

Graftskin, a Human Skin Equivalent, Is Effective in the Management of Noninfected Neuropathic Diabetic Foot Ulcers

A prospective randomized multicenter clinical trial

ARISTIDIS VEVES, MD
VINCENT FALANGA, MD
DAVID G. ARMSTRONG, DPM

MICHAEL L. SABOLINSKI, MD
FOR THE APLIGRAF DIABETIC FOOT
ULCER STUDY

OBJECTIVE — We assessed in a randomized prospective trial the effectiveness of Graftskin, a living skin equivalent, in treating noninfected nonischemic chronic plantar diabetic foot ulcers.

RESEARCH DESIGN AND METHODS — In 24 centers in the U.S., 208 patients were randomly assigned to ulcer treatment either with Graftskin (112 patients) or saline-moistened gauze (96 patients, control group). Standard state-of-the-art adjunctive therapy, which included extensive surgical debridement and adequate foot off-loading, was provided in both groups. Graftskin was applied at the beginning of the study and weekly thereafter for a maximum of 4 weeks (maximum of five applications) or earlier if complete healing occurred. The major outcome of complete wound healing was assessed by intention to treat at the 12-week follow-up visit.

RESULTS — At the 12-week follow-up visit, 63 (56%) Graftskin-treated patients achieved complete wound healing compared with 36 (38%) in the control group ($P = 0.0042$). The Kaplan-Meier median time to complete closure was 65 days for Graftskin, significantly lower than the 90 days observed in the control group ($P = 0.0026$). The odds ratio for complete healing for a Graftskin-treated ulcer compared with a control-treated ulcer was 2.14 (95% CI 1.23–3.74). The rate of adverse reactions was similar between the two groups with the exception of osteomyelitis and lower-limb amputations, both of which were less frequent in the Graftskin group.

CONCLUSIONS — Application of Graftskin for a maximum of 4 weeks results in a higher healing rate when compared with state-of-the-art currently available treatment and is not associated with any significant side effects. Graftskin may be a very useful adjunct for the management of diabetic foot ulcers that are resistant to the currently available standard of care.

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From the Joslin-Beth Israel Deaconess Foot Center and Harvard Medical School (A.V.), Boston, Massachusetts; the Department of Dermatology and Skin Surgery (V.F.), Roger Williams Medical Center, Providence, Rhode Island; the Boston University School of Medicine (V.F.), Boston, Massachusetts; the Department of Surgery (D.G.A.), Southern Arizona Veterans Affairs Medical Center, Tucson, Arizona; and Organogenesis (M.L.S.), Canton, Massachusetts.

Address correspondence and reprint requests to Aristidis Veves, MD, Joslin Beth Israel Deaconess Foot Center, One Deaconess Rd., Boston, MA, 02215. E-mail: aveves@caregroup.harvard.edu.

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Abbreviations: CTDS, customized tridensity sandal; DFU, diabetic foot ulceration; HSE, human skin equivalent.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Diabetic foot ulceration (DFU) is a major problem that significantly impairs the quality of life of the patient, leads to prolonged hospitalization, and may result in a major amputation. Foot problems affect 15–20% of all individuals with diabetes and are the reason for 20% of all diabetes-related hospital admissions and >50,000 lower-extremity amputations per year in the U.S. (1,2). The cost of DFU is also significant; the attributable cost for a middle-aged diabetic man with a new foot ulcer has recently been estimated to be \$27,987 for the first 2 years after diagnosis (3).

Peripheral neuropathy, deformity, and macrovascular disease were initially thought to be the main reasons for either development of foot ulceration or failure to heal the ulcer and subsequent amputation (4,5). However, over the last decade, additional factors have been found to contribute both to the development of and the failure to heal the DFU. These factors include reduced resistance to infection, functional changes in the microcirculation, and abnormalities in the expression and activity of growth factors and cytokines that are involved in the healing process (6–8). Thus, a consensus has developed that wound healing of the DFU is impaired and that failure to heal in the absence of arterial occlusive disease is not necessarily related to failure to off-load the ulcer area caused by either the use of inadequate techniques or poor patient compliance (9). The recent American Diabetes Association Consensus Development Conference on Diabetic Foot Wound Care concluded that DFU has all of the characteristics of a chronic wound and that a better understanding of the effect of diabetes on wound healing is required for the development of successful treatment techniques (9).

Living human skin equivalents (HSEs), which are produced by using tissue-engineering techniques, have been successful in treating chronic wounds, such as venous ulcers (10). Although their precise mode of

action is not known, it is believed that they act by both filling the wound with extracellular matrix and inducing the expression of growth factors and cytokines that contribute to wound healing. Graftskin (Apligraf; Organogenesis, Canton, MA, and Novartis Pharmaceuticals, East Hanover, NJ) is an allogeneic bilayered cultured skin equivalent that is currently available in the U.S. for the treatment of venous ulcers (11,12). Like human skin, Graftskin has both an upper epidermal and a lower dermal layer and contains human skin cells. The dermal layer is formed by human fibroblasts (dermal cells), which organize the provided structural protein and produce additional matrix proteins. The epidermal layer is formed by prompting human keratinocytes (epidermal cells) first to multiply and then to differentiate to replicate the architecture of the human epidermis. Unlike human skin, Graftskin does not contain structures such as blood vessels, hair follicles, or sweat glands or other cell types such as Langerhans' cells, melanocytes, macrophages, or lymphocytes. Graftskin has been shown to produce all cytokines and growth factors that are produced by the normal skin during the healing process (12).

In this study we evaluated the efficacy of Graftskin, a specific HSE, in the management of chronic DFUs in a prospective randomized multicenter large-scale fashion. Our primary hypothesis was that weekly application of Graftskin for a maximum of five applications would increase the wound healing rate in noninfected nonischemic chronic plantar DFUs.

RESEARCH DESIGN AND METHODS

The main inclusion criteria were type 1 or 2 diabetes, age 18–80 years, HbA_{1c} between 6 and 12%, and full-thickness neuropathic ulcers (excluding the dorsum of the foot and the calcaneus). The ulcer was required to be of ≥ 2 weeks duration and the postdebridement ulcer size had to be between 1 and 16 cm². All patients were also required to have dorsalis pedis and posterior tibial pulses that were audible by Doppler. The main exclusion criteria were clinical infection at the studied ulcer site, clinically significant lower-extremity ischemia (as defined by an ankle/brachial index of < 0.65), active Charcot's disease as determined by clinical and radiographic examination, and an ulcer that was of a non-diabetic pathophysiology (e.g., rheumatoid, radiation-related, and vasculitis-related ulcers). Patients with significant medical

conditions that would impair wound healing were also excluded from the study. These conditions included liver disease, aplastic anemia, scleroderma, malignancy, and treatment with immunosuppressive agents or steroids.

Patients were randomized at the end of the screening visit according to a computer-generated randomization schedule provided by the sponsor. Participating patients were informed about the results of the randomization during their next visit. The clinical study protocols and the informed consent that each patient was required to sign was approved by the appropriate institutional review boards for each participating center.

Protocol

This was a multicenter prospective randomized controlled clinical trial that recruited patients from 24 centers. All patients were required to complete a screening period of 7 days, during which time the healing response to saline-moistened gauze, after aggressive debridement, was measured by wound tracings and investigator assessment. Patients whose ulcers responded to saline-moistened gauze during the screening period, as defined by a 30% decrease in the size of the ulcer, were not entered into the study. All patients entered into the treatment phase of the study were evaluated for efficacy by 12 weeks. Patients were then followed for another 3 months for safety evaluations. During this period, the participants were seen once a month.

Graftskin treatment

At study day 0, Graftskin was applied in the clinic setting using a sterile technique. The ulcer was debrided and irrigated with saline before Graftskin was placed directly over the ulcer site. Any excess edge was trimmed to fit the ulcer. After the Graftskin was applied, the site was covered with a layer of saline-moistened Tegapore (3M Health Care, St. Paul, MN), completely covering the ulcer and extending to the normal surrounding skin. Hypoallergenic tape was used to secure the Tegapore to normal skin. The wound was then covered with a layer of dry gauze, a layer of petrolatum gauze (Kendall Health Care Products, Mansfield, MA), and Kling (Johnson & Johnson Medical, Arlington, TX). Patients in the Graftskin group could have Graftskin reapplied at study weeks 1–4 for a maximum of five applications, if required. If Graftskin appeared to cover the wound at these follow-up visits, no

debridement or new Graftskin application was necessary. Complete dressing changes were performed by the investigator at visits scheduled for weeks 1, 2, 3, and 4. During study weeks 1, 2, 3, and 4, both the control and Graftskin-treated patients returned to the investigator for a mid-weekly visit, at which time the complete dressing was changed again. After the mid-weekly visit, the patients changed only the secondary dressing (dry gauze, petrolatum gauze, and Kling) once daily until the next scheduled weekly visit.

Control treatment

The control treatment was selected because saline-moistened gauze has been determined to be the standard of care by the American Diabetes Association (13). The ulcer was covered with a layer of saline-moistened Tegapore that completely covered the ulcer and was secured by hypoallergenic tape. This primary dressing was then covered with a layer of saline-moistened gauze, followed by a layer of dry gauze and a layer of petrolatum gauze, and wrapped with a layer of Kling. The patient changed only the secondary dressing two times a day from day 0 to study week 5. Complete dressing changes were performed by the investigator at visits scheduled for study weeks 1, 2, 3, and 4. During study weeks 1, 2, 3, and 4, midweekly complete dressing changes were performed according to the regimen previously described for the Graftskin group.

Additional dressing changes

Ulcers in both groups that did not heal by study week 5 were covered with a layer of saline-moistened gauze and a layer of petrolatum gauze and were wrapped with a layer of Kling. Patients changed this dressing two times a day as outpatients and continued to be seen weekly by the investigator for study weeks 6–12.

Customized sandals

All patients were instructed to avoid bearing weight on the affected foot throughout the duration of the study and were required to use either crutches or a wheelchair for the first 6 weeks of the study. All patients were additionally fitted with customized tridensity sandals (CTDSs) from the Foot Comfort Center (Denver, CO) at the initiation of the study. The CTDSs were worn throughout the entire study, or for a minimum 4 weeks after the ulcer achieved complete

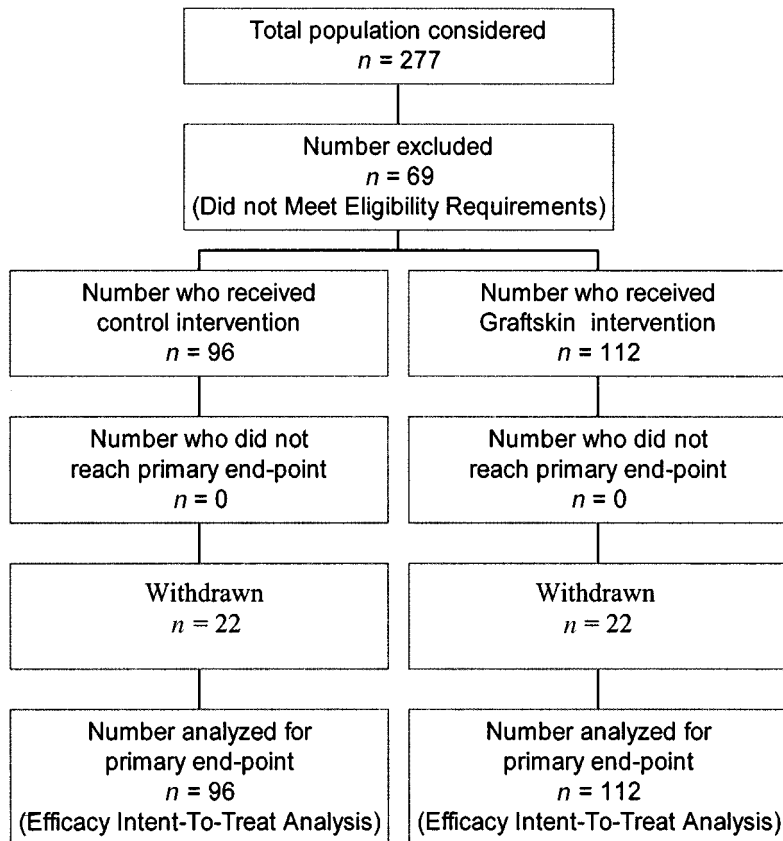


Figure 1—Trial profile.

wound closure. The CTDS was intended to be used as an additional off-loading device in concert with the aforementioned crutches and/or wheelchair. Patients were asked at each visit about their weightbearing status and, if needed, were reinforced to ensure compliance with the non-weight-bearing instructions.

End points

Efficacy parameters were evaluated weekly from study day 0 to study week 12. Complete wound closure was defined as full epithelialization of the wound with the absence of drainage. Wound closure was assessed postdebridement, provided debridement was necessary. Wound closure was also assessed by computerized planimetry of the wound tracing. Secondary end points included improvement in undermining, maceration, exudate, granulation, eschar, and fibrin slough. These points were evaluated by the investigator on each side during every weekly visit. A semiquantitative approach was used

with maceration and exudate graded as nonexistent, mild, moderate, or intense, whereas granulation was graded as covering 0, 0–25, 26–50, 51–75 or 76–100% of the ulcer wound. Eschar and fibrin slough were graded as covering 0, ≤ 50 , and $\geq 50\%$ of the ulcer.

Table 1—Baseline characteristics

Characteristics	Graftskin patients	Control patients
n	112	96
Age (years)	58 \pm 10	56 \pm 10
Sex (M/F)	88/24 (79/21)	74/22 (77/23)
Race (Caucasian/African-American/Hispanic)	77/20/14 (69/18/13)	67/14/13 (70/15/14)
BMI (kg/m ²)	30.9 \pm 6.54	33.1 \pm 7.72
HbA _{1c} (%)	8.6 \pm 1.5	8.6 \pm 1.4
Ankle/brachial index*		
0.65–0.80	10 (8.9)	10 (10.4)
0.80–1.00	50 (35.7)	29 (30.2)
>1.00	59 (52.7)	54 (56.3)
Wound area (cm ²)	2.97 \pm 3.10	2.83 \pm 2.45
Wound duration (months)	11.5 \pm 13.3	11.1 \pm 12.5

Data are means \pm SD or n (%). *Measurements were not performed in three patients in each group.

Safety assessments

Safety was monitored by evaluating the following parameters at the time points indicated: treatment-emergent adverse event reports (each visit after day 0), laboratory assessments (day –7 and week 12 or final study visit), vital signs (day –7 and week 12 or final study visit), and immunological evaluations (day –7, week 4, and week 12 or final study visit).

Statistical analysis

Intention-to-treat analysis was performed, and the complete wound healing rate was evaluated using the Cochran-Mantel-Haenszel test. The median time to closure was estimated by using the Kaplan-Meier lifetable analysis (log-rank test), whereas the estimation of the probability of closure was performed using the Cox's multivariate analysis (Wald χ^2). Multiple regression was performed with the Cox's proportional hazards regression analysis. For all analyses, we used an α value of 0.05.

RESULTS

— During the screening visit, 277 patients were randomized, but 69 were disqualified when they were seen a week later because they did not meet the inclusion/exclusion criteria (Fig. 1). There were 77 reasons that led to the exclusion of these subjects, including wound closure $>30\%$ in 21 (27%), abnormal blood tests and/or HbA_{1c} out of the range in 26 (20%), ulcer characteristics not compatible with inclusion/exclusion criteria in 8 (10%), withdrawal of the informed consent in 6 (8%), osteomyelitis on reviewing the foot X-rays in 5 (6%), infection in 5 (6%), peripheral vascular disease in 3 (4%), active Charcot's disease in 1 (1%), and preexisting medical condition in 1 (1%).

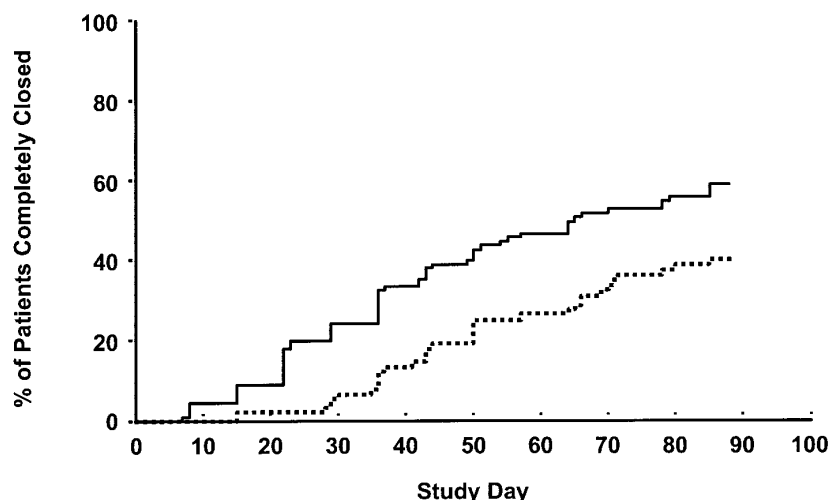


Figure 2—Percentage of wounds healed by study visit. The percentage of wounds that achieved complete healing at each study visit is shown for control (---) and Graftskin-treated (—) wounds. Complete healing was defined as full epithelization in the absence of drainage. Graftskin-treated ulcers received up to five applications of Graftskin during the first 4 weeks of treatment. Control ulcers received moist saline dressings changed two times per day. At the end of the study, a significantly higher percentage of Graftskin-treated patients achieved complete wound healing when compared with patients treated with standard care (56 vs. 38%, $P < 0.01$).

There were no differences in the main characteristics between the patients who were disqualified and those who were enrolled in the study (data not shown).

Of the 208 patients who were enrolled into the treatment phase, 112 were randomized to receive Graftskin treatment, and 96 were randomized to receive control (standard care) treatment. Of the 208 patients, 44 (21%) withdrew before completion of the study at the study month 6 visit, 22 (20%) withdrew from the Graftskin-treated group, and 22 (23%) withdrew from the control group. The reasons for discontinuation before the study month 6 visit were adverse events in 15 (34%), lost to follow-up in 14 (32%), withdrawal of informed consent in 5 (11%), withdrawal based on the investigator judgment in 4 (9%), failure to attend more than two consequent scheduled visits in 2 (5%), noncompliance in 1 (2%), other in 1 (2%), death in 1 (2%), and protocol violation in 1 (2%). However, the data that were collected while the patients were in the study were included in the intention-to-treat analysis.

Efficacy assessment

At baseline, the two groups were similar regarding demographics, type and duration of diabetes, and ulcer size and duration (Table 1). The complete wound healing life table analysis is shown in Fig. 2. By the end

of the study, complete wound healing was achieved in 63 (56%) Graftskin-treated patients—a significantly higher rate when compared with 36 (38%) control subjects ($P = 0.0042$) (Table 2). The odds ratio for complete healing for a Graftskin-treated ulcer compared with a control-treated ulcer was 2.14 (95% CI 1.23–3.74). The Kaplan-Meier median time to complete closure was 65 days for Graftskin—significantly lower than the 90 days observed in the control group ($P = 0.0026$).

Because of the multicenter nature of this trial, each participating center was considered as an independent factor in the analysis. The percentage of Graftskin patients who attained 100% wound closure by or during study week 12 was greater than that of the active control patients, whether the data analyzed over all centers or by individual pooled centers.

To adjust for factors other than treatment that could influence the time to 100% wound closure, a Cox’s proportional hazards regression analysis was used. The fac-

tors examined included pooled center, duration of ulcer (months), baseline ulcer area, glucose control, ulcer location, BMI, presence or absence of a quiescent Charcot deformity, number of ulcers on the target foot, age subgroup, current smoking status, and nutritional status. After adjusting for all factors found in the final model (pooled center, baseline ulcer area, Charcot’s status, and treatment-by-Charcot’s status interaction), treatment was found to have a statistically significant effect on time to closure ($P = 0.0001$). The estimated hazard ratio indicated that an average patient treated with Graftskin had a 1.59-fold better chance for closure per unit time than a patient treated with the active control (95% CI 1.26–2.00).

The average required Graftskin applications per patient during the study were 3.9 (minimum of 1, maximum of 5). More specifically, 10 (9%) patients required one application, 11 (10%) two applications, 15 (13%) three applications, 17 (15%) four applications, and 59 (53%) five applications.

Secondary end points

Between study day 0 and study week 12, both Graftskin and active control groups showed statistically significant improvement in undermining, maceration, exudate, granulation, eschar, and fibrin slough. A statistically significant difference was seen between the two treatment groups with regard to maceration ($P < 0.05$), exudate ($P < 0.05$), and eschar ($P < 0.05$). The study wounds treated with Graftskin showed greater improvement in all of these wound characteristics at study week 12 than wounds treated with control.

Ulcer recurrence

At 6 months, the incidence of ulcer recurrence was similar in the two groups, with 5.9% (3 of 51) in the Graftskin group and 12.9% (4 of 31) in the active control group (NS).

Safety

Because of adverse events, six Graftskin-treated and nine control-treated patients

Table 2—Complete wound closure

Treatment	Kaplan-Meier estimate of time (days) to complete closure			Log-rank test
	Minimum	Median	Maximum	
Graftskin	7	65	88	$P = 0.0026$
Control	15	90	92	

Table 3—Results of safety analysis

	Graftskin	Active control	Fisher's exact test <i>P</i>
<i>n</i>	112	96	
Wound infection at studied ulcer	12 (10.7)	13 (13.5)	0.67
Cellulitis	10 (8.9)	8 (8.3)	1.00
Osteomyelitis	3 (2.7)	10 (10.4)	0.04
Amputations on study limb	7 (6.3)	15 (15.6)	0.028
Reulceration during first 6 months	3 (5.9)	4 (12.9)	0.42

Data are *n* (%).

withdrew before completion of the study. The rate of wound infection, cellulitis, osteomyelitis, and reulceration are shown in Table 3. In summary, there were no differences in all of the measurements except the development of osteomyelitis at the studied ulcer site and amputations at the studied limb, the rates of which were significantly lower in the Graftskin-treated patients.

Immune response

In tests of patients' sera, there were no observations of antibody responses against either bovine type I collagen or the class I HLA antigens on human dermal fibroblasts and human epidermal cells. T-cell-specific responses were not observed against bovine type I collagen, human fibroblasts, or human keratinocytes. There was also no clinical evidence of Graftskin rejection by any patient.

CONCLUSIONS — In the present study, we have shown that the complete wound healing rate was significantly increased in Graftskin-treated patients with chronic plantar DFU when compared with patients treated with the standard state-of-the-art care available at present. This was accompanied by a significant reduction in the median time required to heal the ulcer, and the rate of adverse reaction was similar in the two groups. Thus, the odds ratio for complete healing for a Graftskin-treated patient was 2.14 compared with a patient treated with standard care during the 12-week period of the study.

The treatment of the chronic DFU requires a multidisciplinary approach that involves extensive surgical debridement of the ulcer area, adequate off-loading of the foot, aggressive treatment of infection, and restoration of blood flow in the peripheral arteries when necessary (9,13–15). All of these requirements were very carefully met in this study, and this can be clearly seen in the control group's satisfactory healing rate,

which was similar to the rate that has been reported in previous well-conducted trials (16–20). Furthermore, the observed healing rate was higher than that reported in a meta-analysis that included 10 control groups, further underlining the emphasis that was given to the provision of state-of-the-art standard care to all participants (21). Therefore, treatment with Graftskin should be considered as an adjunct to the currently recommended standard care and not as a substitute for it.

An average of 3.9 Graftskin applications was performed during the study. As complete wound healing was achieved in a considerable number of patients after one or two applications, it may be proved that weekly application is not necessary in all patients. As an alternative, we believe that a single application can be contemplated and the clinician may defer a second application if a successful wound closure rate is achieved to indicate complete wound healing needing no further intervention.

Ulcer recurrence is common in diabetic patients, and a history of previous foot ulceration is a strong predicting factor of the development of foot ulceration in the future (22). In the present study, the rate of reulceration was very satisfactory in both groups, a fact probably related to the support that was provided to the participants. In addition, it is encouraging that the rate in the Graftskin-treated patients was slightly lower than that observed in the control group. This finding may suggest that application of Graftskin results in healed wounds that are at least as viscoelastically resolute as wounds that healed strictly by secondary intention.

Improvements in both our understanding of the wound healing mechanisms and the field of bioengineering have led to the development of various products, such as new wound dressings, growth factors, and living skin equivalents (16–20). Combined usage of these techniques may further improve the wound healing rate, but because they can also be expensive, it is

currently recommended that such procedures should be avoided until the conduction of well-controlled clinical trials (9).

Graftskin has been shown in previous studies not to elicit an immunological response from the host, and this finding was confirmed in this study (10,23). Graftskin was also not associated with any other adverse effects, such as wound infection and cellulitis, when compared with the control group. Furthermore, the rate of osteomyelitis and lower limb amputations (including both minor and major ones) was significantly lower in the Graftskin group. Although these findings were not primary end points in this study, they are encouraging and indicate that Graftskin can prevent the deep tissue infection that leads to amputation. Further studies will be required to confirm these findings.

The healing rate in Graftskin-treated patients was higher in the best recruiting centers, reaching a level of 75% in the best recruiting center (24). This finding indicates that a learning curve exists and that optimal results should be expected after a certain experience has been gained in Graftskin application. It may also reemphasize the need to treat DFU in well-organized centers that have a large volume of patients, allowing the development of a multidisciplinary approach.

It should finally be emphasized that Graftskin application carries a considerable cost and should therefore be reserved for chronic foot ulcers that have failed to respond to the currently available standard care. Studies are being conducted to evaluate the cost efficacy of Graftskin treatment application, and these data will hopefully be available soon. Nevertheless, the staggering cost of foot ulceration as reported by Ramsey et al. (3) indicate that even expensive new modalities may be cost-effective in the long term. That Graftskin reduced the median time required to heal the ulcers by 25 days also supports this suggestion, but the final judgment should be withheld until the data regarding cost efficacy become available.

In summary, in the present study we have shown, in a randomized prospective controlled fashion, that weekly application of Graftskin for a maximum of 4 weeks results in a higher healing rate when compared with state-of-the-art currently available standard treatment and is not associated with any significant side effects. Graftskin may be a very useful adjunct for the management of DFUs that are resistant to the currently available standard of care.

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APPENDIX

Investigators for the Apligraf Diabetic Foot Ulcer Study

Investigators were: Geoffrey M. Habershaw, DPM, Hau T. Pham, DPM, James S. Chrzan, DPM, John M. Giurini, DPM, Thomas Lyons, DPM, and Bary Rosenblum, DPM, of the Deaconess-Joslin Foot Center, Boston, MA; David P. Fivenson, MD, and Michelle Choucair, MD, of the Henry Ford Hospital, Detroit, MI; Joel A. Block, MD, and Margaret Michalska, MD, of the Rush Presbyterian Center for Clinical Studies, Chicago, IL; James W. Snyder, MD, of Southwest Medical Associates, Las Vegas, NV; Richard A. Pollak, DPM, MS, of San Antonio Podiatry Associates, San Antonio, TX; Joel M. Lerner, DPM, and Michael B. Lerner, DPM, of Foot and Ankle Physicians, PA, Union, NJ; James M. Poindexter Jr., MD, of Georgia Vascular Surgery, The Institute for Wound Care, Atlanta, GA; Caroline E. Fife, MD, of the Hermann Center for Wound Healing, Houston, TX; Lloyd E. King Jr., MD, PhD, of Vanderbilt University, Nashville, TN; Michael A. Pfeifer, MD, of the Diabetes and Obesity Center, East Carolina University, Greenville, NC; Anna Falabella, MD, of the University of Miami School of Medicine, Miami, FL; Elliot L. Chaikof, MD, of the Emory University School of Medicine, Atlanta, GA; David Bright, MD, of Medical Partners of Martin County, Stuart, FL; Michael A. Machtinger, DPM, of the VA Medical Center, West Palm Beach, FL; Scott Lipkin, DPM, and David Steed, DPM, of Lehigh Valley Podiatry, Allentown, PA; Kenneth Hershon, MD, and Zevi W. Isseroff, DPM, of North Shore Diabetes & Endocrine Associates, New Hyde Park, NY; Lesley Wong, MD, of the Johns Hopkins Bayview Medical Center, Baltimore, MD; Charles J. Shuman, DPM, and Janette A. Thompson, DPM, of the VA Medical Center, Washington, DC; Lawrence B. Harkless, DPM, of the University of Texas Health Science Center at San Antonio, San Antonio, TX; Brent Nixon, DPM, of the VA Medical Center of Tucson, Tucson, AZ; Robert Mendicino, DPM, of the Foot and Ankle Institute of Western Pennsylvania, Pittsburgh, PA; Mark Lipman, MD, of the Center for Clinical Studies of Infectious Diseases, Sarasota, FL; Joshua M. Bernard, MD, of the University of

South Florida, Tampa, FL; and Peter Sheehan, MD, of the Staten Island University Hospital, Staten Island, NY.

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