Single-stage application of a novel decellularized dermis for treatment-resistant lower limb ulcers: Positive outcomes assessed by SIAscopy, laser perfusion, and 3D imaging, with sequential timed histological analysis

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Manuscript received: February 28, 2013
Accepted in final form: August 17, 2013

DOI:10.1111/wrr.12113

ABSTRACT

We present results of an original clinical study investigating efficacy of a decellularized dermal skin substitute (DCD) as part of a one-stage therapeutic strategy for recalcitrant leg ulcers. Twenty patients with treatment-resistant ulcers underwent hydrosurgical debridement, after which DCD was applied and covered with negative pressure dressings for 1 week. Participants were reviewed on seven occasions over 6 months. 3D photography, full-field laser perfusion imaging, spectrophotometric intracutaneous analysis, and sequential biopsies were used to monitor healing. Mean ulcer duration and surface area prior to DCD placement were 4.76 years (range 0.25–40 years) and 13.11 cm^2 (range 1.06–40.75 cm^2), respectively. Seventy percent of ulcers were venous. Surface area decreased in all patients after treatment (range 23–100%). Mean reduction was 87% after 6 months, and 60% of patients healed completely. Wound bed hemoglobin flux increased significantly 6 weeks after treatment ($p = 0.005$). Histological and immunohistochemical analysis confirmed progressive DCD integration with colonization by host fibroblasts, lymphocytes, and neutrophils, resulting in fibroplasia, reepithelialisation, and angiogenesis, with correlating raised CD31, collagen I, and collagen III levels. Subgroup analysis showed differing cellular behavior depending on wound duration, with delayed angiogenesis, reduced collagen deposition, and smaller reductions in surface area in ulcers present for over 1 year. The stain intensities of immunohistochemical markers including fibronectin, collagen, and CD31 differed depending on depth from the wound surface and presence of intact epithelium. DCD safely produced significant improvement in treatment-resistant leg ulcers. With no requirement for hospital admission, anesthetic, or autogenic skin grafting, this treatment could be administered in hospital and community settings.

INTRODUCTION

Chronic wounds are defined as a break in skin epithelial continuity persisting for longer than 42 days or of frequent recurrence.[1] They display disruption of normal healing processes, resulting in a state of pathological inflammation.[2] Over 90% of such wounds in the lower limb are secondary to venous ulcers, pressure sores, or diabetic ulcers, which collectively consume 1% of the total United Kingdom annual healthcare budget.[3–5] They are common, with the prevalence of active leg ulceration in the UK estimated at 1.5/1000, and approximately 1 in 100 people will suffer during their lifetime. Leg ulceration is a chronic recurring condition, with approximately 50% of patients reporting repeated episodes.[6] With an aging population and increasing prevalence of diabetes, obesity, and atherosclerosis, the incidence and associated cost of leg ulcers are likely to increase in the future.

The most effective current treatment strategies, such as compression bandaging, are expensive, labor-intensive, and slow to show results.[6–8] Sabolinski reported that standard compression therapy healed 20–40% of venous ulcers, while a 2012 Cochrane review found success rates of 30–75%.[9,10] Lees and Cornwall found that 25–50% of venous ulcers persist for longer than 1 year.[6,8] Consequently, there is a need for new, more effective therapies. The past three decades have seen the development and rapid evolution of bioengineered skin substitutes. These materials mimic native skin in terms of structure, consisting of extracellular matrix (ECM) materials, alone (decellular [also known as acellular] products) or in combination with cell types such as fibroblasts and keratinocytes (cellular products).[11] Skin substitutes function as sterile tissue grafts that are applied directly to a prepared wound bed and integrate with surrounding native tissues to actively stimulate cell migration, angiogenesis, and

epithelialization, resulting in accelerated wound healing.[12] Evidence is now emerging for their use in diabetic and venous ulcer management. Indeed, several previous studies showed significantly improved diabetic ulcer healing rates using acellular skin substitutes compared with controls.[13–16] Likewise, a 2010 Cochrane review concluded that cellular skin substitutes used in conjunction with compression bandaging increased venous ulcer healing compared with simple dressings plus compression.[17]

National Health Service Blood and Transplant (NHSBT) decellularized dermis (DCD) is a new form of acellular dermal skin substitute produced from split-thickness skin grafts harvested from cadaveric tissue donors. All epidermal and cellular components from the dermis are removed by NHSBT in a patented sequential process based on a technique developed by Hogg et al.[18] We report results of a prospective 6-month pilot study to assess the safety and efficacy of NHSBT DCD as part of a treatment strategy for chronic treatment-resistant leg ulcers. This unique approach, performed in an outpatient setting, involved hydrosurgical wound debridement, DCD application, and topical negative pressure (TNP) therapy. Unlike other similar studies, treatment of recruited patients did not require general anesthesia, admission to hospital, or significant analgesia. Validated clinical wound measurements were combined with assessment of histological tissue markers and physiological parameters. The combination and correlation of basic scientific findings with clinical outcomes from noninvasive imaging techniques, and biopsy sampling enabled effective assessment of this treatment stratagem.

MATERIALS AND METHODS

Twenty-two patients enrolled in a 6-month pilot study organized and run by a tertiary referral vascular center with dedicated regional leg ulcer services. Approval was granted by the National Research Ethics Service (NRES Committee North West—Greater Manchester South, reference number 10/H1003/99) and the local research and development department (University Hospitals of South Manchester NHS Foundation Trust, reference number 2010BP001). Patients with treatment-resistant ulcers assessed by specialist hospital or community wound services were approached in approved clinics. Treatment resistance was defined as minimal or absent response to appropriate treatment for more than 3 months. Those satisfying inclusion and exclusion criteria (Supporting Information Table S1) underwent arterial duplex scanning of the affected lower limb to confirm appropriate ankle brachial pressure indices (ABPI). Suitable patients gave written consent and were enrolled. Only one ulcer per patient was treated using DCD. All procedures and assessments were performed in outpatient treatment rooms.

Eleven patients of each sex were recruited, with a mean age of 69 years (range 45–92). All were white Caucasian. Sixteen patients had pure venous ulcers, 3 had diabetic ulcers, and 3 had ulcers of mixed etiology, located in various lower limb sites (Supporting Information Table S2). The duration of ulcers prior to DCD treatment varied from 3 months to 40 years with mean duration of 4.76 years.

On day 0, patients were investigated using noninvasive wound imaging techniques including 2D and 3D photography (Eykona Technologies, Oxford, United Kingdom), full-field laser perfusion imaging (FLPI) (Moor Instruments, Axminster, United Kingdom), and spectrophotometric intracutaneous analysis (SIAscopy) (Phycon Medical Inc., Miami, FL).

FLPI provides noninvasive measurement of blood flow (hemoglobin flux) in skin microcirculation and indirect assessment of angiogenesis after DCD treatment. Tissue thickness sampled is typically 1 mm, and capillary diameters of up to 10 μm with flow rates of 0.01–10 mm/second can be detected.[19] SIAscopy enables in vivo quantification of cutaneous oxyhemoglobin, eumelanin, and dermal collagen concentration using a handheld scanner.[20] SIAscopy readings were taken from the center points of ulcers at each assessment.

After noninvasive assessments, a 5-mm full-thickness skin biopsy was taken from the wound margin under local anesthetic before the ulcer was debrided using the Versajet hydrosurgery system (Smith & Nephew, Hull, United Kingdom). The presence of punctuate bleeding confirmed successful debridement and adequate wound bed preparation before DCD placement. A 5×5 cm sheet of DCD was cut to the dimensions of the wound using standard sterile instruments, applied, and fixed in place using liquid skin adhesive (Dermabond; Ethicon, San Lorenzo, Puerto Rico) around the ulcer margin. Once dry, the PICO negative pressure wound system (Smith & Nephew) was applied (Figure 1). It consists of a small portable pump connected via tubing to a dressing overlying the wound, which exerts 80 mmHg pressure across the wound bed for 7 days. Patients were sent home after treatment with a 1-week supply of oral antibiotics recommended by a microbiologist. Patients tolerated the application process well, requiring minimal analgesia. DCD had excellent handling characteristics and provided complete wound coverage in all but three cases, where ulcer area was greater than 25 cm^2. There was macroscopic evidence of DCD incorporation into the wound bed after 7 days of TNP therapy, with full integration noted after 2 weeks (Figure S1).

Patients were followed up 1 week, 2 weeks, 3 weeks, 4.5 weeks, 6 weeks, 4 months, and 6 months after DCD application (Table 1). At each visit, the aforementioned noninvasive imaging modalities were used alongside clinical assessment to gauge wound healing. Measurements of wound surface area and volume were taken prior to biopsy harvesting. Further 5-mm full-thickness skin biopsies were taken at week 3 and week 6 appointments from designated parts of the wound margin. Wound swabs for culture and sensitivities were sent on day 0, week 3, and week 6, as well as any point where wound infection was suspected. TNP dressings were removed after 1 week, and patients returned to preexisting graduated compression bandaging systems or dressings alone as directed by the referring leg ulcer services.

The primary outcome measure was reduction in wound surface area seen after treatment with DCD. Secondary measures included temporal changes in hemoglobin flux, wound volume, concentration of specific dermal proteins, and alterations in cutaneous architecture and morphology.

Two patients were excluded from the study during follow-up. One did not comply with review appointments or adhere to medical advice regarding wound care and hygiene. The second developed critical limb ischemia during the study period despite meeting initial inclusion criteria. Arterial duplex imaging showed significant progression of a previously noted right superficial femoral artery stenosis. ABPI was found to have dropped to 0.55, and while angioplasty did not increase the ABPI, the patient's symptoms improved

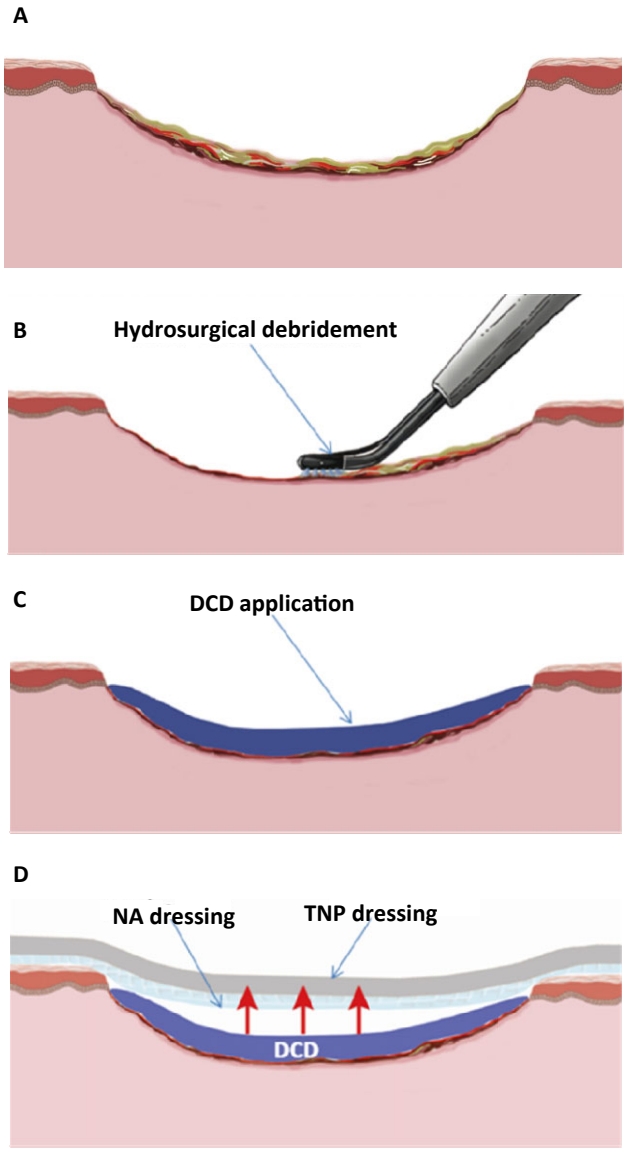

Figure 1. Pilot study therapeutic strategy for treatment-resistant ulcers (A) utilizing hydrosurgical wound debridement (B), decellularized dermis application (C), and negative pressure wound therapy (D). DCD, decellularized dermis; NA, nonadherent; TNP, topical negative pressure.

despite the ulcer remaining static. Only patients who completed follow-up are included in data analyses.

Biopsies

5-mm-diameter full-thickness (extending down to hypodermic fat) skin punch biopsies were obtained aseptically under local anesthetic. They were harvested from 12, 3, and 6 o'clock positions in designated ulcers at day 0, week 3, and week 6 respectively. The 12 o'clock position was defined as the most superior point of the ulcer.

Biopsies were taken at the wound margin so that, macroscopically, half the tissue was ulcerated and half appeared to be normal periulcerous tissue. Specimens were placed in formalin and sectioned in a plane so that transition from normal to ulcerated tissue could be observed during histological assessment (Supporting Information Figure S2).

All biopsies were stained with hematoxylin and eosin (H&E) before being reviewed by a consultant histopathologist who, in a blinded fashion, scored architectural and morphological parameters within the dermis and epidermis. Based on

Table 1. Pilot study time points

Study time point	Actions and wound assessment measures
Day 0	• Wound assessment • 2D and 3D photography • FLPI, SIAscopy and wound swab • 5-mm punch biopsy • Application of DCD
Day 7	• Wound assessment • 2D and 3D photography • FLPI and SIAscopy
Day 14	• Wound assessment • 2D and 3D photography • FLPI and SIAscopy
Day 21	• Wound assessment • 2D and 3D photography • FLPI, SIAscopy, and wound swab • 5-mm punch biopsy
Day 32	• Wound assessment • 2D and 3D photography • FLPI and SIAscopy
Day 42	• Wound assessment • 2D and 3D photography • FLPI, SIAscopy, and wound swab • 5-mm punch biopsy
4 months	• Wound assessment • 2D and 3D photography • FLPI and SIAscopy
6 months	• Wound assessment • 2D and 3D photography • FLPI and SIAscopy • End of study

Treatment involving hydrosurgical wound debridement, DCD application, and topical negative therapy was applied on day 0. Noninvasive measures of wound healing included 2D photography, 3D photography, FLPI, and SIAscopy.
Biopsies from the wound margin and a wound swab were taken on day 0, at 3 weeks, and at 6 weeks.
Patients were followed up on seven occasions over 6 months.
DCD, decellularized dermis; FLPI, full-field laser perfusion imaging; SIAscopy, spectrophotometric intracutaneous analysis.

overall pattern of tissue morphology, cell types present, and their distribution, each sample was scored according to the specific phase (inflammatory, proliferative, or remodeling) of acute wound healing that it most resembled.

Furthermore, specimens were stained for immunohistochemical markers, including collagen I, collagen III, fibronectin, α-smooth muscle actin (α-SMA), and the endothelial cell marker CD31. Tissue Studio software (Definiens, Munich, Germany) was used to analyze stain intensity. Within each section, three discrete areas were defined and analyzed separately in order to compare cellular behavior in different areas of the wound bed (Supporting information Figure S2d). These areas were the papillary dermis underlying the exposed ulcer surface (ulcer edge, UE), the papillary dermis underlying the healing, reepithelialized surface (healing edge, HE), and a deeper region in the reticular dermis (RD).

NHSBT DCD

NHSBT DCD is a tissue allograft licensed for human application/transplantation. All aspects of consent, donation, processing, testing, storage, and supply are approved by the UK Human Tissue Authority. DCD is prepared from skin allografts donated by deceased British human tissue donors. Blood from donors is tested for mandatory markers, including HIV-1 and -2, hepatitis B, hepatitis C, human T-lymphotropic virus, and syphilis. Once approved, cadaveric split-thickness skin grafts are harvested, and sequential treatments are applied to remove the epidermis, as well as cells and cell remnants, from the dermis (Supporting Information Figure S3). The processing protocol has been shown not to adversely affect any of the biological or biomechanical properties of the tissue.[18] Following processing, grafts are terminally sterilized with gamma irradiation in the frozen state, and thus supplied for clinical application as a sterile graft.

Statistics

Our clinical research group was commissioned by NHSBT to complete a pilot study of 20 patients. Follow-up data are available for all outcome measures at each time point after DCD application. All parametric measures were analyzed using repeated-measures analysis of variance (ANOVA) testing for the effects of time, sex, ulcer duration, and all interaction between these terms. Contrasts were used to compare the mean value at week 0 with each of the mean values at subsequent time points. Wound volume data (nonparametric because of the number of zero values) were analyzed with a Friedman test to look for changes with time overall, followed by a series of Wilcoxon matched-pairs signed-ranks tests to compare week 0 results with each of the subsequent time points. A p-value of ≤ 0.05 is considered statistically significant for surface area, volume, FLPI, and SIAscopy data.

ANOVA was applied to immunohistochemistry data reported at weeks 0, 3, and 6, testing for effects of time, tissue section (UE, HE, and RD), gender, ulcer duration, and all pairwise interactions between these terms. A Bonferroni adjustment was made, and the adjusted p-value against which to test individual ANOVA results while maintaining an overall 5% level of significance was 0.001 or less.

RESULTS

Wound surface area

All ulcers decreased in surface area after DCD application (range 23–100%) (Supporting Information Figure S4). Mean reduction was 49.51% after 6 weeks, 80% after 4 months, and 87% after 6 months. Sixty percent of patients treated healed completely with 100% reepithelialization. There was a significant reduction in surface area compared with baseline from 3 weeks onwards ($p = 0.0171$) (Figure 2). Subgroup analysis revealed that ulcers present for longer than 1 year ($n = 11$) showed smaller reductions in surface area than those under 1 year ($n = 9$) in duration ($p = 0.0301$). There was no significant difference in surface area reduction between men and women ($p = 0.5524$). There was one recurrence after complete healing secondary to poor compliance with maintenance compression hosiery.

Wound volume

Complete wound volume data were obtained in only 10 patients because the measuring device only became available midway through recruitment for the study. There were significant changes in wound volume with time, as median volume measurements were significantly lower than week 0 from 4.5 weeks onwards ($p = 0.016$). Mean wound volume reduction after 6 weeks was 62%.

FLPI

There were significant changes in vascularity with time ($p < 0.001$). Mean hemoglobin flux at week 1 was significantly lower than at week 0 ($p = 0.0169$). However, it subsequently increased, and mean values at 6 weeks were significantly greater than baseline ($p = 0.0052$) (Figure 3). Flux values subsequently fell below day 0 values, but this

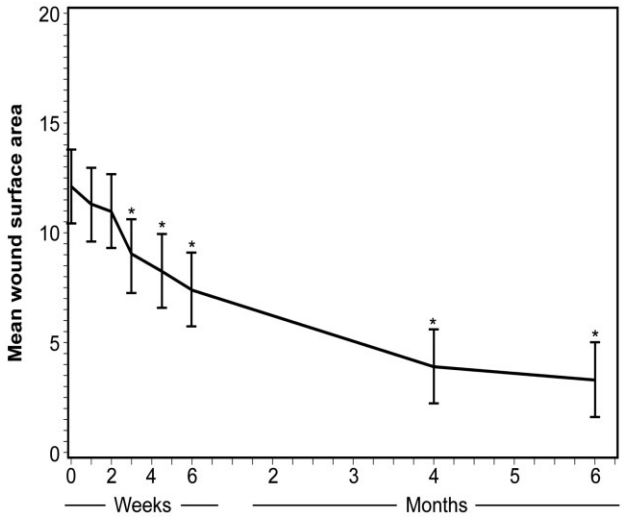

Figure 2. Plot of the mean changes in surface area from baseline, with 95% confidence intervals. $*p \leq 0.05$.

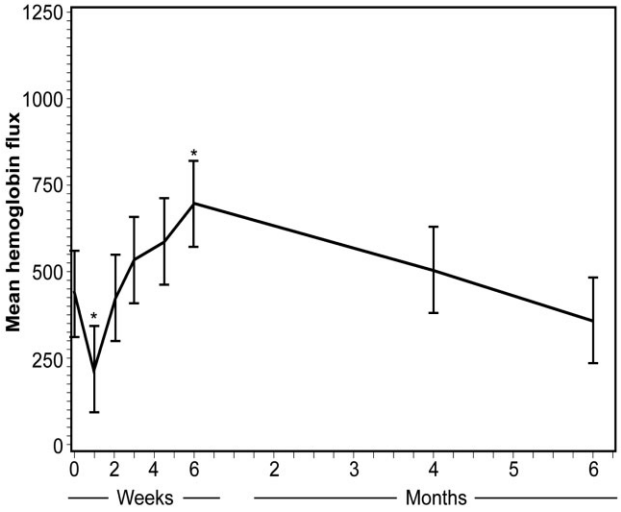

Figure 3. Plot of the mean changes in hemoglobin flux from baseline (in arbitrary units), with 95% confidence intervals. $*p \leq 0.05$.

did not reach significance ($p = 0.3078$). Subgroup analysis showed that ulcers present for more than 1 year before treatment took longer to generate equivalent angiogenic responses than wounds of less than 1 year's duration (Figure 4). Furthermore, ulcers present for less than 1 year showed significantly lower flux values after 6 months compared with day 0 ($p = 0.0148$), whereas those of greater than 1 year's duration did not ($p = 0.2279$).

SIAscopy

There was a significant increase in mean collagen concentration at week 1 compared with day 0 ($p < 0.001$). However, means at subsequent time points were not significantly different from day 0. There was no significant change in mean tissue hemoglobin levels with time. Mean melanin concentration at week 1 was significantly lower than at day 0 ($p = 0.004$); however, means at subsequent time points were not significantly different from day 0.

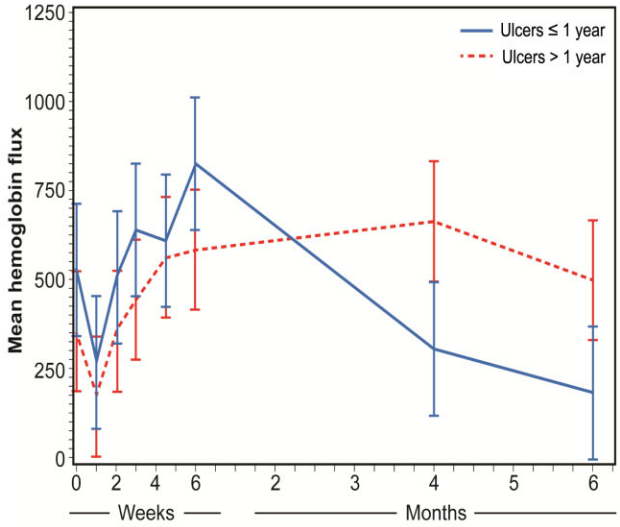

Figure 4. Plot of the mean changes in hemoglobin flux from baseline according to duration of ulcer, with 95% confidence intervals.

Histology

Seventeen patients had biopsies at all three time points, while the remaining three participants had two taken. Consequently, 57 patient samples were stained using H&E. Ninety-five percent of these biopsies were considered suitably representative. Day 0 samples showed signs of chronic inflammation including intracorneal neutrophils, epidermal exocytosis, spongiosis, dermal edema, vascular ectasia, and a predominantly lymphocytic and neutrophilic dermal inflammatory cell infiltrate.

After DCD application, the amount of spongiosis and dermal edema was seen to decrease, while the amount of stromal fibrosis and reepithelialization increased (Figure 5). The type and quantity of inflammatory cells seen in the dermis increased after application of DCD. Histiocyte levels appeared to peak 6 weeks after DCD application, while lymphocyte and plasma cell levels peaked after 3 weeks and then decreased again. Neutrophil levels did not change significantly over time.

DCD was observed in situ in many of the biopsies obtained. Three weeks after DCD application, the graft was commonly colonized by host fibroblasts, lymphocytes, and neutrophils (Figure 6A). There were also examples of giant cells adjacent to the graft. Most significantly, there was strong evidence of neovasculogenesis and vascular ectasia in surrounding dermis (Figure 6B). Furthermore, in most patients the overall impression was of progression from early to later phases of healing when third- and sixth-week biopsy samples were compared.

Immunohistochemistry

Collagen I

At all time points, collagen I levels were highest at the UE and lowest in the RD, though this difference did not reach significance ($p = 0.0085$) (Supporting Information Figure S5). There was an initial decrease in collagen I concentration after 3 weeks, and although levels increased by 6 weeks, this change did not reach significance ($p = 0.0723$). However, subgroup analysis showed that in patients with ulcers present for less than 1 year, the increase in collagen I levels at 6 weeks was significant when compared with those with ulcers present for over a year ($p < 0.001$) (Supporting Information Figure S6). There was no difference with respect to gender ($p = 0.4165$).

Collagen III

Collagen III levels were highest at UE and lowest in RD, though this difference did not reach statistical significance ($p = 0.06$). There was no significant change in collagen III concentration after 3 weeks ($p = 0.5001$), but levels

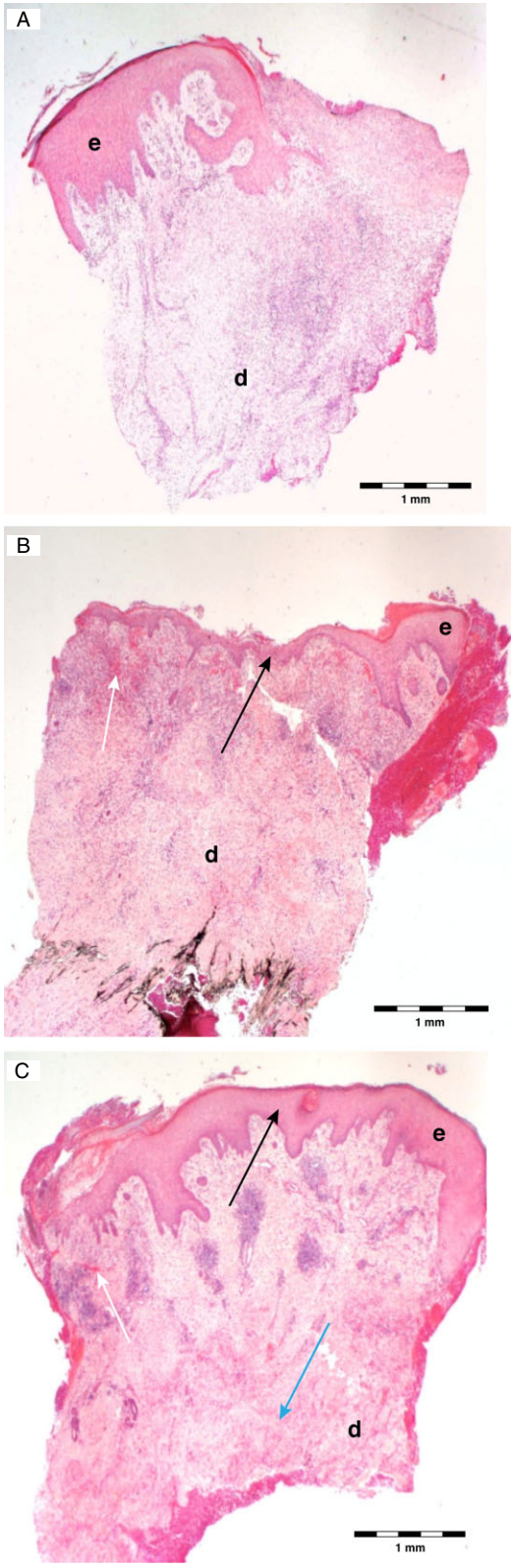

Figure 5. Typical histological appearance of consecutive wound edge biopsies (A, day 0; B, week 3; C, week 6) from the same patient. After treatment there is evidence of reepithelialization (black arrows), reduced dermal edema, and variable inflammatory cell infiltrate. There is also significant neovasculogenesis (white arrows) and stromal fibrosis (blue arrow) over time. d, dermis; e, epidermis.

subsequently increased after 6 weeks ($p = 0.0715$) (Supporting Information Figure S7). Gender and duration of ulceration did not affect collagen III levels ($p = 0.6189$ and $p = 0.012$, respectively).

Fibronectin

There was an initial decrease in fibronectin concentration after 3 weeks, but levels had increased to values greater than on day 0 after 6 weeks, though this did not reach statistical significance ($p = 0.3249$). Ulcer duration ($p = 0.1168$) and

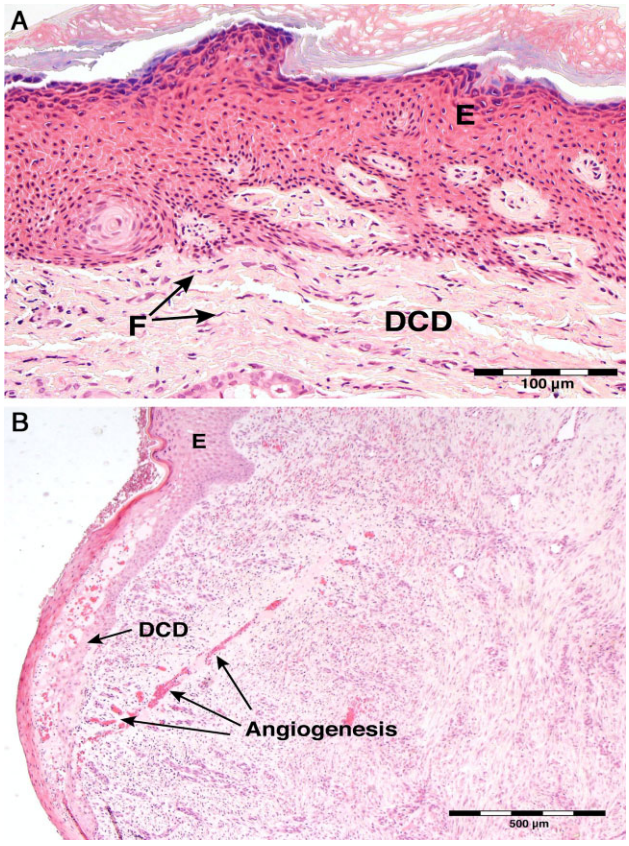

Figure 6. A. Punch biopsy taken 6 weeks after National Health Service Blood and Transplant decellularized dermis (DCD) application, showing DCD in-situ containing host fibroblasts (F) and inflammatory cells. It is covered by new epithelium (E), suggesting that its retained basement membrane is conducive to keratinocyte migration. B. Evidence of active neoangiogenesis in tissue adjacent to DCD, 6 weeks after treatment.

gender ($p = 0.0891$) did not affect mean fibronectin values. However, mean fibronectin values differed significantly between the three sections ($p = 0.0001$), being highest at UE and lowest in HE.

α-SMA

There were no significant interaction terms, and levels did not change significantly with time ($p = 0.6636$). Mean α-SMA was not affected by gender or ulcer duration. However, α-SMA levels were significantly higher at HE than in other regions ($p = 0.0001$).

CD31

None of the interaction terms were significant, though CD31 levels increased 3 weeks after treatment ($p = 0.0374$). CD31 levels were significantly greater at HE and UE than RD after application of DCD ($p < 0.0001$ in both cases) (Supporting Information Figure S8). This correlates with FLPI data. There was no difference in CD31 values based on ulcer duration or sex ($p = 0.1628$ and $p = 0.7845$ respectively).

Wound microbiology and complications

No serious complications were noted during follow-up. Positive wound swabs were obtained, mostly indicating wound colonization. Only 3 patients showed evidence of clinical infection, which was treated successfully with oral antibiotics. The causative organism in each case was *Staphylococcus aureus*. There were no hypersensitivity reactions to DCD.

DISCUSSION

This study has shown significant improvements in treatment-resistant leg ulcers after application of DCD used in combination with hydrosurgical debridement and short-term TNP therapy (Figure 7). All wounds decreased in surface area and volume, with 60% healing completely. There was strong evidence of angiogenesis seen immunohistochemically and on noninvasive imaging. Furthermore, wound biopsies showed histological evidence of host cell migration, proliferation and conversion of chronic ulcers to wounds with characteristics more akin to acute wound healing. Subgroup analysis showed different cellular and molecular behavior in wounds of different duration, with delayed angiogenesis, reduced collagen deposition, and slower reductions in wound surface area seen in ulcers present for more than 1 year. ECM proteins such as fibronectin, collagen, and α-SMA also had differing concentrations throughout the wound bed depending on depth from the surface and presence of intact epithelium.

Chronic wounds result from the combination of systemic and local factors that promote adverse conditions for wound healing. Systemic causes include old age, diabetic hyperglycemia, malnutrition, and tissue hypoxia secondary to arterial or venous insufficiency.[21,22] Local inflammatory stimuli such as bacterial bioburden, necrotic tissue, foreign bodies, and cellular hypoxia result in protease- and reactive oxygen species-rich environments.[21,23,24] There is subsequent down-regulation of protective mechanisms, direct cell membrane damage, and ECM breakdown.[22,24] Existing treatment strategies have variable rates of success but leave significant treatment-resistant populations.[11] Acellular dermal skin substitutes are an evolving therapeutic option with a growing evidence base in chronic wound management.[11] These materials accurately mimic native tissues to promote healing.[25] Their exact mode of action is not fully understood, but fundamentally, they provide the wound bed with a three-

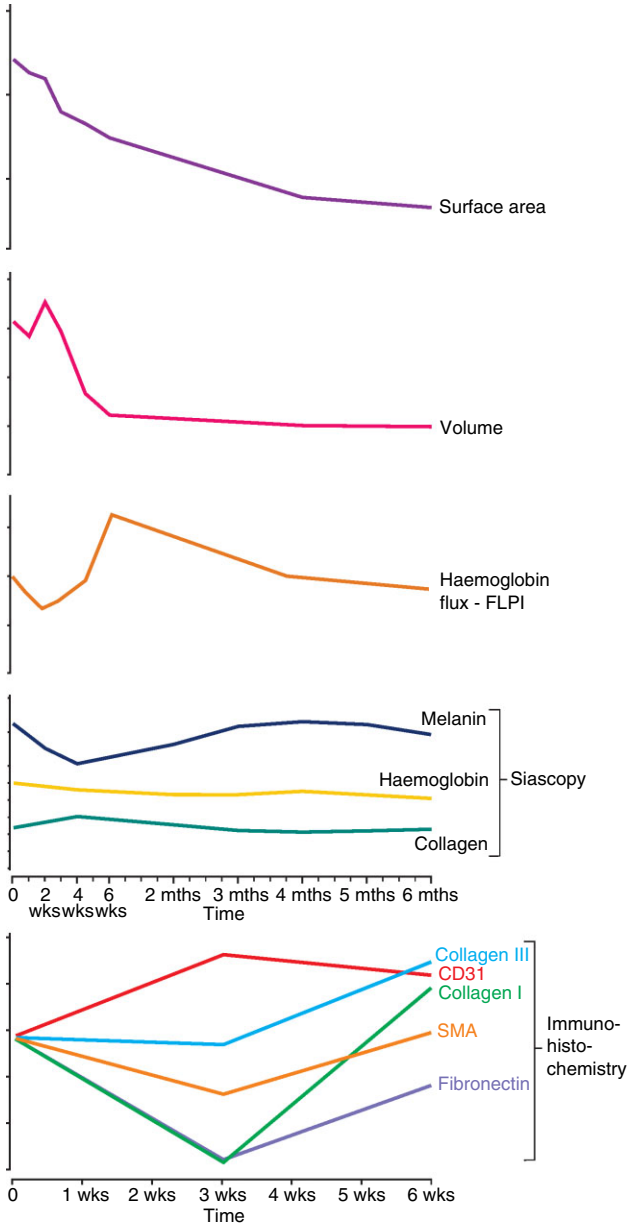

Figure 7. Summary of all wound assessment tools showing trends in outcome measures in the follow-up period after decellularized dermis application. FLPI, full-field laser perfusion imaging; SIAscopy, spectrophotometric intracutaneous analysis; SMA, smooth-muscle actin.

dimensional biomatrix material that forms a template for host cellular infiltration and a physical support to guide the differentiation, proliferation, and positioning of migrating cells, including fibroblasts, polymorphonuclear cells, and endothelial cells within the wound space.[26,27] DCD is derived from human cadaveric skin and therefore has comparable biomechanical properties to the injured tissue. This is crucial, as scaffold characteristics including elasticity, porosity, and biocompatibility have been shown to mediate beneficial cellular activity during healing.[26,27]

In this study we have shown histological evidence of DCD incorporation into host tissues with colonization by fibroblasts, neutrophils, and lymphocytes after 3 weeks. Their recruitment, subsequent migration, and secretion of bioactive molecules promote wound healing through replacement of previously senescent cellular populations that have reduced responsiveness to growth factors as well as limited replicative and synthetic abilities.[28,29] Higher concentrations of fibronectin, collagen I, and collagen III were found in the wound bed, particularly at the UE, 6 weeks post-DCD treatment. This is likely to relate to increased deposition by migrating fibroblasts, which was supported histologically. Dermal fibroblasts have also been implicated in promoting keratinocyte migration across provisional ECM via release of paracrine effectors.[30] This factor, combined with increased fibronectin and an intact basement membrane in DCD, may have contributed to the successful reepithelialization observed during the study. More importantly for patients, this degree of reepithelialization meant that a second treatment such as split-thickness skin grafting was not required.

FLPI and immunohistochemical data suggest that DCD is a potent angiogenic stimulant, with elevated hemoglobin flux and CD31 values after treatment, as well as new vessel development and vascular ectasia noted histologically. In addition to promoting endothelial cell migration and proliferation, DCD induced ECM deposition, which is essential to angiogenesis. Pro-angiogenic effects of ECM are exerted by ECM proteins directly, by protease-induced ECM breakdown products, and by ECM growth factor sequestration.[31] Improved blood flow to chronic wound beds provides enhanced delivery of oxygen and nutrients as well as preventing repeated ischemic reperfusion injury, which is known to promote diabetic and venous ulcer formation.[32]

Ulcers present for greater than 1 year showed smaller reductions in wound surface area, decreased collagen I deposition, and a delayed angiogenic response. This may reflect a greater senescent cell population or global impairment of healing mechanisms in truly chronic wounds. It has been shown previously that wound size and duration are significantly associated with likelihood of wound healing in diabetic and venous ulcers.[33,34] However, we now suggest that this may be due to proportional time-dependent impairment of fibroplasia and angiogenesis in wounds of significant duration.

The SIAscopic data appear to be at odds with outcomes of other investigative modalities. While SIAscopy is a proven diagnostic tool in normal skin and cutaneous lesions such as melanomas, there is minimal published evidence supporting its use in chronic wound assessment.[35] SIAscopic readings are normally based on collagen levels in the papillary dermis and melanin at the dermoepidermal junction. Chronic wound beds lack an epidermis, and this could explain the absence of changes seen in these SIAscopic parameters.

The effect of DCD on treatment-resistant wounds cannot be precisely quantified from this study, with debridement and TNP acting as confounding factors. However, the role of TNP in chronic wounds is uncertain, as studies investigating the beneficial effects of TNP are often methodologically flawed or poorly powered. Indeed, a 2008 Cochrane review identified seven relevant studies involving 205 participants but concluded that TNP did not significantly increase the healing rate of chronic wounds compared with comparators.[36] While some studies have indicated a therapeutic effect in diabetic ulcers, very few have shown any improvement in venous ulcers.[36,37] As 75% of our patient cohort had venous ulcers, the confounding effect of TNP is limited in this study. Similarly, it is questionable whether initial wound bed debridement would produce such sustained benefit in this treatment-resistant cohort when standard chronic wound care doctrine recommends repeated or "ongoing-maintenance" debridement procedures.[38] It is more likely that hydrosurgical debridement provides adequate wound bed preparation for patients to derive greatest benefit from subsequent treatments such as DCD. Consequently, the prolonged improvements shown in this study are thought to derive primarily from the action of DCD in the chronic wound environment.

The treatment strategy employed in this pilot study is unique and has advantages to patients and clinicians. The procedure is well tolerated, and patients commonly reported that ulcer-associated symptoms including pain, discharge, and malodor rapidly reduced or resolved after treatment. It can be performed on an outpatient basis in a treatment room with no requirement for theaters, general anesthetic, or formal admission to hospital. Consequently, the technique could be applied in community and hospital settings. Estimated costs for DCD application on an ulcer measuring 25 cm^2 are approximately £550 (US$869) per treatment, including £230 (US$363) for hydrosurgical debridement, £120 (US$190) for TNP dressings, and DCD priced at £8 (US$13)/$cm^2$. This seems expensive but may be justified by savings incurred through healing patients who had previously shown no improvement with conventional therapies. Furthermore, DCD is significantly less expensive than using skin substitutes containing live cells, such as neonatal human foreskin fibroblasts, and has a much longer shelf life.

The encouraging results of this study are similar to those seen in larger trials by Reyzelman et al. and Mostow et al., who utilized acellular dermal skin substitutes on diabetic and venous ulcers, respectively.[15,39] Furthermore, the study cohort has a similar etiological profile to those in larger population studies of patients with lower limb ulceration, and outcomes may therefore be applicable to this demographic.[8,40] The next phase in the evaluation of NHSBT DCD will be a large multicenter randomized controlled trial and a cost-effectiveness analysis evaluating DCD against current standard treatment regimes such as compression bandaging alone and debridement plus compression bandaging.

In conclusion, we have demonstrated that DCD and its application process safely promoted wound healing in treatment-resistant chronic lower limb ulceration. Reepithelialization, angiogenesis, and granulation tissue formation were demonstrated using noninvasive measures and confirmed with histological and immunohistochemical analysis. This technique could be adapted to both hospital and community settings and represents a novel therapeutic option in the management of chronic lower limb ulceration.

ACKNOWLEDGMENTS

This paper was accepted for oral podium presentation at the Wound Healing Society/Symposium on Advanced Wound Care meeting in Denver, CO, USA, 1–5 May 2013. The work also received a prize at The European Tissue Repair Society meeting held in Athens in October 2012.

The authors acknowledge the support of Richard Lomas, Akila Chandresekar, and Paul Rooney, as representatives of National Health Service Blood and Transplant (NHSBT), who provided decellularized dermis (DCD) for this study. In particulaaar, we are grateful to Frances Seeney, the Principal Statistician at NHSBT-Organ Donation & Transplantation team based in Bristol for statistical advice and support. We acknowledge Anthony Odell and Helen Berry from Tissue Regenix (York, UK), who provided funding toward this work. The authors would also like to gratefully thank NHS senior managers (in post at the time of the study), as without their support this study would not have been possible, Julian Hartley (CEO of UHSM NHS Foundation Trust at Wythenshawe Hospital in Manchester) and Karen Zaman (Directorate Manager of Burns, Plastic and Reconstructive Surgery).

Source of Funding: This study was funded in part by National Health Service Blood and Transplant (NHSBT) and Tissue Regenix (York, UK). This study involves an NHSBT-derived product that has been licensed to Tissue Regenix. All Versajet and PICO consumables were generously provided by Smith & Nephew (Hull, UK).

Conflict of Interest: The authors declare they have no competing financial interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's Web-site:

Figure S1. Macroscopic evidence of decellularized dermis (DCD) graft integration in the weeks after DCD application.

Figure S2. Full thickness punch biopsies are taken from the ulcer periphery (A), resulting in tissue samples with an appearance as illustrated in B. They are processed, sectioned, and stained, enabling histological analysis (C) Sections are split into three dermal components to enable detailed analysis of immunohistochemical staining of tissue in response to decellularized dermis (DCD) grafting (D). HE, healing edge; UE, ulcer edge; RD, reticular dermis; d, dermis; e, epidermis.

Figure S3. Hematoxylin and eosin staining of NHSBT decellularized dermis showing its decellularized or acellular nature. It functions as an extracellular matrix protein scaffold to guide the influx of host cells and exert positive influence on their differentiation and secretory profile.

Figure S4. A 92-year-old woman with venous ulceration of right tibial crest present for 18 months prior to treatment with decellularized dermis (A). B–F show changes in the clinical appearance of the leg ulcer after therapy.

Figure S5. Changes in collagen I levels over time and in different sections of the dermis after application of DCD. RD, reticular dermis; HE, healing edge; UE, ulcer edge.

Figure S6. Change in collagen I levels with time after application of decellularized dermis in patients, showing variation depending on the duration of ulcers prior to grafting.

Figure S7. Changes in collagen III levels over time and in different sections of the dermis after application of decellularized dermis. RD, reticular dermis; HE, healing edge; UE, ulcer edge.

Figure S8. Changes in CD31 levels over time and in different sections of the dermis after application of decellularized dermis. RD, reticular dermis; HE, healing edge; UE—ulcer edge.

Table S1. Pilot study inclusion and exclusion criteria.

Table S2. Study cohort demographics.