

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

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 compared 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 compared 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

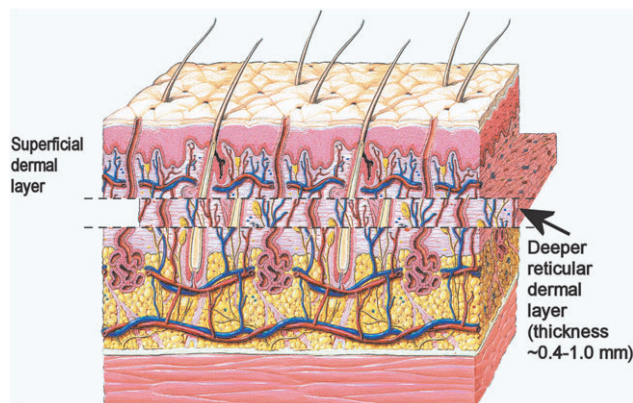


Figure 1 Comparison of superficial and deep reticular cut dermal graft layers.

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.

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

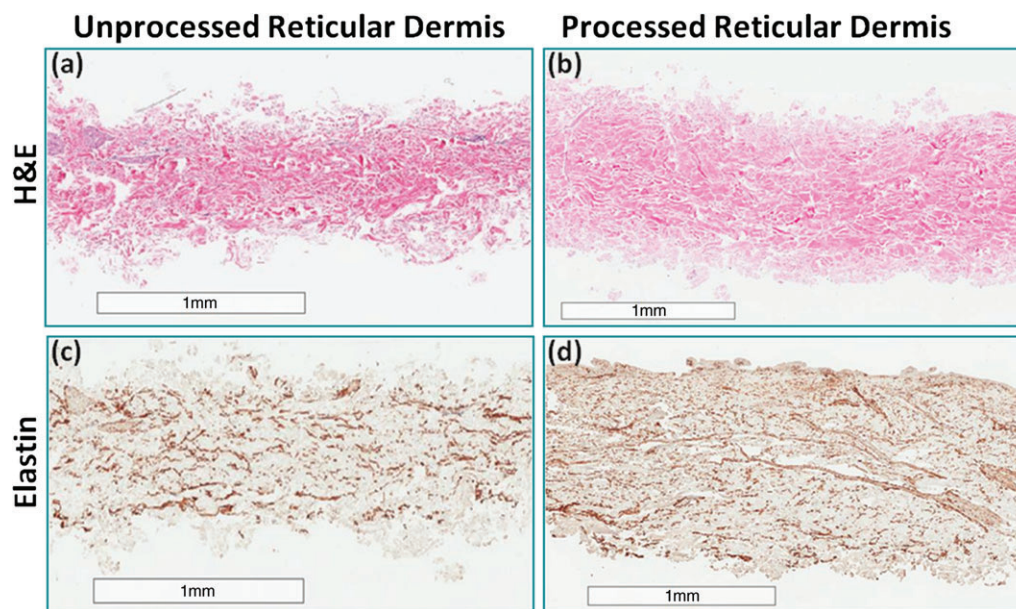


Figure 2 Aseptic processing preserves inherent architecture of the tissue and the key matrix protein, elastin, similar to unprocessed reticular dermis (magnification 2X). Key: (a) unprocessed reticular dermis with H&E stain; (b) processed reticular dermis with H&E stain; (c) unprocessed reticular dermis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

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 cm². • 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 than 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 cm² • 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

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 <0.05 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 (4.7 cm²) compared with the SOC group (2.7 cm²) (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*=0.00028) (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*=0.00036 (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*=0.00036) (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*=0.00014).

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).

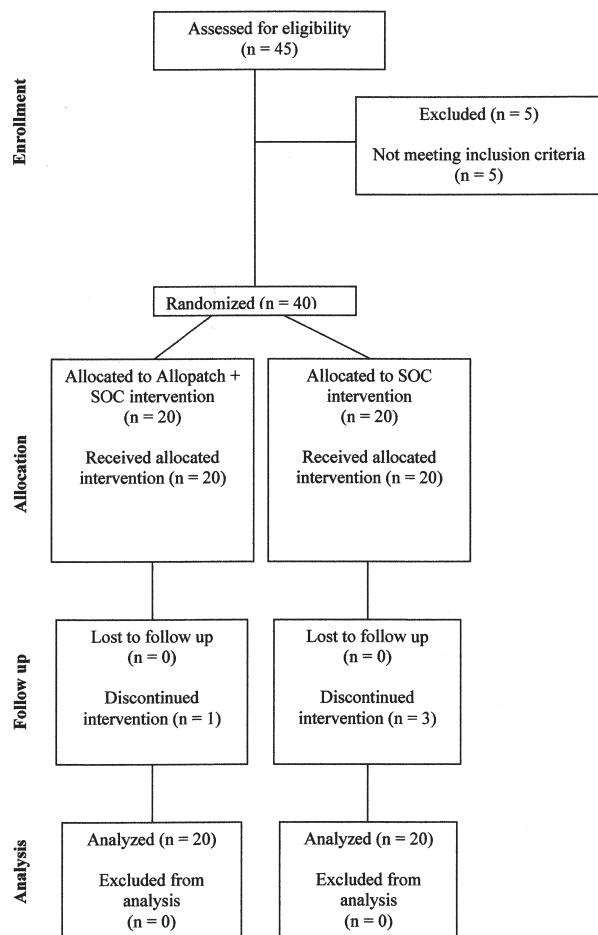


Figure 3 Flow chart of trial participants.

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

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.

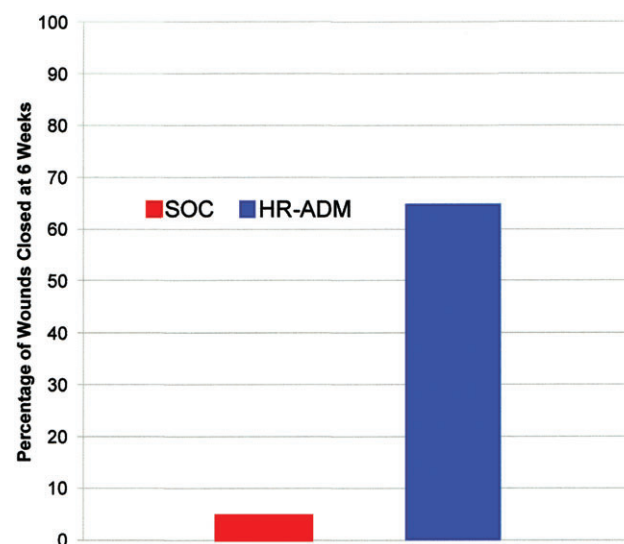


Figure 4 Bar graph showing complete wound healing at 6 weeks between the HR-ADM and SOC group versus the SOC group.

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

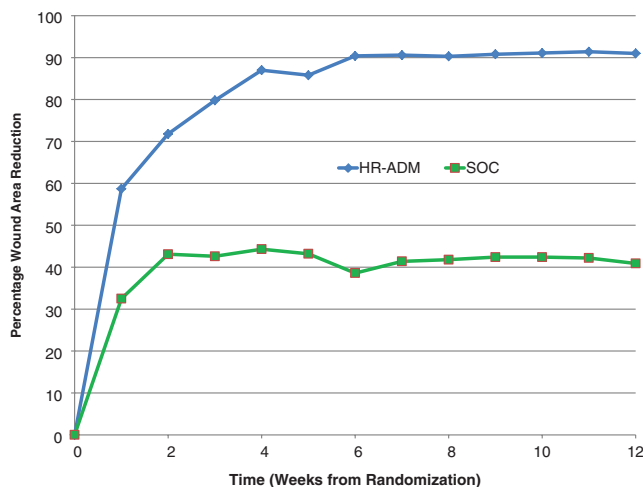


Figure 5 Percentage of wound area reduction by week and treatment group.

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

Covariate	P*	HR	95% CI	
			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 cm².

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 ($P=0.00028$). 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 ($P=0.00036$). 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 ($P=0.0003$) (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,

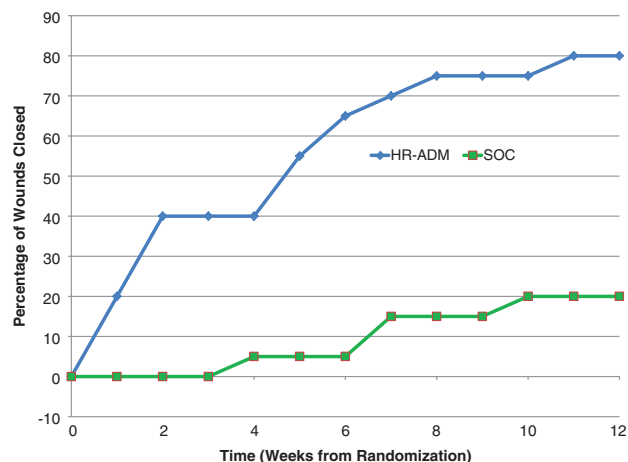


Figure 6 Percentage of wounds closed by week and treatment group.

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 ($P=0.006$). 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 ($P=0.029$). There was also a statistically significant difference between groups in time to heal within 12 weeks ($P=0.023$) 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

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 than 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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REFERENCES

- Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and trends in diabetes among adults in the United States, 1988-2012. *JAMA* 2015;**314**:1021-9.
- Herman WH, Rothberg AE. Prevalence of diabetes in the United States: a glimmer of hope? *JAMA* 2015;**314**:1005-7.
- Margolis DJ, Malay DS, Hoffstad OJ, Leonard CE, MaCurdy T, de Nava KL, Tan Y, Molina T, Siegel KL. *Incidence of diabetic foot ulcer and lower extremity amputation among medicare beneficiaries, 2006 to 2008: Data Points #2*. Data Points Publication Series [Internet]. Rockville: Agency for Healthcare Research and Quality (US), 2011.
- Margolis DJ, Kantor J, Berlin JA. Healing of diabetic neuropathic foot ulcers receiving standard treatment. *Diabetes Care* 1999;**22**:692-5.
- Margolis DJ, Gelfand JM, Hoffstad O, Berlin JA. Surrogate end points for the treatment of diabetic neuropathic foot ulcers. *Diabetes Care* 2003;**26**:1696-700.
- Sheehan P, Jones P, Caselli A, Giurini JM, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Diabetes Care* 2003;**26**:1879-82.
- Snyder RJ, Cardinal M, Dauphinée DM, Stavosky J. A post-hoc analysis of reduction in diabetic foot ulcer size at 4 weeks as a predictor of healing by 12 weeks. *Ostomy Wound Manage* 2010;**56**:44-50.
- Lavery LA, Barnes SA, Keith MS, Seaman JW, Armstrong DG. Prediction of healing for postoperative diabetic foot wounds based on early wound area progression. *Diabetes Care* 2008;**31**:26-9.
- Valenzuela-Silva CM, Tuero-Iglesias ÁD, García-Iglesias E, González-Díaz O, Del Río-Martín A, Yera Alos IB, Fernández-Montequín JJ, López-Saura PA. Granulation response and partial wound closure predict healing in clinical trials on advanced diabetes foot ulcers treated with recombinant human epidermal growth factor. *Diabetes Care* 2013;**36**:210-5.
- Harding K, Kirsner R, Lee D, Mulder G, Serena T. *An expert working group review. International consensus: acellular matrices for the treatment of wounds*. London: Wounds International, 2010.
- Irion G. *Comprehensive wound management, 2nd edn*. Thorofare: Slack, Inc., 2010:3-14.
- Mast BA. The skin. In: Cohen IK, Diegelmann RF, Lindblad WJ, editors. *Wound healing: biochemical and clinical aspects*. WB Saunders: Philadelphia, 1992:344-55.
- Wilgus TA. Regenerative healing in fetal skin: a review of the literature. *Ostomy Wound Manage* 2007;**53**:16-31; quiz 32-3.
- Dunkin CS, Pleat JM, Gillespie PH, Tyler MP, Roberts AH, McGrouther DA. Scarring occurs at a critical depth of skin injury: precise measurement in a graduated dermal scratch in human volunteers. *Plast Reconstr Surg* 2007;**119**:1722-32; discussion 1733-4.
- Rolfe KJ, Grobelaar AO. A review of fetal scarless healing. *ISRN Dermatol* 2012;**2012**:698034.
- Cuono C, Langdon R, McGuire J. Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. *Lancet* 1986;**1**:1123-4.
- Munster AM, Smith-Meek M, Shalom A. Acellular allograft dermal matrix: immediate or delayed epidermal coverage? *Burns* 2001;**27**:150-3.
- Woo KY, Sibbald RG. A cross-sectional validation study of using NERDS and STONEES to assess bacterial burden. *Ostomy Wound Manage* 2009;**55**:40-8.
- Wendelken ME, Berg WT, Lichtenstein P, Markowitz L, Comfort C, Alvarez OM. Wounds measured from digital photographs using photodigital planimetry software: validation and rater reliability. *Wounds* 2011;**23**:267-75.
- Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, Elbourne D, Egger M, Altman DG. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *J Clin Epidemiol* 2010;**340**:e1-37.
- Falanga V, Sabolinski M. A bilayered living skin construct (APLIGRAF) accelerates complete closure of hard-to-heal venous ulcers. *Wound Repair Regen* 1999;**7**:201-7.
- Veves A, Falanga V, Armstrong DG, Sabolinski ML. Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. *Diabetes Care* 2001;**24**:290-5.
- Marston WA, Hanft J, Norwood P, Pollak R. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. *Diabetes Care* 2003;**26**:1701-5.
- Reyzelman A, Crews RT, Moore JC, Moore L, Mukker JS, Offutt S, Tallis A, Turner WB, Vayser D, Winters C, Armstrong DG. Clinical effectiveness of an acellular dermal regenerative tissue matrix compared to standard wound management in healing diabetic foot ulcers: a prospective, randomised, multicentre study. *Int Wound J* 2009;**6**:196-208.
- Zelen CM, Gould L, Serena TE, Carter MJ, Keller J, Li WW. A prospective, randomised, controlled, multi-centre comparative effectiveness study of healing using dehydrated human amnion/chorion membrane allograft, bioengineered skin substitute or standard of care for treatment of chronic lower extremity diabetic ulcers. *Int Wound J* 2015;**12**:724-32.
- Zelen CM, Serena TE, Gould L, Le L, Carter MJ, Keller J, Li WW. Treatment of chronic diabetic lower extremity ulcers with advanced therapies: a prospective, randomised, controlled, multi-centre comparative study examining clinical efficacy and cost. *Int Wound J* 2016;**13**:272-82.

27. Ruszczak Z. Effect of collagen matrices on dermal wound healing. *Adv Drug Deliv Rev* 2003;**55**:1595–611.
28. Deakin NO, Turner CE. Paxillin inhibits HDAC6 to regulate microtubule acetylation, Golgi structure, and polarized migration. *J Cell Biol* 2014;**206**:395–413.
29. Kirsner RS, Bohn G, Driver VR, Mills JL Sr, Nanney LB, Williams ML, Wu SC. Human acellular dermal wound matrix: evidence and experience. *Int Wound J* 2015;**12**:646–54.
30. Brigido SA, Boc SF, Lopez RC. Effective management of major lower extremity wounds using an acellular regenerative tissue matrix: a pilot study. *Orthopedics* 2004;**27**(1 Suppl):s145–9.
31. Brigido SA. The use of an acellular dermal regenerative tissue matrix in the treatment of lower extremity wounds: a prospective 16-week pilot study. *Int Wound J* 2006;**3**:181–7.