

Human Fibroblast-Derived Dermal Substitute: Results from a Treatment Investigational Device Exemption (TIDE) Study in Diabetic Foot Ulcers

Robert A. Warriner III, MD, FACA, FCCP, FCCWS, ABPM/UHM; Matthew Cardinal, ME;
on behalf of the TIDE Investigators

ABSTRACT

OBJECTIVE: To gain experience in the use of Dermagraft (Advanced Biohealing Inc, La Jolla, California), a human fibroblast-derived dermal substitute (HFDS), for the treatment of nonhealing diabetic foot ulcers (DFUs).

STUDY DESIGN: An open label, noncontrolled, multicenter clinical trial of HFDS in the treatment of DFU was conducted. Subjects with DFUs underwent sharp debridement of the study ulcer and were prescribed an off-loading device. All of the subjects enrolled received applications of HFDS, beginning at day 0 and applied weekly thereafter, along with saline gauze or polyurethane foam dressings from day 0 to week 20. A maximum of 8 HFDS applications was allowed.

MAIN OUTCOME MEASURES: The primary and secondary end points of the study were complete wound closure by weeks 12 and 20, respectively.

MAIN RESULTS: A total of 23 centers screened 91 subjects, and 18 centers enrolled an intent-to-treat (ITT) population of 62 subjects. For the ITT population, 27 (44%) subjects healed by week 12, and 32 (52%) healed by week 20. Fifty-one subjects (82%) completed the study to week 12, and 46 subjects (74%) completed the entire 20-week study; wound closure rates in these groups were 59% and 70%, respectively. Median time to healing was 13 weeks. The overall incidence of at least 1 adverse event (44%, 27/62) was typical for this subject population. No adverse events were attributable to HFDS.

CONCLUSION: Data from this study support the safety and efficacy of HFDS in the treatment of nonhealing DFUs.

KEYWORDS: human fibroblast-derived dermal substitute, nonhealing diabetic foot ulcers, TIDE study

ADV SKIN WOUND CARE 2011;24:306-11

INTRODUCTION

Diabetes is a national and global epidemic that can lead to a number of debilitating complications with significant adverse outcomes.^{1,2}

Peripheral neuropathy and vascular disease are common complications of diabetes; these comorbidities are frequently a conduit to diabetic foot ulceration.^{3,4} A diabetic foot ulcer (DFU) is a multifarious problem, often complicated by biomechanical stress to the insensate foot, susceptibility to bacterial colonization, a compromised immune and vascular system, and hyperglycemia.⁵⁻⁷ In some cases, DFUs precede subcutaneous tissue infection, osteomyelitis, or tissue necrosis that can result in morbid outcomes, such as lower-extremity amputation (LEA).⁸⁻¹⁰ During their lifetime, 15% of people with diabetes will experience a DFU, and between 14% and 24% of those with a DFU will require amputation.¹¹

Diabetes and DFUs are major economic and social burdens for both the individual and national/global healthcare system. In 2007, the total cost of diabetes in the United States was estimated to be \$174 billion; peripheral vascular disease and its complications, including DFUs, were major contributors to higher healthcare resource usage and longer hospital admissions.¹² Studies of patients with diabetes showed that those with chronic DFUs scored significantly lower than those without ulcers in quality of life and social function measures.^{13,14} Patients with DFUs that eventually heal incur lower direct healthcare costs than do patients with unhealed ulcers, whereas patients requiring LEAs incur the highest costs.¹⁵ When combined with DFU prevention strategies, an aggressive DFU treatment regimen that promotes faster healing is essential to help avoid extensive medical costs.¹⁶

Normal healing of an acute wound follows a series of events, starting with hemostasis, proceeding through an inflammatory stage, and progresses to closure of the lesion through formation of granulation tissue, reepithelialization, and, finally, remodeling.¹⁷ DFUs frequently become chronic. A chronic wound is clinically defined as one that has not proceeded through the normal healing process in a timely manner or has not produced a sustained anatomic and functional repair.¹⁸

Diabetes influences the normal healing process at the molecular level.¹⁹ Several key factors that contribute to impaired wound healing in patients with diabetes include inadequate blood

Robert A. Warriner III, MD, FACA, FCCP, FCCWS, ABPM/UHM, is Chief Medical Officer, Diversified Clinical Services, Jacksonville, Florida. Matthew Cardinal, ME, is Senior Clinical Research Assistant, Advanced BioHealing, Inc, La Jolla, California. **Acknowledgment:** This study was funded by Advanced BioHealing, Inc. Submitted December 20, 2010; accepted February 9, 2011.

Table 1.**TREATMENT CHARACTERISTICS**

Treatment Variables	All Patients (n = 62)	Healed by Week 12 (n = 27)	Unhealed by Week 12 (n = 35)
Discontinued study	16 (25.8%)	0 (0%)	16 (45.7%)
Primary offloading device			
Orthotics/custom shoe	40 (64.5%)	20 (74.1%)	20 (57.1%)
Crutches/walker	9 (14.5%)	4 (14.8%)	5 (14.3%)
Wheelchair	13 (21.0%)	3 (11.1%)	10 (28.6%)
Dressing changes, per week	3 (2–6)	3 (2–4)	4 (2–6)
Dressing type			
Polyurethane foam	30 (48.4%)	16 (59.3%)	14 (40.0%)
Saline gauze	32 (51.6%)	11 (40.7%)	21 (60.0%)
Study ulcer infection type			
Infection (local wound)	6 (9.7%)	1 (3.7%)	5 (14.3%)
Cellulitis	5 (8.1%)	0 (0%)	5 (14.3%)
Osteomyelitis	2 (3.2%)	0 (0%)	2 (5.7%)
Any study ulcer infection	12 (19.4%)	1 (3.7%)	11 (31.4%)
Noninfection adverse event	15 (24.2%)	1 (3.7%)	14 (40.0%)

Variables are represented as mean \pm SD, median (first-third quartile), or n (%).

supply, infection, fibroblast senescence, inhibited keratinocyte migration, reduction of growth factors, and absence of normal protein matrix in the dermis.²⁰ Keratinocytes may accumulate at the periphery of chronic wounds, but they are unable to move onto the wound surface, ultimately inhibiting epithelialization.²¹

Despite the progress in understanding the biology of chronic wounds, there is still a need for wound care treatment regimens that address the underlying deficiencies of chronic DFUs.²² Recent advances in tissue engineering have produced a human

fibroblast-derived dermal substitute (HFDS) that may address this need.^{23,24} The usage of dermal substitutes has become more prevalent in DFU treatment protocols, given an objective analysis of impaired healing.^{25,26} However, there is still a desire for more efficacy data from clinical studies on such therapies.²⁷ The following data are the results of a Treatment Investigational Device Exemption (TIDE) clinical study on the use of HFDS in the treatment of chronic DFUs.

RESEARCH DESIGN AND METHODS

HFDS (Dermagraft; Advanced BioHealing Inc, La Jolla, California) is a cryopreserved fibroblast-derived dermal substitute composed of fibroblasts, extracellular matrix, and a bioabsorbable scaffold. Human fibroblast cells are cultured in vitro onto a bioabsorbable polyglactin mesh. As the cells proliferate across the mesh, they secrete human dermal collagen, matrix proteins, growth factors, and cytokines to create a 3-dimensional human dermal substitute containing metabolically active, living cells. HFDS does not contain macrophages, lymphocytes, blood vessels, or hair follicles. The proposed mode of action of HFDS is reepithelialization by facilitating the restoration of the dermal bed.

This study was an open-label, multicenter, controlled evaluation of HFDS in the treatment of DFUs. The TIDE study on HFDS was conducted concurrently with a pivotal clinical trial, which resulted in Food and Drug Administration (FDA) approval of Dermagraft for the treatment of DFUs.²⁸ Thus, there was no control or placebo-treated group in the TIDE study because it was not designed for treatment comparisons. The goals of the TIDE study were to gain

Table 2.**TIDE STUDY INCLUSION AND EXCLUSION CRITERIA****Inclusion Criteria**

- Patient is ≥ 18 years.
- Patient has a current diagnosis of type 1 or type 2 diabetes mellitus.
- Patient's ulcer extends through the dermis and into subcutaneous tissue (granulation tissue may be present).
- Patient's wound (after debridement) is free of necrotic debris, exhibits no signs of clinical infection, and appears to be composed of healthy vascularized tissue.
- The patient, in the opinion of the investigator, has adequate circulation to the foot as evidenced by a palpable pulse on the study foot (either dorsalis pedis or posterior tibial artery) and/or Doppler ultrasound measurement.
- If patient is capable of bearing children, she is using a medically accepted means of birth control, and she tests negative on a serum pregnancy test.
- Patient and caregiver are willing to participate in the clinical study and can comply with the follow-up regimen.
- If a patient is treated with warfarin (Coumadin) or heparin, a prothrombin time or partial thromboplastin time (PTT) test result must be reviewed with the primary care physician and found to be within the targeted therapeutic range.
- In addition, the study investigator must determine that the patient's wound can be safely debrided without undue risk of bleeding.
- Patient or his/her legal representative has read and signed the IRB-approved informed consent form before treatment.

Exclusion Criteria

- Patient has clinical evidence of gangrene on any part of the affected foot.
- Patient's ulcer is over a Charcot deformity of the midfoot or over the tarsal bones—talus, distal calcaneus, navicular, and cuboid.
- The patient's ulcer is due to a nondiabetic etiology.
- Patient's ulcer has tunnels or sinus tracts that cannot be completely debrided.
- Patient's ulcer has increased in size by $\geq 50\%$ during the screening period.
- Patient has 1 or more medical condition(s), including renal, hepatic, hematologic, neurological, or immune disease that, in the opinion of the Investigator, would make the patient an inappropriate candidate for this wound healing study.
- Patient has or has had a malignant disease (other than facial basal cell carcinoma) not in remission for ≥ 6 months.
- Patient has severe malnutrition as evidenced by albumin < 2.0 .
- Patient has known alcohol or drug abuse.
- Patient's random blood sugar reading is > 450 mg/dL.
- Patient's urine ketones are noted to be "small, moderate, or large."
- Patient is receiving oral or parenteral corticosteroids or immunosuppressive or cytotoxic agents or is anticipated to require such agents during the course of the study.
- Patient has a history of bleeding disorder.
- Patient has AIDS or is known to be infected with HIV.
- Patient has previously received treatment with HFDS.
- Patient's ulcer is accompanied by cellulitis, osteomyelitis, or other clinical evidence of infection.

experience in the use of HFDS in a broader patient population than was being studied in the pivotal trial, to expand healthcare provider access to HFDS before FDA approval, and to support safety and efficacy data generated in the pivotal study. The protocol was approved by the institutional review board (IRB) at each center, and all patients gave written informed consent.

At the screening visit, baseline clinical variables (such as demographics, ulcer characteristics) were recorded. Study ulcers received sharp debridement and wound dressings consisting of a non-adherent interface. The ulcers were then dressed with a saline-moistened gauze to fill the ulcer followed by dry gauze or a polyurethane foam dressing. Both dressing types were covered by adhesive tape. Dressing choice was at the discretion of the investigators. In addition to dressings, patients received a prescription for an off-loading device to relieve pressure at the ulcer site (Table 1). Patients were entered into the study if they continued to meet the inclusion/exclusion criteria 1 week later at day 0 (Table 2).

Patients enrolled in the trial received 1 application of HFDS at day 0, then 1 application each week, up to a maximum of 8 applications over the course of the 20-week study. At each visit, patients received sharp debridement to remove necrotic or hyperkeratinized tissue. Wound dressing type and the number of dressing changes during the previous week were recorded at each visit. The investigators instructed each patient to avoid weight bearing on the study leg as much as possible and to use the prescribed off-loading device whenever ambulatory. Patients were evaluated weekly until they had complete wound closure (defined as full epithelialization of the wound with the absence of drainage), reached the week 20 visit without complete closure, or discontinued.

The primary end point of the study was complete wound closure by week 12. Secondary end points were complete wound closure by week 20 and time (weeks) to healing. Safety end points included all adverse events, adverse device effects, unanticipated device effects, infections involving the study ulcer/leg, and surgical procedures involving the study ulcer/leg.

Baseline variables recorded during the trial were evaluated versus the 12-week ulcer healing end point using binary logistic regression. Student *t* test and analysis of variance for normally distributed variables, Kruskal-Wallis tests for nonnormal data, χ^2 tests for categorical variables, and regressions were conducted at $\alpha \leq .05$ using MATLAB Statistics Toolbox (The Mathworks Inc, Natick, Massachusetts) and Minitab 15.1 (Minitab Inc, State College, Pennsylvania). Data management and statistical analysis were conducted by the Clinical Research Department at Advanced BioHealing, Inc.

RESULTS

A total of 33 centers obtained IRB approval and were qualified to participate in the study. Of these centers, 23 centers screened 91

patients, and 18 centers enrolled a total of 62 patients (Figure 1). The original study investigator agreements allowed for the enrollment of up to 5 patients per center. Four centers signed subsequent agreements to enroll up to 5 additional patients. No individual center enrolled more than 9 patients. Patients were enrolled in the study between February 1998 and October 2001, with the study terminating upon FDA approval of Dermagraft.

Treatment characteristics, baseline variables, and study results are listed in Table 1, 3, and 4, respectively. Of the 62 patients enrolled, 16 (26%) discontinued prior to the end of the 20-week

Figure 1.
TIDE STUDY ENROLLMENT AND OUTCOMES

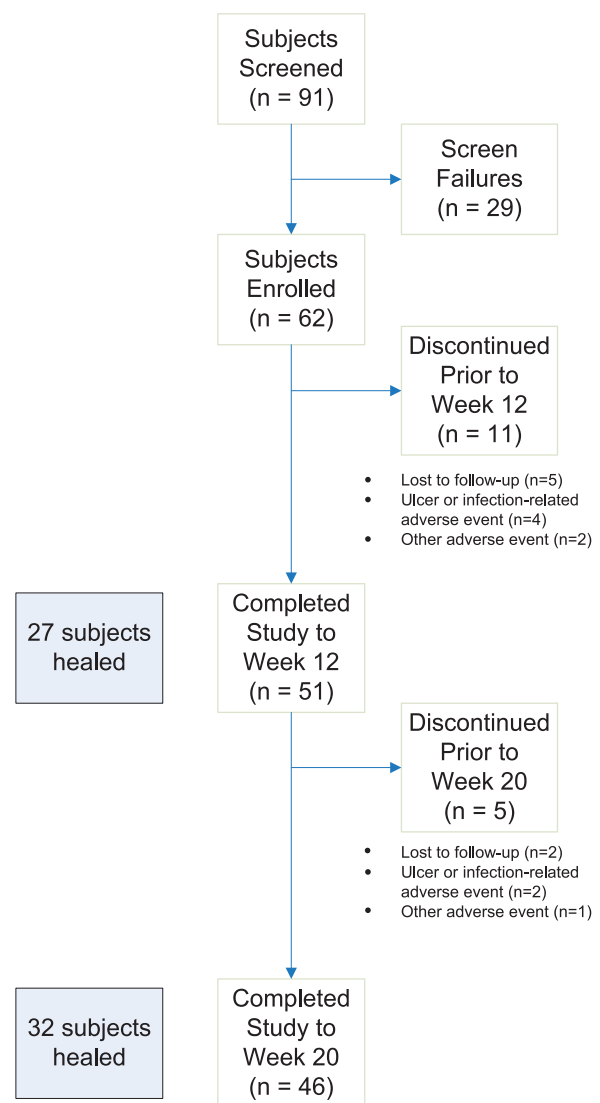


Table 3.

BASELINE VARIABLES: PATIENT DEMOGRAPHICS AND ULCER CHARACTERISTICS

Demographic Variables	All Patients	Healed by Week 12	Unhealed by Week 12
	(n = 62)	(n = 27)	(n = 35)
Age, y	61.9 ± 12.9	61.7 ± 15.1	61.9 ± 11.1
Sex,			
Male	36 (58.1%)	14 (51.9%)	22 (62.9%)
Female	26 (41.9%)	13 (48.1%)	13 (37.1%)
Body mass index, kg/m ²	29.9 (26.5–35.0)	28.2 (25.8–33.9)	30.8 (27.7–35.6)
Blood pressure—systolic	134.9 ± 19.7	131.0 ± 20.0	137.8 ± 19.3
Blood pressure—diastolic	78.2 ± 10.9	74.6 ± 9.2	80.9 ± 11.4
Pulse, beats/min	77.68 ± 10.89	76.3 ± 10.1	78.7 ± 11.5
Respiration, breaths/min	20 (16–22)	20 (17–23)	20 (16–20)
Tobacco use,			
Yes	6 (9.7%)	2 (7.4%)	4 (11.4%)
Palpable pulses (dorsalis pedis and posterior tibial),			
Both pulses	52 (83.9%)	25 (92.6%)	27 (77.1%)
One pulse only	10 (16.1%)	2 (7.4%)	8 (22.9%)
First DFU, Yes	45 (72.6%)	20 (74.1%)	25 (71.4%)
No. of total DFUs at screening,			
1	44 (71.0%)	21 (77.8%)	23 (65.7%)
≥2	18 (29.0%)	6 (22.2%)	12 (34.3%)
No. of DFUs, study foot,			
1	55 (88.7%)	26 (96.3%)	29 (82.9%)
≥2	7 (11.3%)	1 (3.7%)	6 (17.1%)
Study ulcer position,			
Medial	18 (29.0%)	9 (33.3%)	9 (25.7%)
Midline	20 (32.3%)	7 (25.9%)	13 (37.1%)
Lateral	24 (38.7%)	11 (40.7%)	13 (37.1%)
Study ulcer location,			
Toe	12 (19.4%)	7 (25.9%)	5 (14.3%)
Forefoot	33 (53.2%)	13 (48.1%)	20 (57.1%)
Heel	16 (25.8%)	7 (25.9%)	9 (25.7%)
Dorsum	1 (1.6%)	0 (0%)	1 (2.9%)
Study ulcer duration, wk	26 (16–52)	24 (15–52)	26 (19–52)
Ulcer area—day 0, cm ²	2.1 (1.0–5.2)	1.5 (0.9–3.2)	2.7 (1.0–13.8)

Variables are represented as mean ± SD, median (first-third quartile), or n (%).

study. Most patients who discontinued had an adverse event (14/16, 88%) requiring treatment that warranted withdrawal from the study (such as a surgical procedure that altered the study ulcer). One patient who had an exceptionally large study wound received permission from the IRB and the FDA to have an additional 8 applications of HFDS over the study period.

The overall incidence of adverse events was 44% (27/62). There were 13 study ulcer-related adverse events (ie, local wound infection, osteomyelitis, and cellulitis) in 12 patients, a per-patient incidence rate of 19% (12/62). One patient discontinued from the study because of a fatal adverse event. The percentage of patients who underwent a surgical procedure involving the study ulcer was 6% (4/62). There were no adverse device effects or unanticipated device effects reported during the study.

The percentage of patients in the intent-to-treat population with complete wound closure by week 12 was 44% (27/62). By week 20, an additional 5 patients reached complete wound closure (32/62, 52%). After censoring patients without wound

closure by week 20, median time to healing was 13 weeks, with a first quartile of 8 weeks. Healing rates for patients completing the study are shown in Figure 2. For the 46 subjects (74%) who completed the entire 20-week study, wound closure rates were 59% (27/46) and 70% (32/46) at weeks 12 and 20, respectively.

Baseline variables were fitted to a binary logistic regression model on healing by week 12 using forward selection procedures with an entry requirement of $\alpha < .10$ (Table 5). No baseline variables were found to be significantly correlated to wound healing outcomes. Higher diastolic blood pressures and larger ulcer sizes were associated with decreased healing rates, although these associations were not statistically significant ($P = .065$).

DISCUSSION

The patient with a DFU presents a challenging medical, economic, and social problem. Optimal preventive care includes the following key elements: annual examination of the feet by healthcare providers, subsequent examination of high-risk feet at each

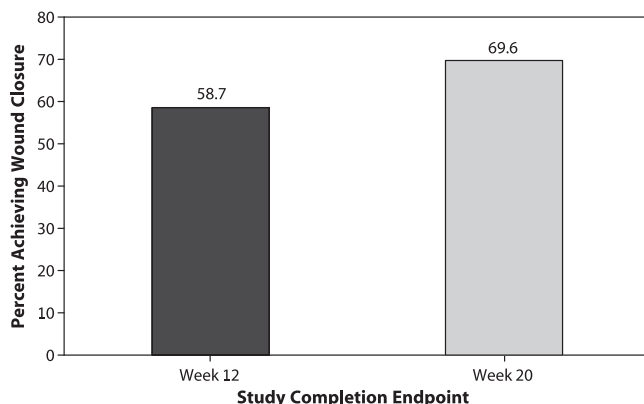
Table 4.**TIDE STUDY OUTCOMES****Efficacy Results**

Complete wound closure by week 12	27 (43.5%)
Complete wound closure by week 20	32 (51.6%)
Time to wound closure, wk	1st Quartile = 8 Median = 13
Safety results	
Study ulcer infection type	
Infection (local wound)	6 (9.7%)
Cellulitis	5 (8.1%)
Osteomyelitis	2 (3.2%)
Any study ulcer infection	12 (19.4%)
Surgical procedure to study ulcer	4 (6.5%)

Variables are represented as mean \pm SD, median (first-third quartile), or n (%).

patient visit, patient education about daily self-care of the feet, use of proper footwear, and optimal glycemic control with appropriate diet and medication. Even with good diabetes management, many patients will have chronic foot ulcers. Proper wound care is essential and includes debridement, infection control, and appropriate custom-fitted orthotics or other methods of off-loading of the wound area. The longer the ulcer persists, the greater the possibility that the patient will develop a serious infection that can lead to hospitalization and possible amputation.

The pathophysiology of a DFU is complicated. Those ulcers that have adequate blood supply and a sufficient number of viable cells that are capable of providing the appropriate protein matrix and necessary growth factors should heal. Ulcers that do not heal acutely and become chronic have been shown to have critical deficiencies in these (degraded wound bed matrix and senescent fibroblasts) or other parameters (eg, patient compliance with off-

Figure 2.**TIDE STUDY OUTCOMES: SUBJECTS COMPLETING STUDY TO WEEK 20 AND ACHIEVING WOUND CLOSURE**

n = 51 (Wk 12); n = 46 (Wk 20)

Table 5.**BINARY LOGISTIC REGRESSION ON ULCER HEALING AT WEEK 12, FORWARD SELECTION (ENTRY REQUIREMENT OF $\alpha < .10$) FITTING OF BASELINE DEMOGRAPHIC AND ULCER VARIABLES**

Predictors	<i>P</i> ^a	Odds Ratio	Odds Ratio 95% CI
Blood pressure—diastolic	.065	0.95	(0.89–1.00)
Ulcer area—day 0 (cm ²)	.064	0.89	(0.79–1.01)

Abbreviation: CI, confidence interval.

^aSignificance at $\alpha \leq .05$.

loading regimens). Studies on HFDS have produced clinical and laboratory data that suggest it may address the issue of fibroblast senescence and matrix degradation.²⁹ The HFDS described herein provides fibroblasts that deposit matrix proteins, produce cytokines, and secrete growth factors, which facilitate angiogenesis and keratinocyte migration.^{30–32} It also provides a preformed collagen, fibronectin, and glycosaminoglycan matrix that can facilitate the migration of the patients' epithelial cells to close the wound.²¹

Previous reports of clinical studies on HFDS for the treatment of DFUs support the theory that chronic DFUs are in need of wound healing therapies that address cellular deficiencies.^{33–36} The pivotal clinical study, which was conducted concurrently with the study reported here, was a multicenter, controlled randomized clinical trial in which 314 patients were treated with HFDS plus conventional therapy or conventional therapy alone (debridement, saline-moistened gauze, and off-loading footwear).²⁸ The proportion of patients who achieved complete wound closure by week 12 was 30% for the HFDS group and 18.3% for the control group. These data were statistically significant ($P = .023$) and resulted in approval of HFDS by the FDA for the treatment of DFUs.

The treatment strategy chosen for the TIDE study followed a similar, although less strict, protocol including weekly debridement of the ulcer site, application of HFDS, use of either a foam dressing or saline-moistened gauze, and a prescription for an off-loading device. Although most patients were allowed to be ambulatory, they were instructed to avoid weight bearing on the study leg as much as possible and to use the prescribed device whenever ambulatory. The results of the TIDE study, with a 12-week healing rate of 44%, support the efficacy results of the pivotal study on HFDS. A similar safety profile with respect to the incidence of study ulcer infections was also observed in the TIDE study.

This study demonstrated that HFDS was safe to use and was not associated with the development of any specific adverse event. With respect to the occurrence of adverse events in general, the incidence was in the expected range for this patient population. The differences between the rates of ulcer infection and

cellulitis reported in the TIDE and pilot study on HFDS were negligible, and the rate of osteomyelitis diagnosis was lower (although not statistically significant) in the TIDE study. There were no adverse events reported in this study that were believed to be caused by the use of HFDS, nor were there any unanticipated device effects.

Implantation of a human dermal substitute onto an allogeneic host may be suspected to initiate an immune response leading to its rejection. However, there was no evidence of rejection of HFDS in this study. This is believed to be due to the inherent properties of HFDS. It is derived from neonatal fibroblasts, which have undeveloped HLA tissue markers. Furthermore, fibroblasts from the dermis are relatively nonantigenic, and laboratory tests have shown that a fibroblast-secreted, 3-dimensional extracellular matrix, such as HFDS, does not express HLA-DR, which is necessary for lymphocytic activation.³⁷ Therefore, HFDS is not expected to cause an immune reaction.

The HFDS TIDE study and its results have some shortcomings. The primary deficiency is that the study was not a randomized trial between HFDS and a control or placebo group. The findings reported here are applicable only to HFDS treatment and do not detail efficacy versus another treatment. There were no exclusion criteria regarding ulcer area and duration; however, the median ulcer size (2.1 cm²) and duration (26 weeks) are consistent with chronic, nonhealing DFUs.

In conclusion, HFDS has been shown in this multicenter, prospective study to be clinically effective based on the prospectively defined trial end points. The proportions of patients achieving complete wound closure were 44% and 52% by weeks 12 and 20, respectively, and the median time to healing was 13 weeks. The use of HFDS in the treatment of DFUs was also shown to be safe. No adverse device effects were reported in this study. The data from this study support the current evidence of HFDS as a safe and effective treatment for DFUs. ●

REFERENCES

- Centers for Disease Control and Prevention. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention; 2011. http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf. Last accessed May 3, 2011.
- Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, et al. The global burden of diabetic foot disease. *Lancet* 2005;366:1719-24.
- McNeely MJ, Boyko EJ, Ahroni JH, et al. The independent contributions of diabetic neuropathy and vasculopathy in foot ulceration. How great are the risks? *Diabetes Care* 1995;18:216-9.
- Reiber GE, Vileikyte L, Boyko EJ, et al. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diabetes Care* 1999;22:157-62.
- Mustoe T. Understanding chronic wounds: a unifying hypothesis on their pathogenesis and implications for therapy. *Am J Surg* 2004;187:65S-70S.
- Marston W. Risk factors associated with healing chronic diabetic foot ulcers: the importance of hyperglycemia. *Ostomy Wound Manage* 2006;52:26-39.
- Armstrong DG, Lavery LA, Harkless LB. Validation of a diabetic wound classification system. The contribution of depth, infection, and ischemia to risk of amputation. *Diabetes Care* 1998;21:855-9.
- Moulik PK, Mtonga R, Gill GV. Amputation and mortality in new-onset diabetic foot ulcers stratified by etiology. *Diabetes Care* 2003;26:491-4.
- Jeffcoate WJ, Chipchase SY, Ince P, et al. Assessing the outcome of the management of diabetic foot ulcers using ulcer-related and person-related measures. *Diabetes Care* 2006;29:1784-7.
- Lavery LA, Armstrong DG, Wunderlich RP, et al. Risk factors for foot infections in individuals with diabetes. *Diabetes Care* 2006;29:1288-93.
- American Diabetes Association. Consensus development conference on diabetic foot wound care, April 7-8, 1999, Boston, Massachusetts. *Diabetes Care* 1999;22:1354-60.
- American Diabetes Association. Economic costs of diabetes in the U.S. in 2007. *Diabetes Care* 2008;31:1-20.
- Vileikyte L. Diabetic foot ulcers: a quality of life issue. *Diabetes Metab Res Rev* 2001;17:246-9.
- Ragnarson Tennvall G, Apelqvist J. Health-related quality of life in patients with diabetes mellitus and foot ulcers. *J Diabetes Complications* 2000;14:235-41.
- Prompers L, Huijberts M, Schaper N, et al. Resource utilisation and costs associated with the treatment of diabetic foot ulcers. Prospective data from the Eurodiale Study. *Diabetologia* 2008;51:1826-34.
- Ragnarson Tennvall G, Apelqvist J. Health-economic consequences of diabetic foot lesions. *Clin Infect Dis* 2004;39:S132-9.
- Loots MA, Lamme EN, Mekkes J, et al. Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. *J Invest Dermatol* 1998;111:850-7.
- Lazarus G, Cooper DM, Knighton DR, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. *Arch Dermatol* 1994;130:489-93.
- Blakytyn R, Jude E. The molecular biology of chronic wounds and delayed healing in diabetes. *Diabet Med* 2006;23:594-608.
- Hehenberger K, Heilborn K, Brismar K, et al. Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal dermal fibroblasts treated with high glucose is associated with increased formation of L-lactate. *Wound Rep Regen* 1998;6:135-41.
- Mansbridge JN, Liu K, Pinney RE, et al. Growth factors secreted by fibroblasts: role in healing diabetic foot ulcers. *Diabetes Obes Metab* 1999;1:265-79.
- Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet*. 2005;366:1736-43.
- Wong T, McGrath JA, Navsaria H. The role of fibroblasts in tissue engineering and regeneration. *Br J Dermatol*. 2007;156:1149-55.
- Ehrenreich M, Ruzcjak Z. Update on tissue-engineered biological dressings. *Tissue Eng* 2006;12:2407-24.
- Dinh TL, Veves A. Treatment of diabetic foot ulcers. *Dermatol Ther* 2006;19:348-55.
- Brem H, Sheehan P, Rosenberg HJ, et al. Evidence-based protocol for diabetic foot ulcers. *Plast Reconstr Surg* 2006;117:193S-209S; discussion 210S-211S.
- Hinchliffe RJ, Valk GD, Apelqvist J, et al. A systematic review of the effectiveness of interventions to enhance the healing of chronic ulcers of the foot in diabetes. *Diabetes Metab Res Rev* 2008;24:S119-44.
- Marston WA, Hanft J, Norwood P, Pollak R. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers. *Diabetes Care* 2003;26:1701-5.
- Gentzkow GD, Jensen JL, Pollak RA, et al; The Dermagraft Diabetic Ulcer Study Group. Improved healing of diabetic foot ulcers after grafting with a living human dermal replacement. *Wounds* 1999;11:77-84.
- Pinney E, Liu K, Sheehan B, et al. Human three-dimensional fibroblasts cultures express angiogenic activity. *J Cell Physiol* 2000;183:74-82.
- Jiang WG, Harding KG. Enhancement of wound tissue expansion and angiogenesis by matrix-embedded fibroblast (Dermagraft), a role of hepatocyte growth factor/scatter factor. *Int J Mol Med* 1998;2:203-10.
- Newton DJ, Khan F, Belch JJ, et al. Blood flow changes in diabetic foot ulcers treated with dermal replacement therapy. *J Foot Ankle Surg* 2002;41:233-7.
- Gentzkow GD, Iwasaki SD, Hershon KS, et al. Use of Dermagraft, a cultured human dermis, to treat diabetic foot ulcers. *Diabetes Care* 1996;19:350-4.
- Edmonds ME, Foster AV, McColgan M. 'Dermagraft': a new treatment for diabetic foot ulcers. *Diabet Med* 1997;4:1010-11.
- Eaglstain WH. Dermagraft treatment of diabetic ulcers. *J Dermatol* 1998;25:803-4.
- Hanft JR, Surprenant MS. Healing of chronic foot ulcers in diabetic patients treated with a human fibroblast-derived dermis. *J Foot Ankle Surg* 2002;41:291-9.
- Kern A, Liu K, Mansbridge J. Modification of fibroblast γ -interferon responses by extracellular matrix. *J Invest Dermatol* 2001;117:112-8.