Human reticular ADMs (HR-ADMs; AlloPatch Pliable, Musculoskeletal Transplant Foundation, Edison, N.J.) was processed aseptically (Fig. 1) without terminal sterilization at Musculoskeletal Transplant Foundation (Edison, N.J.). The tissue was decellularized and disinfected with peracetic acid–based solution and every lot was assessed as per ⟨USP-71⟩ Sterility Tests. The papillary dermis was prepared in a similar method as a comparison. Both reticular and papillary layers were cut to final tissue specifications of 0.4–1.0 mm thick.

ADM Structure

Prehydrated papillary and reticular dermis samples (n = 3 donors) were fixed in 10% formalin, embedded in paraffin, sectioned into 5 μm thick cross-sections, and stained for hematoxylin and eosin (H&E) by Premier Laboratory, LLC (Boulder, Colo.).

Scanning electron microscopy (SEM) imaging was performed to visualize the microstructure of HR-ADM in comparison to papillary dermis (n = 3 donors). Samples were fixed in 10% formalin for 24 hours, rinsed with water twice for 15 minutes, and dehydrated in 50%, 70%, 80%, 95%, and 100% ethanol successively for 15 minutes each. These dry samples were coated under vacuum using

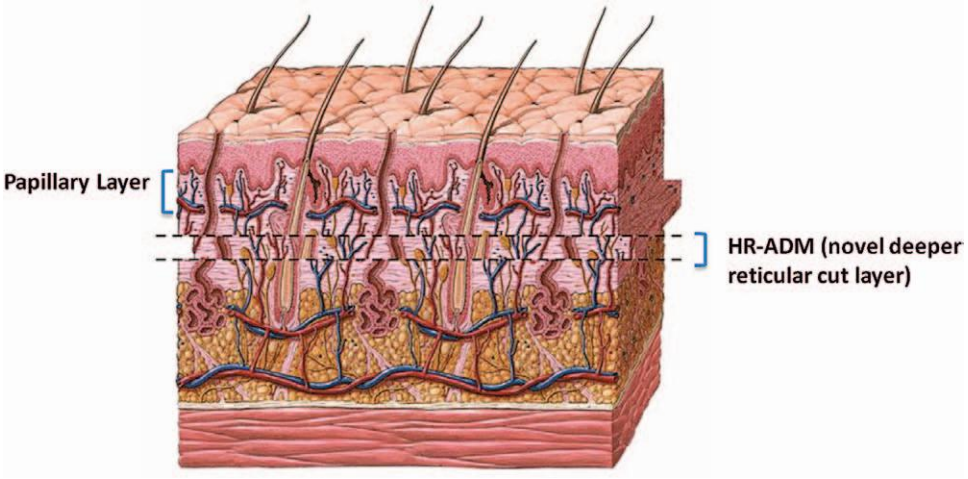

Fig. 1. HR-ADM, a novel deeper cut reticular dermis layer, present below the papillary dermis layer.

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a Balzer MED 010 evaporator (Technotrade International, Manchester, NH) with platinum alloy to a thickness of 25 nm and immediately flash carbon coated under vacuum. Samples were examined in a JSM-5910 SEM (JEOL USA, Inc., Peabody, Mass.) at an accelerating voltage of 25 kV. Imaging was conducted at 250×.

The material porosity (n = 3 donors) was determined through gravimetric method assuming the material is close to density of collagen (1.34 g/cm^3), as collagen is the largest component of dermal tissue. The density of the tissue (using a ratio of dry and wet tissue densities) was calculated as per Loh and Choong.[27] The pore size range was evaluated by mercury intrusion porosimeter (Quantachrome, Fla.) using standard techniques. Mercury is forced into the dermal sample under high pressure through the porosimeter. The pressure needed to force mercury into the sample is inversely proportional to the pore size.

Biomechanical Characterization

Biomechanical properties of dermal tissue were evaluated using a MTS 858 Mini-Bionix tensile testing system (MTS, Eden Prairie, Minn.) with a calibrated 1 kN load cell. Dermal grafts (n = 3 donors) were cut into multiple specimens (4–12) using a custom punch shaped and sized to match the type V (microtensile testing) specimen specified by the American Society for Testing and Materials (ASTM) D638 guidelines for evaluating material properties (3.18 mm width; 0.4–1.0 mm thick). Sample thickness was measured and then loaded into tensile grips. Specimens were pulled under tensile load at a rate of 50.8 mm/min until failure. Ultimate tensile stress (UTS), Young's modulus, and the percentage elongation at break were examined and normalized to cross-sectional area.

Matrix Protein Characterization

Immunohistochemistry staining was performed at HistoTox Labs, Inc. (Boulder, Colo.). Operators were blinded to HR-ADMs (sample 1) and unprocessed reticular dermis (sample 2) for collagens I, III, IV, and VI and elastin. The levels of GAG and HA in HR-ADMs were compared with unprocessed reticular dermis (n = 3 donors). GAGs were quantified using the Blyscan GAG assay (Biocolor Life Science Assays; Carrickfergus, UK/Fisher Science, Houston, Tex.). Samples were extracted in papain (125 μg/mL in 0.1 M phosphate buffer) for 2 hours at 65°C and centrifuged (10,000 rpm; 10 minutes). The dye-binding assay was performed and absorbances were read at 656 nm. HA was quantified using an enzyme-linked immunosorbent assay (Corgenix, Broomfield, Colo.). Samples were extracted (24 hours, 4°C in 1 M sodium chloride and sodium bicarbonate solution), homogenized for 5 minutes in a bullet blender (Next Advance, N.Y.), and centrifuged (10,000 rpm; 10 minutes). Absorbances were read at 450 nm.

Enzymatic Degradation

HR-ADM samples were air dried overnight, weighed (21–25 mg), and rinsed in 0.9% saline solution. Samples (n = 3 donors) were then enzymatically digested (6 hours, 37°C water bath) in a collagenase type 1A (6.65 U/mL final enzyme solution; Sigma, St. Louis, Mo.) and thermolysin (15 U/mL final enzyme solution; Sigma, St. Louis, Mo.) solution in tricine buffer (pH 7.5). The filtered extract was mixed with ninhydrin (0.016 g/1 mL solution)–hydrindantin (0.0024 g/1 mL solution) (Sigma, St. Louis, Mo.) in ethylene glycol monoethyl solution and 4 N sodium acetate buffer (pH 5.5) that reacts with the released amino acids, producing a deep purple color proportional to the amount of peptides released. The standard curve was established with L-leucine (stock solution 2.0 mg/mL; Sigma, St. Louis, Mo.) and sample absorbances were read at 570 nm. The controls were crosslinked[28] and denatured dermis samples. Unprocessed dermal tissue was crosslinked (16 hours, room temperature) with 0.025% glutaraldehyde (Sigma, St. Louis, Mo.) solution, followed by a 2-hour rinse step to remove residual glutaraldehyde. The denatured condition (representing harsh chemical processing) was prepared by crosslinking as above and then boiling (at 100°C) the rinsed samples for 5 minutes. These samples were digested as stated above, reacted, and read at 570 nm.

Cell Behavior Characterization

Normal human dermal fibroblasts (NHDFs; Lonza, Walkersville, Md.) were cultured (0.2 million cells/7 mm disk) on HR-ADMs in fibroblast growth medium (FGM-2) (Lonza) at 37°C and 5% CO_2 in a humidified atmosphere. Cell attachment and matrix production were assessed over time (0, 7, 14 days). H&E, collagen IV, and fibronectin staining were performed by IHC World, LLC (Ellicott City, Md.), with standard histology techniques. Human umbilical vascular endothelial cells (HUVECs) were cultured (0.2 million cells/7 mm disk) on HR-ADMs to examine angiogenic capacity through tubular formation (CD31, AbCam, Cambridge, Mass.) and secretion of functional angiogenic factor, von Willebrand Factor (vWF; AbCam, Cambridge, Mass) on adhered cells through 4', 6-diamidino-2-phenylinadole (Life Technologies, Carlsbad, Calif) staining. Confocal imaging (Rutgers University, Piscataway, N.J.) was performed to visualize tubular network formation and vWF secretion.

Statistical Analysis

All values are reported as average and SD. A student t-test (unpaired) was used to compare mechanical evaluation of dermal tissue (HR-ADM versus papillary), and enzymatic degradation analysis (unprocessed dermis to HR-ADM, crosslinked and denatured), with $P < 0.05$ being considered significant.

RESULTS

Unique Feature of HR-ADM

H&E staining of HR-ADM revealed an open, uniform architecture (no orientation or polarity; Fig. 2). In contrast, the papillary graft was asymmetrical (epidermal-facing side was dense versus the open dermal-facing side), resulting in directionality (distinct orientation or polar-

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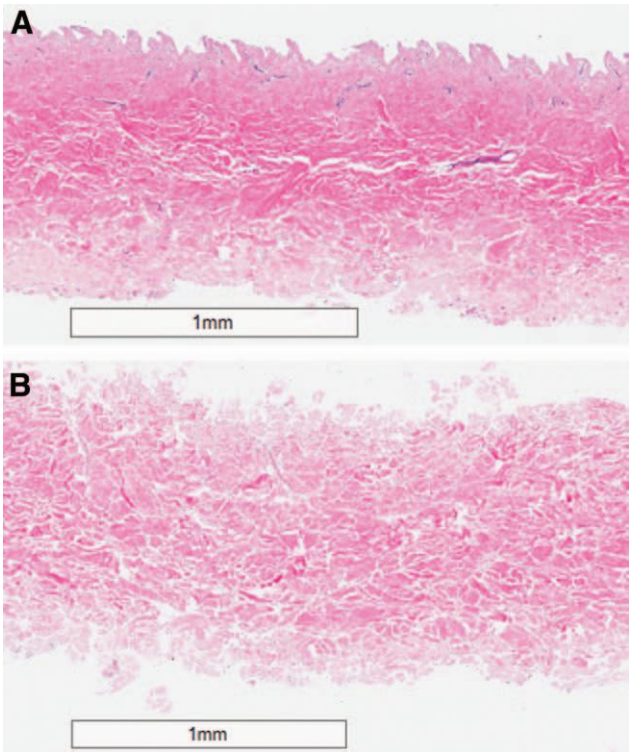

Fig. 2. A, Papillary Dermis is asymmetrical. B, HR-ADM is symmetrical. H&E revealed that the papillary dermis has an asymmetrical matrix structure, whereas the HR-ADM is symmetrical with a more uniform and open structure (magnification 2×). The papillary dermis is dense collagen on one side and loose collagen on the other side (distinct orientation present). The HR-ADM is uniform throughout and similar on both sides of the graft (no sidedness or orientation).

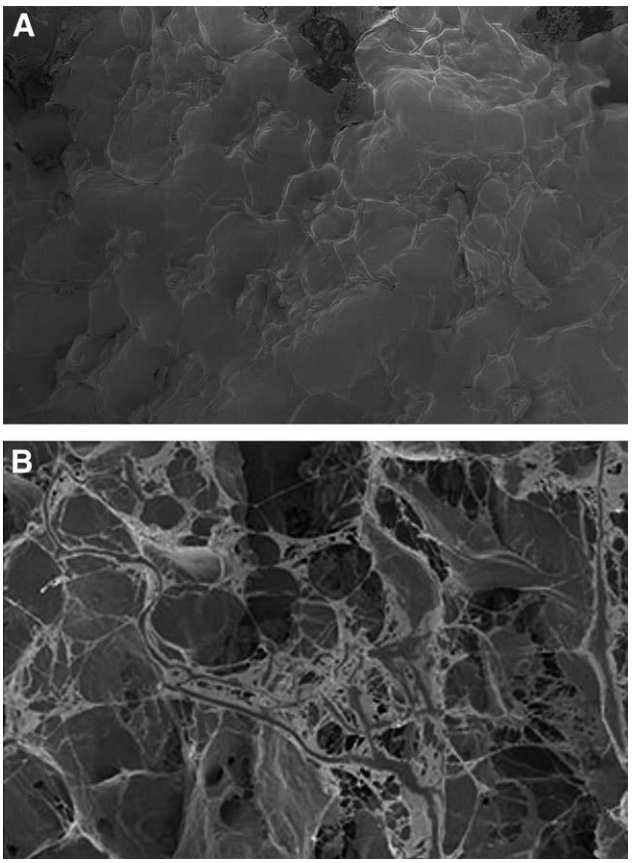

Fig. 3. A, Papillary Dermis. B, HR-ADM. SEM imaging displays the microstructure of HR-ADM and papillary dermis. The papillary dermal graft has a dense appearance at the epidermal facing side, whereas HR-ADM has open, porous appearance on the papillary facing side (magnification at 250×).

ity). Higher magnification (20×) images clearly demonstrated the consistent open, interconnected network of HR-ADM compared with the asymmetrical papillary dermis (**See PDF**, **Supplemental Digital Content 1**, which reveals distinct structural differences between papillary and reticular dermal structures, ***http://links.lww.com/PRSGO/A274***). Additionally, SEM imaging (Fig. 3) confirmed the open architecture present in HR-ADMs compared with papillary dermis. The porosity of HR-ADM was 88% ± 4% and of papillary dermis was 82% ± 6%. The pore size range as determined by mercury intrusion for HR-ADMs was 2.7–500 μm, whereas that determined for papillary dermis was 0.8–500 μm (Table 1). This open, interconnected network in HR-ADMs was seen in Figure 2, and this pore size range supports cell infiltration as evidenced in Figure 7.

Biomechanical Characterization

The HR-ADM thickness was 0.8 ± 0.2 mm (n = 4 donors; 4–8 samples/donor), whereas the papillary dermis thickness was 0.7 ± 0.1 mm (n = 3 donors; 8–12 samples/donor). HR-ADMs exhibited lower UTS values (7 ± 2 MPa) and Young's modulus (6 ± 1 MPa) compared with the papillary dermis UTS (14 ± 3 MPa) and Young's modulus (15 ± 3 MPa). HR-ADMs had significantly lower UTS (P = 0.03) and Young's modulus (P = 0.019) values compared with papillary dermis (Table 1). These lower biomechanical properties of HR-ADMs were similar to those reported for fetal porcine dermis as an elastic biomaterial comparison. The percentage elongation at break was significantly greater (P = 0.03) for HR-ADM (131% ± 15%) compared with papillary dermis (104% ± 2%), and is expected in an elastic scaffold. Generally,

Table 1. Biomechanical Properties of HR-ADM and Papillary Dermal Grafts

Material Properties	Papillary Dermis	HR-ADM	Fetal Porcine Dermis*
Porosity, %	82 ± 6	88 ± 4	Not reported
Pore size, μm	0.8–500	2.7–500	Not reported
Measured thickness, mm	0.7 ± 0.1	0.8 ± 0.2	Not reported
Ultimate tensile strength, MPa	14 ± 3	7 ± 2	2.1 ± 0.3
Young's modulus (stiffness), MPa	15 ± 3	6 ± 1	5.9 ± 1.5
% elongation at break, mm/mm	104 ± 2	131 ± 15	Not reported

The ultimate tensile strength and Young's modulus (stiffness) of HR-ADM and papillary dermis were compared to fetal porcine dermis.[37] HR-ADM demonstrates significantly lower tensile strength (P = 0.03) and Young's modulus (P = 0.019) and higher percentage elongation at break (P = 0.03) compared to papillary dermis.

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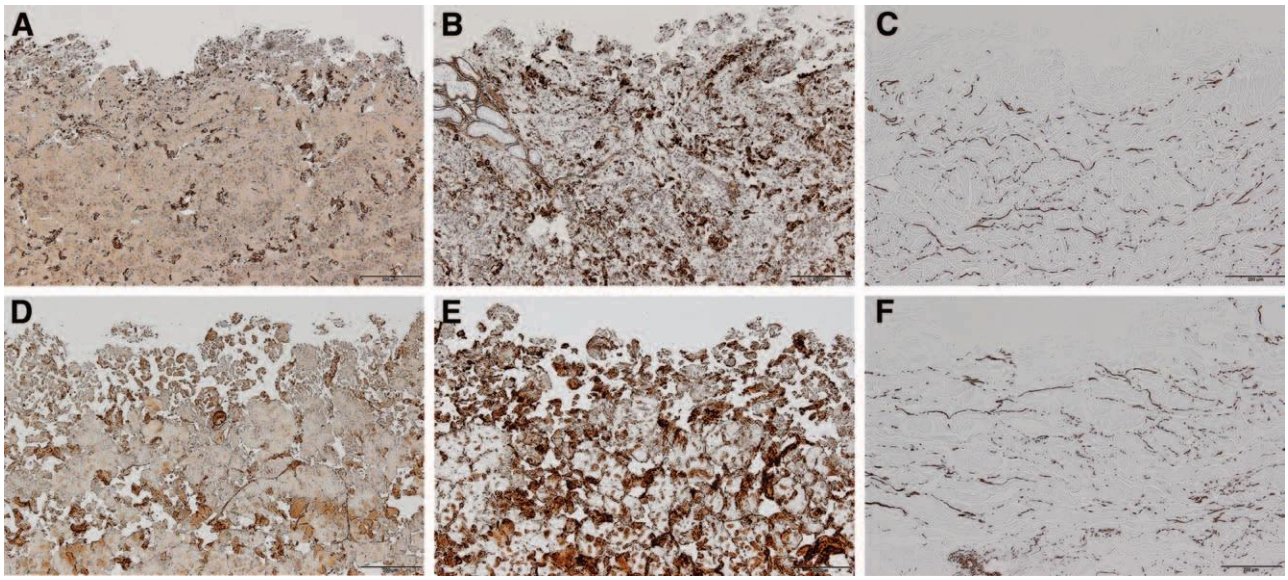

Fig. 4. Immunohistochemistry staining of unprocessed reticular dermis (A) Collagen I, (B) Collagen IV, (C) Elastin and HR-ADM (D) Collagen I, (E) Collagen IV, (F) Elastin. Aseptically processed HR-ADM revealed retention of collagen types I and VI and elastin as compared to unprocessed reticular dermis (magnification, 10×). All images were taken from the papillary facing side. Similar observations were found on the deep dermal facing side.

open porous scaffolds under tension align first, stretch, and break, whereas dense scaffolds, which have some orientation, load first and then break resulting in lower percentage elongation at break. Therefore, the biomechanical testing confirmed that the HR-ADMs are flexible structures, exhibiting low stiffness and increased elasticity.

Native ECM Components Preserved

Immunohistochemistry staining qualitatively revealed the retention of organized collagen types I and VI and elastin (Fig. 4) in unprocessed reticular dermis and HR-ADMs after aseptic processing (**See PDF**, **Supplemental Digital Content 2**, which demonstrates collagen III and IV retention, ***http://links.lww.com/PRSGO/A275***). Although there was some reduction in staining intensity for collagen III, the majority of the ECM components in HR-ADMs are similar to unprocessed tissue. Additionally, GAGs and HA were present and quantified in unprocessed reticular dermis (2.5±0.1 mg/g; 1000±88 μg/g) and retained in HR-ADMs (2.7±0.6 mg/g; 272±51 μg/g), respectively (Fig. 5). Although lower HA levels were present in the processed HR-ADM as compared with the unprocessed sample, a considerable amount of HA is retained. These critical ECM components provide an organized architecture to support cellular activities.

Enzymatic Degradation

To verify that aseptic processing preserves the native dermal components, enzymatic degradation studies examined the release of peptides in unprocessed and processed tissue samples along with controls (crosslinked, denatured dermis) representing process-altered tissue. Peptide release

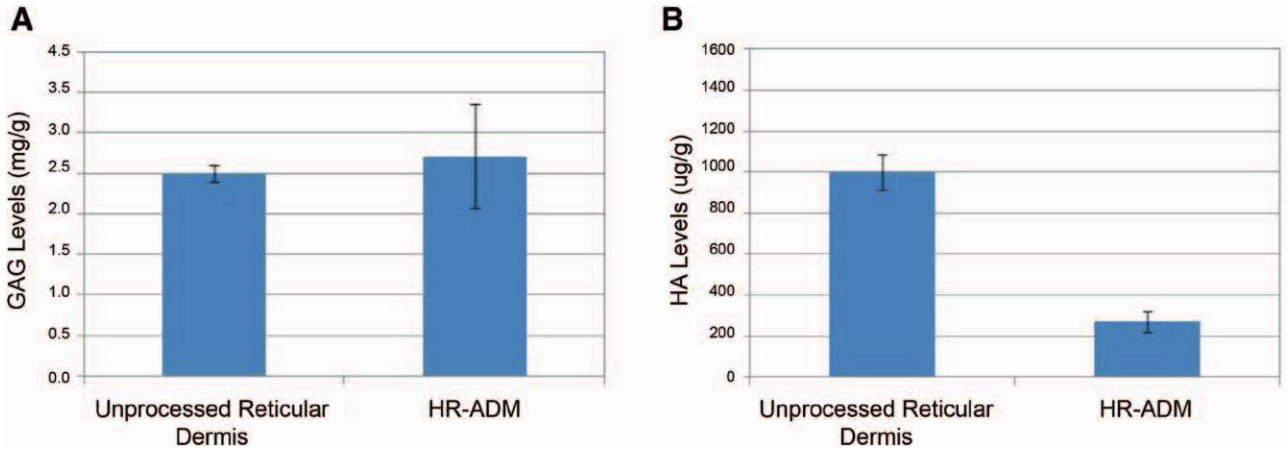

Fig. 5. A, Glycosaminoglycans (mg/g) and hyaluronic acid (μg/g; B) are present in aseptically processed HR-ADM compared to unprocessed reticular dermis.

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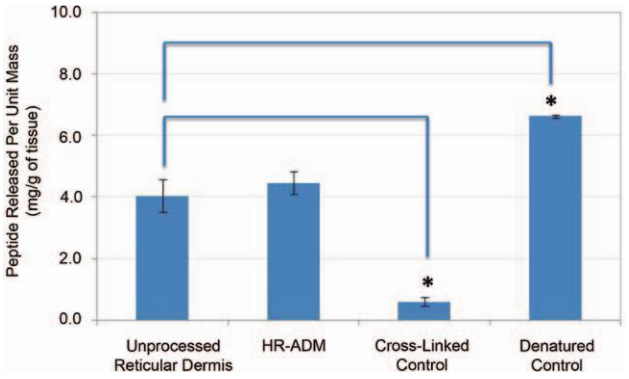

Fig. 6. Peptide release varied according to dermal processing methods. Aseptically processed HR-ADMs demonstrated similar peptide release profile compared to native, unprocessed reticular dermis. Crosslinked dermis with 0.025% glutaraldehyde[28] renders the matrix more resistant to degradation, with significantly (P = 0.004) lower peptide release, whereas denatured dermis yielded greater degradation of dermal components, with significantly (P = 0.013) greater peptide release.

for aseptically processed HR-ADMs (4.5±0.4 mg/g of tissue) was similar (no significant difference) to that for unprocessed reticular tissue (4.0±0.5 mg/g of tissue; Fig. 6). Crosslinking dermis resulted in significantly (P = 0.004) lower peptide (0.6±0.1 mg/g of tissue) release (resistance to degradation) due to the crosslinked collagen structure, whereas denatured dermis yielded significantly (P = 0.013) higher peptide (6.6±0.6 mg/g of tissue) release (degraded collagen); both reflecting altered tissue components. Therefore, aseptic processing preserves the native dermal components, whereas other processing methods may alter it.

In Vitro Cell Studies

Histological and confocal imaging demonstrated that both NHDFs and HUVECs readily attached to HR-ADMs. Histology analysis of NHDFs revealed cell attachment and infiltration into the graft (Fig. 7). Immunohistochemistry analysis confirmed fibroblasts secreted an abundance of collagen IV in a multilayered network on top and within the open HR-ADM network as early as day 7 (**See PDF**, **Supplemental Digital Content 3**, which displays fibronectin secretion and day 14 images, ***http://links.lww.com/PRSGO/A276***). HUVECs also readily attached (4', 6-diamidino-2-phenylinadole staining) with endothelial marker CD31, highlighting distinct, sustained tubular, network formation, and vWF, which is secreted by functional endothelial cells and confirming angiogenic capacity (Fig. 8). Similar observations were found on both sides of the HR-ADM. Both fibroblasts and endothelial cells are functional on HR-ADMs by attaching and secreting matrix proteins, which support granulation and angiogenic activities.

DISCUSSION

ADMs are used to protect the wound surface, maintain hydration, and provide a conducive microenvironment

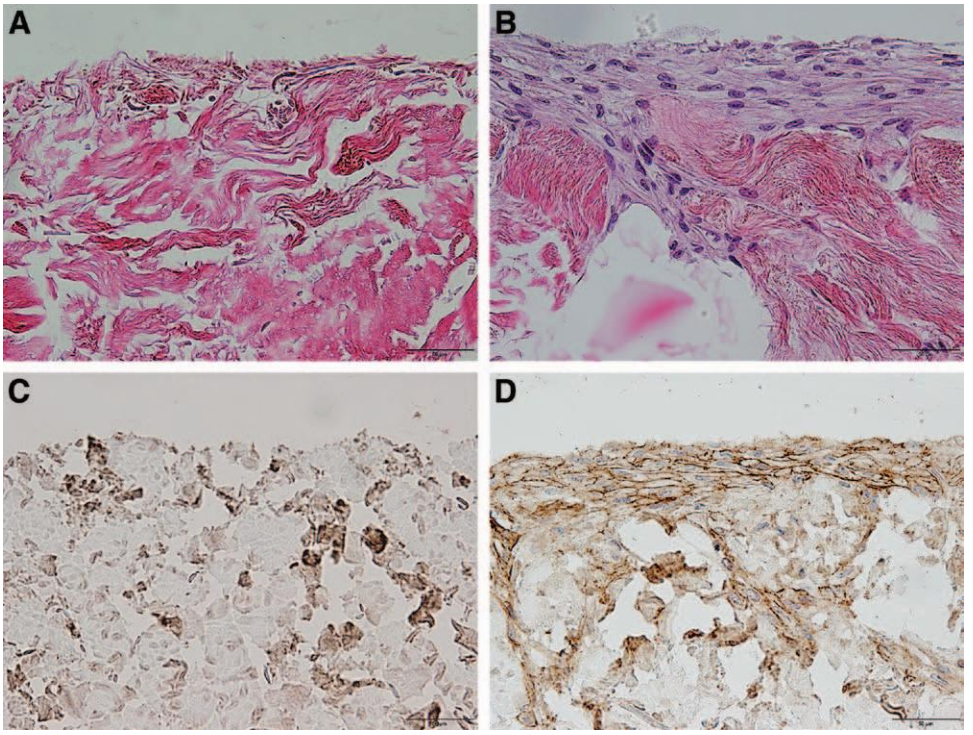

Fig. 7. H&E Histology demonstrated (A) HR-ADM alone and (B) NHDFs cultured on HR-ADMs at day 7 where cells readily attached and infiltrated within the graft (magnification 40×). Immunohistochemistry imaging revealed that (C) HR-ADM only and (D) NHDFs secreted an abundance of collagen IV on top and within the open, interconnected graft as early as day 7. Results were similar on both sides of the HR-ADM. These secreted ECM components support granulation activities.

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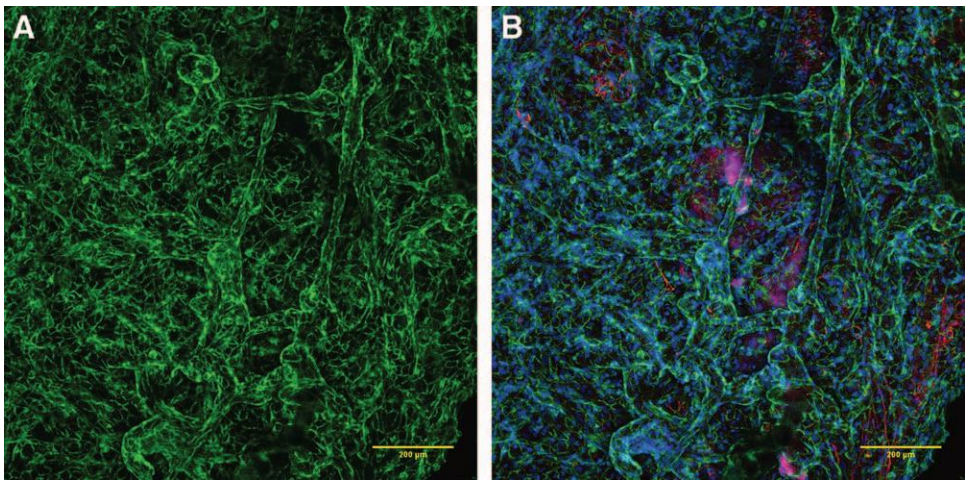

Fig. 8. Confocal imaging of HUVECs cultured for 10 days on HR-ADMs at 10× magnification. A, Endothelial cell marker, CD31 (green), revealed distinct, sustained tubular network formation. B, Adhered HUVECs shown by 4', 6-diamidino-2-phenylinadole staining (blue nuclei) have secreted vWF (red) which also verifies angiogenic capacity of functional endothelial cells. Similar observations were found on both sides of the HR-ADM.

for dermal repair and regeneration.[2,6,18,21,29] Traditionally, these matrices are obtained from the papillary dermal layer and are processed by methods that can alter the native dermal architecture and tissue quality, thereby impacting host engraftment and tissue remodeling. The novel dermal graft (HR-ADM) obtained from the deep reticular dermis layer used in this study was aseptically processed, and this preserved the native architecture and key ECM components that facilitate graft integration.

Histological analysis of the papillary dermis revealed an asymmetrical network (dense on one side, open on the other); this architectural polarity within the papillary region has been reported previously.[1] The heterogeneous nature can impact cell infiltration and native tissue remodeling. The novel HR-ADM provided a uniform, open network, ensuring a homogeneous framework. The absence of any graft asymmetry or orientation can be beneficial in the clinical setting, facilitating the ease of use.

It is well known that open scaffold architectures modulate cell–matrix interactions and augment cellular activities and newly formed tissue.[30,31] Increasing porosity can significantly improve cellular infiltration and tissue integration,[30] whereas the intrinsic mechanical properties (stiffness, elongation) can regulate cellular behavior (proliferation, cell–matrix integration).[32–34] This study demonstrated that HR-ADMs had an open, interconnected network with elastic biomechanical properties that are similar to fetal skin and significantly lower than papillary dermis. From literature, papillary dermis exhibited biomechanical properties which are in alignment with our study; similar Young's modulus (18.4 MPa) for an ADM,[35] obtained from papillary dermis, and UTS values (22 ± 8 MPa[36] and 13–30 MPa[13]) were also observed. In contrast, HR-ADMs behaved similarly to fetal porcine tissue having low biomechanical properties: UTS (2.1 ± 0.3 MPa) and Young's modulus (5.9 ± 1.5 MPa).[37] Exhibiting similar biomechanical properties to fetal porcine tissue may culminate in reduced scar formation.[37–39] As human wounds heal, the stiffness has shown to increase from 18 to 40 kPa,[40] indicating wound bed fibrosis and scarring.[41,42] Now, depending on clinical applications, different biomechanical properties are necessary. In the wound setting, graft strength is not critical, whereas the elasticity, flexibility, and conformability to the wound topography and irregular wound sizes are advantageous. Hence, HR-ADMs provide a promising elastic scaffold for wound repair.

This study also demonstrated that aseptic processing preserved ECM components important for wound healing, including collagens and elastin. They provide a stable, organized structure along with signaling cues that facilitate wound healing.[4,12] Collagens are instrumental in supporting the wound healing phases[8,22]; homing inflammatory cells[3,7]; supporting fibroblast attachment/granulation[10,43]; and facilitating keratinocyte migration.[44] Fetal fibroblasts have been shown to express more collagen III than collagen I and promote a more reticular deposition of fibers in a basket-weave orientation, which can assist in minimizing scar formation.[45,46] This type of reticular collagen network can help promote regeneration versus repair and minimize scarring; that is distinctly different from disorganized, parallel bundles of collagen that cause scarring.[38] Elastin provides scaffold elasticity and also mediates cellular activities by regulating the activity of TGFβs and its presence/organization minimizes scar formation.[47–49] Therefore, the preserved organized, basket-weave collagen and elastin structure present in HR-ADMs may promote regenerative healing.

GAGs and HA, which are important biological components for both adult and fetal wound healing,[20,50] are also retained in HR-ADMs. Exogenous addition of HA has reduced scar formation in adults by harnessing local growth factors and modulating cell behavior.[51,52] GAGs also have an effect on chronic inflammatory response and fibrotic encapsulation, which otherwise may progress to implant

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failure or scarring.[53] GAGs and HA can influence the ECM structure, assembly, and hydration[38]; impact inflammation[50,51]; and foster granulation and protect cells from free-radical damage.[25] The retention of both GAGs and HA in HR-ADMs is predictive of clinical utility in facilitating wound healing.

These key biological ADM properties are beneficial to be retained through the processing steps to remove bioburden and minimize immunogenicity. Terminal sterilization and harsh chemical treatments can modify the scaffold structure and degradation characteristics by resident enzymes found in wounds.[54–56] Furthermore, these treatments can damage the collagen structure and other bioactive components, hampering cell adhesion and cell–ECM interactions.[54,55] Crosslinking agents (such as glutaraldehyde[28]) strengthen scaffold biomechanical properties; however, they reduce the ability of cell–matrix interactions by impairing the collagen structure and cell-binding sites, yielding poor clinical properties.[26] Consequently, tissue processing strategies must balance bioburden reduction and cell removal with maintenance of scaffold integrity. This study verified aseptic processing retained the native dermal components. Furthermore, enzymatic degradation of HR-ADM yielded similar peptide release compared with unprocessed tissue, whereas crosslinking or denaturing dermis significantly altered peptide release.

Further evidence that aseptic tissue processing preserved the native architecture and biological components comes from in vitro fibroblast and endothelial cell studies in HR-ADMs. The open architecture of HR-ADMs (2.7–500 μm pore size range) and retained ECM components supported cell attachment and infiltration. Both cell types readily attached and were functional on the HR-ADMs by secreting an abundance of new matrix proteins (collagen IV, fibronectin, vWF) on top and within the graft. The secreted matrix proteins are critical in supporting granulation and angiogenesis[4,5,57,58] and stimulate and guide other cellular responses.[10,59]

In summary, aseptically processed HR-ADMs provide a unique, biologically and mechanically advantageous scaffold for wound repair. The in vitro findings are supported by the clinical findings where HR-ADMs combined with standard of care performed significantly better than standard of care alone in the treatment of chronic diabetic foot ulcers.[60] Further in vitro studies are needed to characterize the cell behavior and functionality of these biologically and mechanically stable novel reticular dermal grafts in a chronic setting.

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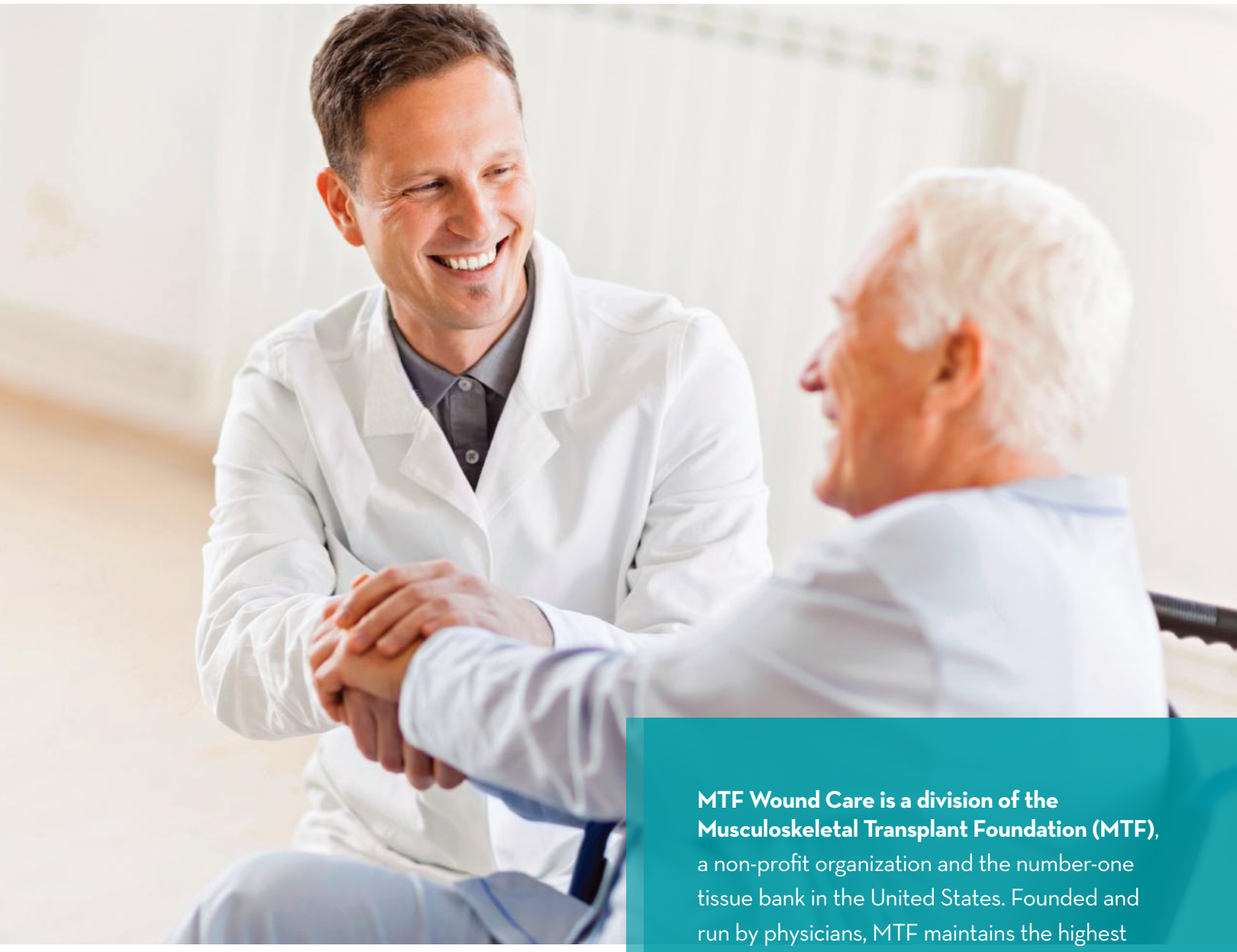

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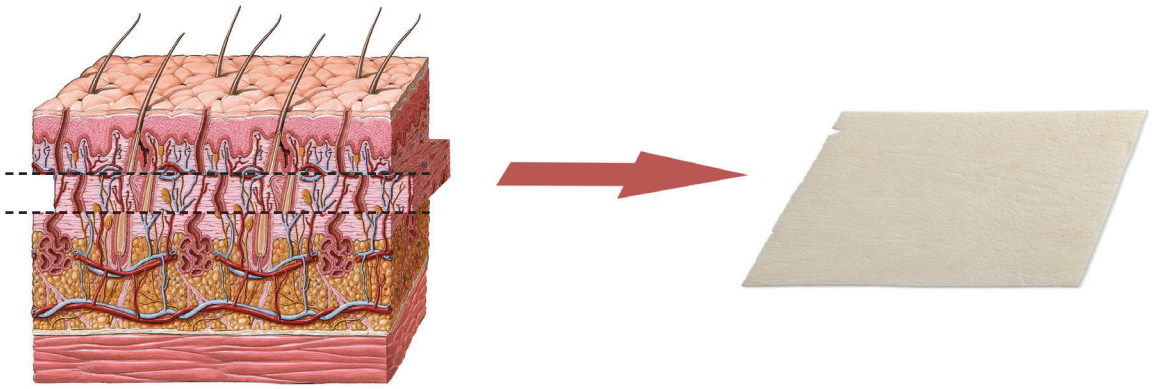

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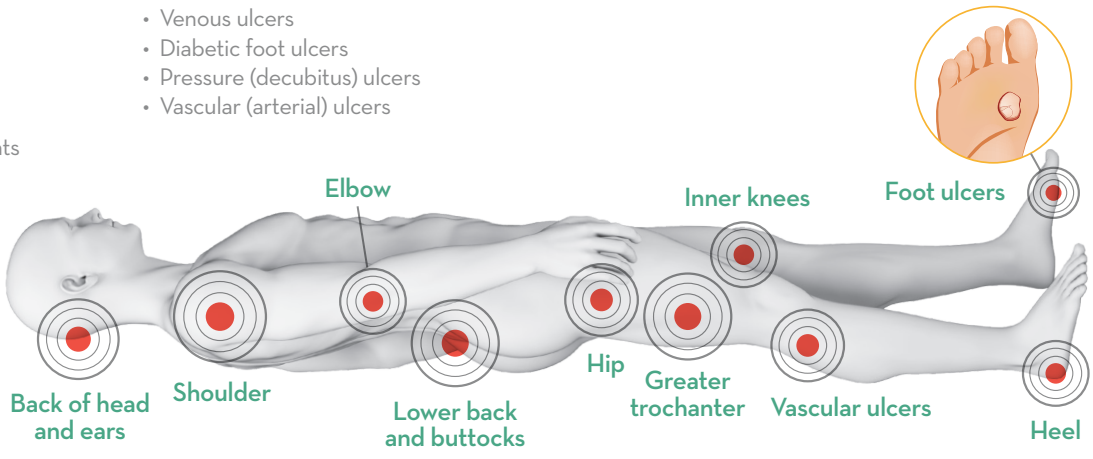

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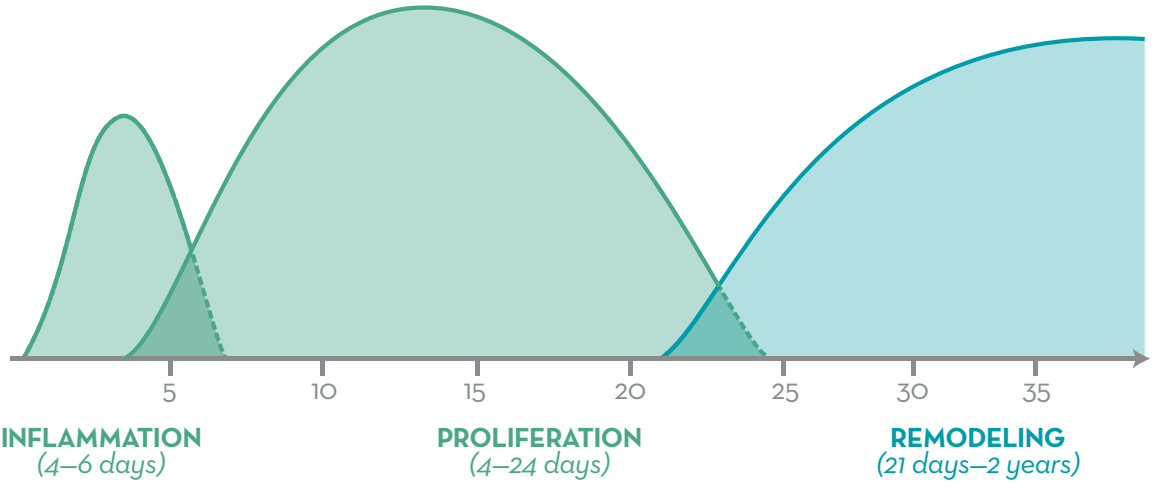
Normal wound healing consists of three consecutive and overlapping phases:[1,2,3]



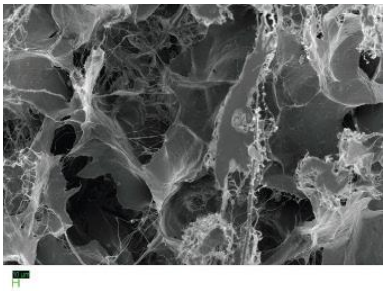
AlloPatch Pliable contains key biological components known in literature to facilitate in these phases and support long-term remodeling

ALLOPATCH PLIABLE'S OPEN ARCHITECTURE...

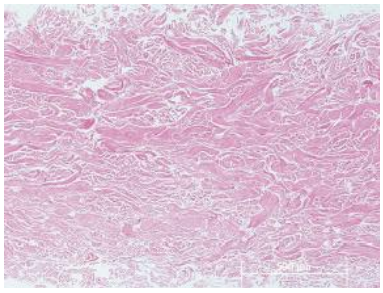
means quicker incorporation and vascularization[4] in the inflammation and proliferation phase, where host cells can easily adhere to both sides.

To continue to support the proliferation phase, AlloPatch Pliable offers 5X greater cell attachment on the epidermal side; 2X for dermal compared to competitive ADMs. **Greater cell attachment = faster tissue incorporation.[4]**

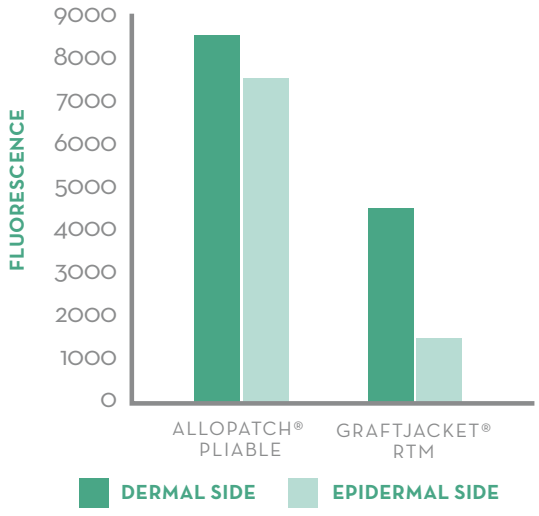
Open collagen architecture



Uniform structure



Greater Fibroblast Attachment



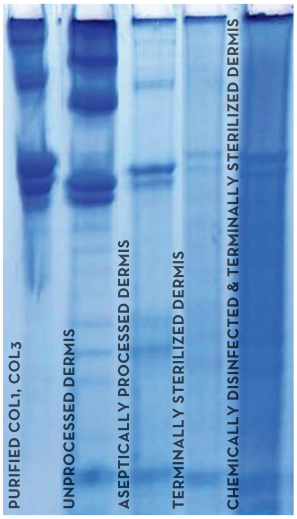
5X greater cell attachment on epidermal side
2X greater attachment on dermal side
Cell Attachment = faster tissue incorporation[4]

GraftJacket® is a registered trademark of Wright Medical Technology

ALLOPATCH PLIABLE'S ASEPTIC PROCESSING...

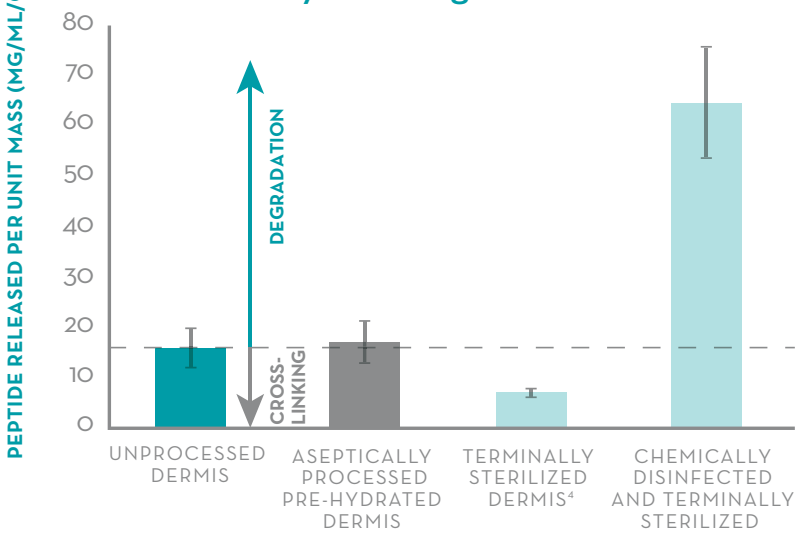
preserves the natural tissue scaffold and the integrity of the tissue in the remodeling phase. This means the graft is more stable and supports long-term remodeling at similar rates as the unprocessed, native tissue.

SDS-PAGE gel electrophoresis



In aseptically processed dermis, protein integrity is preserved.

Enzymatic Degradation



Competing products, which are processed with harsh chemicals and terminal sterilization, may experience severe alterations to the native matrix.

VERSATILE AND EASY TO USE.

Ready, right out of the package. With AlloPatch Pliable, there's no rehydration needed. It comes pre-hydrated right in the package, so it's ready when you are, with no lost OR time.

Available in multiple sizes to optimally match wound size, with little or no waste.

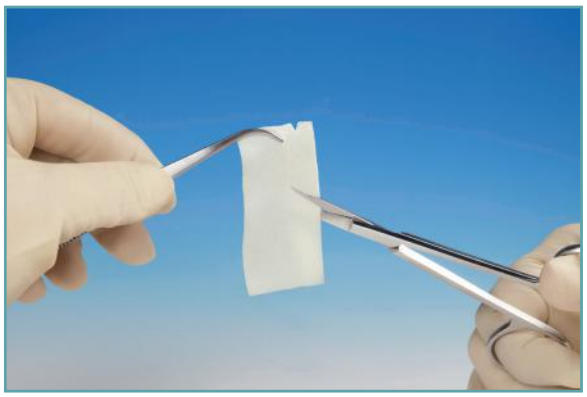
Stores easily. No freezing or refrigeration needed. AlloPatch Pliable needs only ambient storage and lasts up to three years on the shelf.

Flexible, thin sheet conforms to anatomy and maintains surface area contact.

ORDERING INFORMATION AND SERVICE

Size	Total square cm	Thickness	Quantity	Order number
1.5cm x 1.5cm	2.25	0.4mm–1.0mm	1 each	WC1515
2cm x 2cm	4	0.4mm–1.0mm	1 each	WC0202
4cm x 4cm	16	0.4mm–1.0mm	1 each	WC0404
4cm x 8cm	32	0.4mm–1.0mm	1 each	WC0418

HCPCS code	Description
Q4128	AlloPatch HD, per sq cm



MTF REIMBURSEMENT SUPPORT

The Pinnacle Health Group, Inc.
MTF@thepinnaclehealthgroup.com
1-866-369-9290

MTF CUSTOMER SERVICE

MTFOP@mtf.org
1-800-433-6576

MTF WOUND CARE

MTF Wound Care ▪ 125 May Street, Edison NJ 08837 ▪ (800) 946-9008 ▪ +1 (732) 661-0202 ▪ mtfwoundcare.org

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Mktg-1093 Rev. 1

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*Data on file at MTF

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

ESTABLISHMENT REGISTRATION AND LISTING FOR HUMAN CELLS, TISSUES, AND CELLULAR AND TISSUE-BASED PRODUCTS (HCT/Ps)

(See reverse side for instructions)

1. REGISTRATION NUMBER
(FDA Establishment Identifier)

FEI: 3001236616

2. REASON FOR SUBMISSION

a. ☐ INITIAL REGISTRATION / LISTING
b. ☐ ANNUAL REGISTRATION / LISTING
c. ☒ CHANGE IN INFORMATION
d. ☐ INACTIVE

VALIDATION--FOR FDA USE ONLY 1
VALIDATED BY FDA:14-APR-2015
DISTRICT: New Jersey
PRINTED BY FDA:15-JUL-2015

PART I - ESTABLISHMENT INFORMATION

3. OTHER FDA REGISTRATIONS

a. BLOOD FDA 2830 NO. _____

b. DEVICES FDA 2891 NO. FEI: 0002249062

c. DRUG FDA 2656 NO. _____

4. PHYSICAL LOCATION (Include legal name, number and street, city, state, country, and post office code)

Musculoskeletal Transplant Foundation

Edison Corporate Center
125 May St. Suite 300
Edison, New Jersey 08837

a. PHONE 732-661-0202 EXT

b. ☐ SATELLITE RECOVERY ESTABLISHMENT
(MANUFACTURING ESTABLISHMENT FEI NO._____

c. ☐ TESTING FOR MICRO-ORGANISMS ONLY

5. ENTER CORRECTIONS TO ITEM 4

6. MAILING ADDRESS OF REPORTING OFFICIAL (Include institution name if applicable, number and street, city, state, country, and post office code)

Musculoskeletal Transplant Foundation
Attn: Joel Osborne
Edison Corporate Center
125 May St. Suite 300
Edison, New Jersey 08837

a. PHONE 732-661-0202 EXT

7. ENTER CORRECTIONS TO ITEM 6 b. PHONE

8. U.S. AGENT

a. E-MAIL

9. REPORTING OFFICIAL'S SIGNATURE

a. TYPED NAME Joel C. Osborne
b. E-MAIL ra_licenses@mtf.org
c. TITLE Vice President, RA d. DATE 13-APR-2015

PART II - PRODUCT INFORMATION

10. ESTABLISHMENT FUNCTIONS AND TYPES OF HCT / Ps

Types of HCT / Ps	Establishment Functions: Recover	Screen	Test	Package	Process	Store	Label	Distribute	11. HCT/Ps DESCRIBED IN 21 CFR 1271.10	12. HCT/Ps REGULATED AS MEDICAL DEVICES	13. HCT/Ps REGULATED AS DRUGS OR BIOLOGICAL DRUGS	14. PROPRIETARY NAME(S)
a. Bone		X		X	X	X	X	X	X	X		*** See full text on next page
b. Cartilage		X		X	X	X	X	X	X			
c. Cornea												
d. Dura Mater												
e. Embryo ☐ SIP ☐ Directed ☐ Anonymous												
f. Fascia		X		X	X	X	X	X	X			
g. Heart Valve												
h. Ligament		X		X	X	X	X	X	X			
i. Oocyte ☐ SIP ☐ Directed ☐ Anonymous												
j. Pericardium		X		X	X	X	X	X	X			
k. Peripheral Blood Stem ☐ Autologous ☐ Family Related ☐ Allogeneic												
l. Sclera												
m. Semen ☐ SIP ☐ Directed ☐ Anonymous												
n. Skin		X		X	X	X	X	X	X			*** See full text on next page
o. Somatic Cell Therapy Products ☐ Autologous ☐ Family Related ☐ Allogeneic												
p. Tendon		X		X	X	X	X	X	X			
q. Umbilical Cord Blood ☐ Autologous ☐ Family Related ☐ Allogeneic												
r. Vascular Graft												
s. Amniotic Membrane		X		X	X	X	X	X	X			*** See full text on next page
t. Adipose Tissue		X		X	X	X	X	X	X			
u.												
v.												

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

ESTABLISHMENT REGISTRATION AND LISTING FOR HUMAN CELLS, TISSUES, AND CELLULAR AND TISSUE-BASED PRODUCTS (HCT/Ps)
(See reverse side for instructions)

1. REGISTRATION NUMBER
(FDA Establishment Identifier)

FEI: 3001236616

ADDITIONAL INFORMATION:

Proprietary Names:

A. Bone: DBX Mix, DBX Paste, DBX Putty, Conform Sheet, Luminary CC-ALIP, Luminary T-PLIF, Luminary PLIF, ARCH ODL, VerteFill, Conform Flex, ENACT

N. Skin: FlexHD Structural Plus, FlexHD Pliable Perforated,FlexHD Structural, AlloPatch Pliable

Proprietary Name(s):

a. Bone	Conform Putty, Conform Cube, Trinity Evolution, Trinity ELITE, DBX, DBX Strip, DBX Inject, AFT, Allofix, Dental DBX
n. Skin	DermaMatrix, FlexHD, FlexHD Diamond, Allopatch HD, BellaDerm, PerioDerm, FlexHD Pliable
Amniotic Membrane	AmnioClear, VersaShield, Revitalon, AmnioBand, Enhance

PATIENT RECORD

Tissue recipient records must be maintained by the consignee and transplant facility for the purpose of tracing tissue post transplantation. A TissueTrace® Tracking Form and peel-off stickers have been included with each package of tissue. Please record the patient ID, name and address of the transplant facility, allograft tissue information (using the peel-off stickers) and comments regarding the use of the tissue on the TissueTrace Tracking Form. Alternatively a system for electronic submission may be used and sent to MTFTTC@ScerIS.com. ***Within the United States*:** Once completed, the bottom page of the form should be returned to MTF using the self-addressed mailer. Copies of this information should be retained by the transplant facility for future reference. ***Outside of the United States*:** Once completed, the bottom page of the form should be returned to the local allograft representative or provider. Copies of this information should be retained by the hospital for future reference.

Reference: Current MTF policies and procedures are in compliance with current FDA, AATB and other regulatory requirements.

Definitions of Label Symbols

 **See IFU**

 **Do Not Reuse**

Processed By:

125 May Street
Edison, NJ 08837
USA

Within the United States: 1.800.433.6576
Outside of the United States: +1.732.661.0202

All recovery, processing and distribution costs were paid for by MTF, a non-profit organization.

CAUTION: Restricted to use by a physician, dentist and/or podiatrist.

MTF tissue forms and products are protected by one or more issued or licensed United States patents. A list of patents on available tissues and related technologies may be found on the MTF web site www.mtf.org.
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CTO: 100024

PI –112 Rev1, 01/2015
RM –2065

READ BEFORE USING

AlloPatch™ Pliable
Allograft Dermal Matrix

DONATED HUMAN TISSUE

CAUTION: TISSUE IS FOR SINGLE PATIENT USE ONLY.
Aseptically Processed. Passes USP <71> Sterility Tests.
Not Terminally Sterilized. Do Not Sterilize.

THIS TISSUE WAS RECOVERED FROM A DECEASED DONOR FROM WHOM LEGAL AUTHORIZATION OR CONSENT HAS BEEN OBTAINED. THIS RECOVERY WAS PERFORMED USING ASEPTIC TECHNIQUES. PROCESSING AND PACKAGING WERE PERFORMED UNDER ASEPTIC CONDITIONS. TERMINAL STERILIZATION AGENTS WERE NOT USED IN THE PROCESS.

DESCRIPTION

AlloPatch Pliable is donated human allograft dermis minimally processed to remove dermal cells and is packaged in an ethanol solution. AlloPatch Pliable tissue is processed from deep cut tissue from which the epidermal layer has been physically removed. The process utilized preserves the extracellular matrix of the dermis. The resulting allograft serves as a framework to support cellular repopulation and vascularization at the surgical site.

INDICATIONS FOR USE

AlloPatch Pliable is processed to remove cells while maintaining the integrity of the matrix with the intent to address the issues of the specific and nonspecific inflammatory responses. AlloPatch Pliable is used as a wound care scaffold for the replacement of damaged or inadequate integumental tissue such as diabetic foot ulcers, venous leg ulcers, pressure ulcers, or for other homologous use.

ADVERSE EFFECTS

Possible adverse effects of using human skin include but are not limited to:

- Local or systemic infection
- Dehiscence and/or necrosis due to poor revascularization
- Specific or nonspecific immune response to the graft

***Within the United States*: Adverse outcomes attributable to the tissue must be promptly reported to MTF. *Outside of the United States*: Adverse outcomes attributable to the tissue must be promptly reported to your local representative.**

PRECAUTIONS

Conditions that could potentially inhibit integration of AlloPatch Pliable include, but are not limited to:

- Fever
- Uncontrolled diabetes
- Pregnancy
- Low vascularity of the surrounding tissue
- Local or systemic infection
- Mechanical trauma
- Poor nutrition or poor general medical condition
- Dehiscence and/or necrosis due to poor revascularization
- Inability to cooperate with and/or comprehend post-operative instructions
- Infected or nonvascular surgical sites

CAUTIONS

Do not sterilize. Do not freeze. No known sensitizing agents are present in this tissue. AlloPatch Pliable is packaged in an ethanol solution and must be rinsed in a sterile solution prior to implantation. Care should be taken when using AlloPatch Pliable in conjunction with electrical equipment. NOTE: No β-lactam antibiotics are used during the processing of tissue in AlloPatch Pliable.

Extensive medical screening procedures have been used in the selection of all tissue donors for MTF (please see Donor Screening and Testing). Transmission of infectious diseases such as HIV or hepatitis, as well as a theoretical risk of the Creutzfeldt-Jakob (CJD) agent, may occur in spite of careful donor selection and serological testing.

ALLOGRAFT INFORMATION

AlloPatch Pliable is composed of an acellular dermal matrix. During tissue processing and packaging, this allograft was tested and showed no evidence of microbial growth, complying with the requirements of USP <71> Sterility Tests. **Do not subject allograft to additional sterilization procedures.**

Dispose of excess or unused tissue and all packaging that has been in contact with the tissue in accordance with recognized procedures for discarding regulated medical waste materials.

INSTRUCTIONS FOR USE

Standard accepted operative practices should be followed. AlloPatch Pliable is packaged in a sterilized foil pouch that is designed to be passed directly into the sterile field.

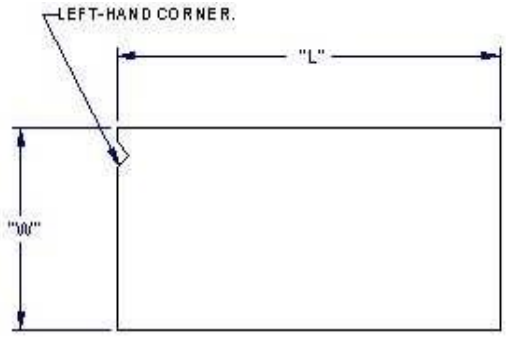
1. Peel back the outer Tyvek Package and pass the inner foil pouch to the sterile field.
2. Remove AlloPatch Pliable from the inner-foil pouch using sterile gloves/forceps and immediately rinse in a sterile solution prior to implantation.
3. Once the tissue has been removed from the inner pouch, discard the pouch and packaging solution outside of the sterile field and away from electrosurgical equipment.
4. AlloPatch Pliable may be aseptically trimmed to fit the dimensions of the application site. The tissue can be shaped with scissors or scalpel. At this point, the AlloPatch Pliable is ready for application in the surgical site.

Note: Ensure the wound site has been debrided and prepared prior to graft placement.

Orientation

In order to discern the dermal side from the epidermal side, note that in most instances the epidermal side may have more pigmentation than the dermal side. For further verification, add a drop of blood to both sides of the graft and rinse with sterile saline. The dermal side will appear red and the epidermal side will appear pink.

To ensure proper orientation of AlloPatch Pliable, position it so that the indicating notch is in the upper left-hand side of the tissue, facing left. This will assure that the epidermal side is facing up.



DONOR SCREENING & TESTING

Prior to donation, the donor's medical/social history is screened for medical conditions or disease processes that would contraindicate the donation of tissues in accordance with current policies and procedures approved by the MTF Medical Board of Trustees.

Donor blood samples taken at the time of recovery were tested by a facility that is CLIA certified and registered with the FDA. The donor blood samples were tested for:

- Hepatitis B surface antigen
- Hepatitis B core antibody
- Hepatitis C antibody
- HIV-1/2 antibody
- Syphilis
- HIV -1 (NAT)
- HCV (NAT)

All infectious disease tests were negative. This allograft tissue has been determined to be suitable for transplantation.

The infectious disease test results, consent, current donor medical history interview, physical assessment, available relevant medical records to include previous medical history, laboratory test results, autopsy and coroner reports, if performed, and information obtained from any source or records which may pertain to donor suitability, have been evaluated by an MTF physician and are sufficient to indicate that donor suitability criteria current at the time of procurement, have been met. This tissue is suitable for transplantation. The donor suitability criteria used to screen this donor are in compliance with the FDA regulations published in 21 CFR Part 1271 Human Cells, Tissues, and Cellular and Tissue Based Products, as applicable. All procedures for donor screening, serologic and microbiologic testing meet or exceed current standards established by the American Association of Tissue Banks.

PACKAGING & LABELING

AlloPatch Pliable is aseptically packaged in a sterilized hermetically sealed foil pouch. The foil pouch containing AlloPatch Pliable is inside a sealed sterilized Tyvek pouch. The Tyvek pouch is sealed, labeled and then placed inside an envelope. This allograft must not be used under any of the following circumstances:

- If the container seal is damaged or not intact or has any physical damage;
- If the container label or identifying bar code is severely damaged, not legible or is missing; or
- If the expiration date shown on the container label has passed.

Once a container seal has been compromised, the tissue shall be either transplanted, if appropriate, or otherwise discarded.

STORAGE

AlloPatch Pliable should be stored at ambient temperature. No refrigeration or freezing is required. It is the responsibility of the transplant facility or clinician to maintain the tissue intended for transplantation in the appropriate recommended storage conditions prior to transplant.