HYAFF 11–Based Autologous Dermal and Epidermal Grafts in the Treatment of Noninfected Diabetic Plantar and Dorsal Foot Ulcers

A prospective, multicenter, controlled, randomized clinical trial

Carlo Caravaggi, md[1]
Roberto De Giglio, md[1]
Chiara Pritelli, md[1]
Manuela Sommaria, md[1]
Sergio Dalla Noce, md[1]
Ezio Faglia, md[2]
Manuela Mantero, md[2]
Giacomo Clerici, md[3]
Pietro Fratino, md[3]
Luca Dalla Paola, md[4]
Giulio Mariani, md[5]
Roberto Mingardi, md[6]
Alberto Morabito, phd[7]

OBJECTIVE — To evaluate the clinical efficacy and safety of HYAFF 11–based autologous dermal and epidermal grafts in the management of diabetic foot ulcers.

RESEARCH DESIGN AND METHODS — A total of 79 patients with diabetic dorsal (n = 37) or plantar (n = 42) ulcers were randomized to either the control group with nonadherent paraffin gauze (n = 36) or the treatment group with autologous tissue-engineered grafts (n = 43). Weekly assessment, aggressive debridement, wound infection control, and adequate pressure relief (fiberglass off-loading cast for plantar ulcers) were provided in both groups. Complete wound healing was assessed within 11 weeks. Safety was monitored by adverse events.

RESULTS — Complete ulcer healing was achieved in 65.3% of the treatment group and 49.6% of the control group (P = 0.191). The Kaplan-Meier mean time to closure was 57 and 77 days, respectively, for the treatment versus control groups. Plantar foot ulcer healing was 55% and 50% in the treatment and control groups, respectively. Dorsal foot ulcer healing was significantly different, with 67% in the treatment group and 31% in the control group (P = 0.049). The mean healing time in the dorsal treatment group was 63 days, and the odds ratio for dorsal ulcer healing compared with the control group was 4.44 (P = 0.037). Adverse events were equally distributed between the two groups, and none were related to the treatments.

CONCLUSIONS — The autologous tissue-engineered treatment exhibited improved healing in dorsal ulcers when compared with the current standard dressing. For plantar ulcers, the off-loading cast was presumably paramount and masked or nullified the effects of the autologous wound treatment. This treatment, however, may be useful in patients for whom the total off-loading cast is not recommended and only a less effective off-loading device can be applied.

Diabetes Care **26:2853–2859, 2003**

From the [1]Centre for the Study and Treatment of Diabetic Foot Pathology, Ospedale di Abbiategrasso, Milan, Italy; the [2]Policlinico Multimedica, Sesto San Giovanni, Milan, Italy; the [3]Centro per la Prevenzione e la Cura del Piede Diabetico–Fondazione Maugeri, Pavia, Italy; the [4]Casa di Cura Villa Berica, Vicenza, Italy; the [5]Divisione Medicina, Ospedale San Carlo, Milan, Italy; the [6]Ospedale San Bortolo, Vicenza, Italy; and the [7]Institute of Medical Statistics and Biometry, University of Milan, Milan, Italy.

Address correspondence and reprint requests to Dr.Carlo Caravaggi, Centre for the Study and Treatment of Diabetic Foot Pathology, Ospedale di Abbiategrasso (MI), Piazza Mussi, 1, 20081 Abbiategrasso (MI), Italy. E-mail: carlo.caravaggi@fastwebnet.it.

Received for publication 25 February 2002 and accepted in revised form 12 July 2003.

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

The current standard treatment for foot ulcers consists of debridement, treatment of infection, pressure relief, and arterial revascularization, if required (1). The risk of infection to the deep tissues and bone structures depends on how long the skin lesion remains unhealed. Pressure off-loading has been demonstrated to be of paramount importance in the healing of plantar neuropathic ulcers in short amounts of time (2,3). There are many reports of high percentage rates of plantar ulcer healing in 6–10 weeks under a total contact cast (2–9). This technique of pressure relief is now widely recognized as the "gold standard" in diabetic foot ulcer care in terms of quality of pressure off-loading and time to healing (10).

In the last few years the use of modern dressing technology has opened the way to a more physiological approach to the repair process, providing an optimal, moist wound environment and good control of exudate. Even so, the use of nonadhesive paraffin-impregnated dressings is currently considered a standard care measure. Only very recently have allogenic skin substitutes been made available through tissue engineering techniques, thus making it possible to significantly reduce healing times in neuropathic diabetic plantar foot ulcers. Dermagraft (Smith & Nephew, Hull, U.K.) (11,12) has been studied in patients with diabetic foot ulcers. In a recent study, Pollak et al. (13) studied 281 patients with diabetic foot ulcers to compare Dermagraft with conventional treatment. The study results showed that 50.8% of the Dermagraft-treatment group exhibited complete wound healing at 12 weeks as opposed to 31.7% in the control group (P = 0.006). At week 32, there was continued improvement in healing compared with control subjects (57.7 vs. 42.4%, P = 0.039).

Graftskin (Apligraf; Novartis), a bilayered living human skin equivalent (14,15), was compared in a large, randomized study by Veves et al. (16) with saline-moistened gauze in 208 patients. At the 12-week follow-up, 63 (56%) patients treated with the human skin equivalent and 36 (38%) control patients were healed ($P = 0.0042$). The Kaplan-Meier median time to closure was 65 days for the human skin equivalent and 90 days for control treatment ($P = 0.0026$).

Regranex (Beclapermin; Ortho-McNeil Pharmaceuticals), a recombinant human platelet-derived growth factor-BB–based treatment, has been compared with placebo gel in 382 patients with diabetic foot ulcers (17). Results showed a greater incidence of closure (50 vs. 35% of patients; $P = 0.007$) and a decrease in the median time to healing (86 vs. 127 days; $P = 0.013$). Steed et al. (18) found 48% of the recombinant human platelet-derived growth factor group to have healed compared with 25% of the placebo group ($P = 0.01$).

In recent years, grafts formed from autologous fibroblasts and keratinocytes grown on a scaffold made from the benzyl ester of hyaluronic acid have been developed (19). The benzyl ester of hyaluronic acid is an ideal material for this purpose, as it is biocompatible and resorbable and integrates with ulcer tissues (20–22). In a recent observational study, autologous fibroblast and keratinocyte grafts cultivated on this hyaluronic acid–derived scaffold (Hyalograft 3D and Laserskin Autograft, respectively; Fidia Advanced Biopolymers, Abano Terme, Italy) were evaluated by our group in 60 patients suffering from diabetic foot ulcers (23). A healing rate of 91.3% was observed in a mean healing time of 72.7 ± 48.2 days. No treatment-related side effects were observed. These findings have been corroborated by Faglia et al. (24) on diabetic ulcers, Hollander et al. (25) in a study on patients with extensive, traumatic, soft tissue defects, and by Harris et al. (26) in a burn study.

The promising results from these studies encouraged us to assess the efficacy of autologous fibroblasts and keratinocytes in a prospective, randomized, multicenter study. The selected comparator was nonadherent paraffin gauze, based on the meta-analysis of standard treatments conducted by Margolis et al. (27).

RESEARCH DESIGN AND METHODS

This was an open, stratified, randomized, and controlled multicenter study recruiting patients with diabetic foot ulcers from six centers in Italy. The ethics committee of each center reviewed and approved the study protocol.

The main inclusion criteria were type 1 or type 2 diabetes, an ulcer ≥ 2 cm^2 on plantar surface or dorsum of the foot without signs of healing for 1 month, Wagner score 1–2, $TcPO_2$ ≥ 30 mmHg, and ankle brachial pressure index (ABPI) ≥ 0.5. The main exclusion criteria were ulcers with clinical infection, exposed bone, osteomyelitis diagnosed by radiography, inability to tolerate an off-loading cast, and poor-prognosis diseases. The study involved a screening visit in which eligible patients with either a dorsal or plantar ulcer were identified, enrolled, and randomized into one of two treatment groups. Written informed consent was obtained from all patients, and all baseline data and tests results were recorded. Randomization was done by telephone, and the randomization list was generated and held by the sponsor.

Patients in the treatment group (autologous grafting) were also subjected to HIV, hepatitis C virus, and HbsAg blood tests and a skin biopsy. Before the study and throughout the study period, all ulcers were subjected to an aggressive and extensive debridement to remove necrotic tissue and to control infection. In case of wound infection during the study period, an appropriate antibiotic therapy was prescribed.

The application of the standard dressing began at the screening visit for both treatment groups. After 15 days of run in (i.e., at visit 1) all patients with an ulcer area <1 cm^2 were excluded from the study. Patients with an ulcer area ≥ 1 cm^2 received either autologous fibroblasts on Hyalograft 3D (treatment group) or continued treatment with the nonadherent paraffin gauze (control group). All patients attended weekly visits for 11 weeks or until the ulcer healed, whichever came first. At each weekly visit a detailed description of the wound bed, an ulcer area tracing (performed using a transparent plastic grid, Op-Site; Smith & Nephew), and a photograph of the wound were recorded. The wound area was later calculated by computerized morphometric measurement. If clinical signs of infection were evident, a swab for bacteriological analysis was taken, and the microbiological results were recorded. Wound infection was treated with appropriate systemic antibiotics.

Autologous graft treatment

A skin biopsy (1–2 cm^2, 0.8 mm deep) was taken from each patient randomized to the treatment group and sent to the TissueTech Autograft laboratory (Fidia Advanced Biopolymers, Abano Terme, Italy) for fibroblast and keratinocyte cell culturing. Human fibroblasts and keratinocytes were isolated and propagated for subsequent passaging for ~14 days. The cells were then seeded on two distinct biodegradable scaffolds composed entirely of a benzylic ester of hyaluronic acid; these were a three-dimensional nonwoven scaffold and a lamina containing laser-drilled microperforations to allow for migration of keratinocytes onto the wound bed, respectively. Starting on day 8 from seeding, the dermal grafts or the epidermal sheets were ready for transplantation (19–26). Patients in the treatment group received autologous fibroblasts on Hyalograft3D, which was grafted onto the debrided and cleansed wound and covered with nonadherent paraffin gauze and a secondary dressing made of sterile cotton pads and gauze. If a second graft was required, the wound was cleansed with physiologic solution and a second graft applied.

Approximately 7–10 days after Hyalograft3D grafting, the ulcer received autologous keratinocytes grown on Laserskin that was covered and dressed as before. If required, a second autologous keratinocyte graft was permitted. The graft and retaining nonadherent paraffin gauze had to be left on for ≥ 7 days before removal. The secondary dressing could be changed after 5 days from graft application or earlier if the wound was exuding heavily. Seven days after grafting, all patients in the treatment group, as instructed by the investigator, changed the nonadherent paraffin gauze every 2 days at home after cleaning the ulcer with physiologic solution.

Control treatment

Patients in the control group were treated with nonadherent paraffin gauze (Jelonet; Smith & Nephew) covered with a traditional absorbent secondary dressing of sterile cotton pads and gauze. Control

Table 1—*Details of discontinued patients*

Center/ patient	Reason for discontinuation	Group	Ulcer location	Description	Severity	Relationship to treatment:
1/6	Serious AE	Control	Dorsal	Exposition of first metatarsal head	Severe	Unlikely
1/7	Serious AE	Control	Plantar	Phlegmon right foot	Severe	Unlikely
1/13	Serious AE	Control	Dorsal	Phlegmon	Severe	Unlikely
1/17	Serious AE	Control	Dorsal	Infection of dorsal ulcer left foot	Severe	Unlikely
1/37	Investigator decision	Control	Plantar	—	—	—
1/41	Serious AE	Control	Dorsal	Osteomielitis of fifth metatarsal right foot	Severe	Unlikely
1/18	Serious AE	Treated	Plantar	Phlegmon in region of the ulcer	Severe	Not clear
2/4	Serious AE	Treated	Dorsal	Fracture of the right femoral head	Severe	Unlikely
2/23	Serious AE	Treated	Dorsal	Restenosis after peripheral transcutaneous angioplasty	Moderate	Unlikely
2/3	Protocol violation	Control	Plantar	Patient did not attend the 77th-day visit	—	—
2/13	Protocol violation	Control	Dorsal	Patient did not attend two consecutive visits	—	—
2/19	Protocol violation	Control	Plantar	Patient did not attend two consecutive visits	—	—
2/21	Protocol violation	Control	Plantar	Area <1 cm^2 at baseline	—	—
2/14	Protocol violation	Treated	Dorsal	Patient did not attend two consecutive visits	—	—
2/16	Protocol violation	Treated	Dorsal	Patient did not attend two consecutive visits	—	—
2/18	Protocol violation	Treated	Dorsal	Area <1 cm^2 at baseline	—	—
2/9	Protocol violation	Treated	Plantar	Area <1 cm^2 at baseline	—	—
6/1	Protocol violation	Treated	Plantar	Patient did not attend the 77th-day visit	—	—

AE, adverse event.

group visits and dressing changes were scheduled as in the treatment group.

Plantar ulcers

All patients with plantar ulcers in both groups received pressure relief of the affected limb(s) using a nonremovable fiberglass off-loading cast (28) with a window to permit ulcer inspection and conduct dressing changes.

Dorsal ulcers

All patients with dorsal ulcers in both treatment groups received therapeutic shoes (rigid sole and dorsal closure with Velcro) specifically designed to comfortably fit the dressed foot. For all patients in this study, the essentials of foot ulcer care, namely debridement, adequate pressure relief, and treatment of infection, were provided as required by current international guidelines (1).

End points

The primary efficacy parameters (percentage of healed ulcers and time to closure, i.e., complete re-epithelialization without residual exudate or crusting) were evaluated by the investigators at every weekly visit. The secondary efficacy parameters included presence of fibrous slough and necrotic tissue, appearance of granulation tissue, maceration, presence and amount of exudate (graded as absent, slight, moderate, or copious), and presence of odor and infection. Pain intensity and frequency were recorded using a visual analog scale (range = 0–10).

Statistical analysis

The sample size was calculated at 78 subjects to detect any statistically significant differences in the rate of ulcer healing between the treatment and control groups. This was based on $\alpha = 0.05$, $\beta = 95\%$, and an estimated mean healing time of 30 days with 70% healing in the treatment group and 30% in the control group. Intention-to-treat analysis was performed on all patients who were randomized and included after the run-in period.

The difference in continuous variables was analyzed by Student's *t* test and qualitative variables by Fisher's exact test. The median time to closure was estimated by using the Kaplan-Meier life table approach (log-rank test). All analyses were performed two-tailed, with an α value of 0.05. The statistical analysis was conducted using SAS statistical software (SAS, Cary, NC). An additional meta-analysis of the studies of Apligraf, Dermagraft, and Regranex by Pollak et al. (13), Veves et al. (16), and Wieman et al. (17), respectively, and of our study was performed using the method of Der Simonian and Laird (29).

RESULTS — A total of 82 patients with foot ulcers were screened and randomized to one of the two study groups. Only 79 patients with ulcers were included in the intention-to-treat analysis of the study and were analyzed because during the run-in period, two ulcers were excluded due to an area <1 cm^2 and one because of severe acute ischemia of the foot. Of the plantar ulcers included in the run-in period, 72.5% remained ≥ 2 cm^2 and of the dorsal ulcers, 67.6% remained ≥ 2 cm^2. It was not felt that this healing rate influenced the study results.

Of the 79 ulcers analyzed, 36 were assigned to the control group (16 dorsal and 20 plantar) and 43 to the treatment group (21 dorsal and 22 plantar). Overall, 37 ulcers were dorsal and 42 were plantar. In the control group, 10 patients withdrew before completion of the study, 5 because of serious adverse events, 1 based on investigator decision, and 4 for protocol violations. In the treatment group, eight patients withdrew before completion of the study, three because of serious adverse events and five for protocol violations. Details of discontinued patients are presented in Table 1.

Primary efficacy parameters

At baseline the two groups were similar in regard to clinical characteristics (Tables 2 and 3). At the end of the study (i.e., 11

Table 2—*Baseline characteristics*

	Total population	Dorsal ulcers	Plantar ulcers
Control group			
n	36	16	20
Diabetes			
Type 1	3 (8.0)	2 (12.0)	1 (5.0)
Type 2	33 (92.0)	14 (88.0)	19 (95.0)
$TcPo_2$ (mmHg)	48.5 (20.5)	44 (15.5)	49.5 (21)
Ankle brachial index	0.7 (0.22)	0.75 (0.4)	0.7 (0.22)
HbA_{1c} (%)	8.1 ± 2.25	7.9 ± 1.52	8.3 ± 2.75
Treatment group			
n	43	21	22
Diabetes			
Type 1	9 (20.93)	5 (24.0)	4 (18.0)
Type 2	34 (79.07)	16 (76.0)	18 (82.0)
$TcPo_2$ (mmHg)	48.0 (24.0)	42.0 (14.0)	60.0 (20.0)
Ankle Brachial Index	0.73 (0.3)	0.7 (0.36)	0.8 (0.2)
HbA_{1c} (%)	7.9 ± 2.13	7.6 ± 1.88	8.2 ± 2.33

Data are *n* (%); median (interquartile range) for $TcPo_2$, ankle brachial index, and HbA_{1c}; or means ± SD.

weeks) complete wound healing was achieved in 65.3% of the treatment group ulcers versus 49.6% of the control group ulcers ($P = 0.191$, log-rank test). The Kaplan-Meier median time for complete ulcer healing was 57 and 77 days for the treatment and control groups, respectively.

In the plantar ulcer subgroup, the number of ulcers healed at the final visit in the treatment group was 55% (12 of 22) versus 50% (10 of 20) in the control group ($P = 1.00$). The Kaplan-Meier median time to complete closure of plantar ulcers was 57 days for the treatment group and 58.5 days for the control group (Fig. 1).

In the dorsal ulcer subgroup, the number of ulcers healed at the end visit in the treatment group (14 of 21, 66.7%) was significantly higher when compared with the control group (5 of 16, 31.25%) ($P = 0.049$). The odds ratio for complete healing for a dorsal ulcer in the treatment group compared with the control group was 4.44 (95% CI 1.09–17.7, $P = 0.037$). The Kaplan-Meier mean time to complete closure in the dorsal ulcer population was 63 days for the treatment group and was not available for the control group because complete closure was not apparent at 77 days, the final scheduled visit (Fig. 1).

Of the 43 ulcers in the treatment group, 83.7% ($n = 36$) and 16.3% ($n = 7$) received one or two grafts of autologous fibroblasts on Hyalograft3D, respectively, and 95.4% ($n = 41$) and 4.6% ($n = 2$) received one or two grafts of autologous keratinocytes on Laserskin, respectively. Applications of first and subsequent grafts, as appropriate, were unremarkable.

In addition to the intention-to-treat analysis, a per-protocol analysis was conducted on the data available from those patients who completed the study to assess the robustness of the outcomes. Per-protocol analysis was done on the completed set comprising 61 ulcers, i.e., 26 ulcers in the control group and 35 in the treatment group (18 ulcers were not followed according to the protocol). Complete wound healing was achieved in 63.7% of the treatment group ulcers versus 50% of the control group ulcers ($P = 0.332$, log-rank test) with a median time for complete ulcer healing of 59 days for the treatment group and >77 days for the control group.

The four clinical studies that were included in the meta-analysis (Table 4) were considered for determining a pooled effect of the treatment (difference in wound healing proportion, WD = 0.1164, $P = 0.002$), with no statistical significance emerging from the heterogeneity χ^2 test (heterogeneity = 4.71 − degrees of freedom [df] = 4, $P = 0.319$). Thus, after observing the behavior of the two subgroups in our study, only the group with dorsal ulcers obtained any statistically significant benefit from the treatment.

Secondary efficacy parameters

Secondary efficacy parameters (presence of fibrous slough, necrotic tissue, granu-

Table 3—*Clinical characteristics of the ulcers at baseline*

	Total population	Dorsal ulcers	Plantar ulcers
Control group			
n	36	16	20
Area of ulcer (cm_2)	6.2 ± 7.58	8.3 ± 9.67	4.5 ± 4.86
Depth of ulcer (mm)	8.0 ± 5.46	9.9 ± 6.04	6.3 ± 4.35
Duration of ulcer (months)	4.0 (6.0)	4.0 (10.0)	3.5 (6.0)
Localization of the ulcer (*n*)			
Forefoot	24	13	11
Midfoot	7	1	6
Hindfoot	2	1	1
Not specified	3	1	2
Treatment group			
n	43	21	22
Area of ulcer (cm^2)	5.3 ± 6.76	4.6 ± 5.74	5.9 ± 7.69
Depth of ulcer (mm)	6.1 ± 5.68	8.2 ± 6.57	4.0 ± 3.81
Duration of ulcer (months)	4.0 (10.0)	4.0 (3.0)	4.0 (10)
Localization of the ulcer (*n*)			
Forefoot	31	18	13
Midfoot	7	3	4
Hindfoot	3	—	3
Not specified	2	—	2

Data are means ± SD or median (interquartile range).

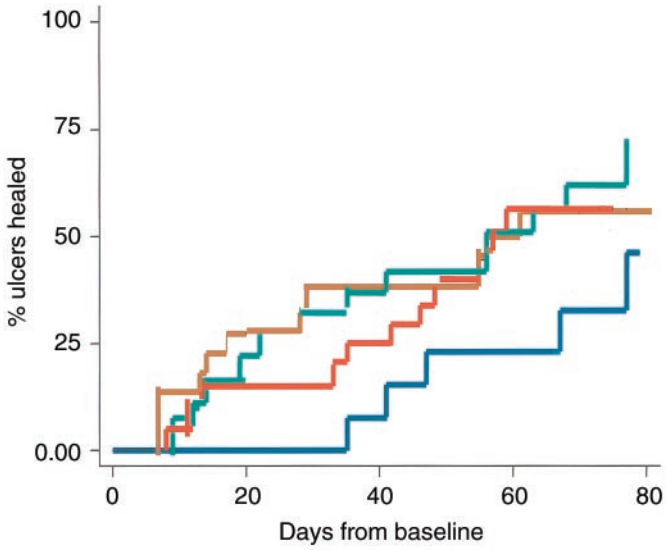

Figure 1—*Percentage of wounds healed by study visit and by subpopulation of randomization: Kaplan-Meier survival plot. Green line, treatment group (Hyalograft3D + Laserskin) with plantar ulcer; yellow line, control group (nonadherent paraffin gauze) with plantar ulcer; red line, treatment group (Hyalograft3D + Laserskin) with dorsal ulcer; blue line, control group (nonadherent paraffin gauze) with dorsal ulcer.*

lation tissue, maceration, exudate, odor, infection, and pain symptomatology) were analyzed, and both groups showed an improvement in these parameters, the treatment group showed greater improvement than the control group as far as exudate presence. At the end of the study, exudate was absent in 86 and 69.4% of the treatment versus control groups, respectively, with a statistically significant difference in dorsal ulcers. It was absent in 31.3 versus 71.4% (visit 7, $P = 0.036$) and in 50 versus 90.5% (visit 12, $P = 0.013$) in the control and treatment groups, respectively. For those ulcers that did not heal during the study period, a mean percentage reduction in size from baseline was recorded for each treatment group. For plantar ulcers in this category, the mean reduction ± SD was −64.7 ± 34.7% and −61.1 ± 26.0% for the control and treatment groups, respectively ($P = 0.823$). For the dorsal ulcers that did not heal, the mean reduction was −32.9 ± 35.1% in the control group and −68.0 ± 37.3% in the treatment group ($P = 0.072$).

Safety

Twenty-two adverse events were reported from the 82 randomized patients (26.8%). These events were equally distributed between the two groups. Of these, 17 (10 in the control group and 7 in the treatment group) were classified as serious adverse events. The most frequent adverse events included wound infection, inflammation, and worsening of ischemia. Nine subjects (six in the control group and three in the treatment group) were withdrawn from the study because of serious adverse events. The grade of event was rated "severe" in eight cases (36.4%), "moderate" in six (36.4%), and "low" in eight (36.4%). No adverse event was determined to be related to any of the products used in the study. Due to the low number of adverse events, no statistical comparison was made.

CONCLUSIONS — This first, randomized, multicenter, controlled clinical study on autologous tissue-engineered products shows promising results for the treatment group. Statistically significant differences in the treatment outcomes were demonstrated for the dorsal ulcer subgroup. These conclusions are based on the intention-to-treat analysis; a per-protocol analysis was conducted (but not completely reported here) to assess the robustness of the results. Both analyses showed ostensibly similar outcomes. The safety and tolerability were excellent; no adverse event related to the study treatment occurred. The safety profile was, as such, closely comparable with that reported for other tissue-engineered treatments for diabetic foot ulcers.

The number of ulcers healed in the dorsal population of the treatment group was significantly higher when compared with that of the control group. Different results were obtained in the plantar ulcer group, in which the autologous grafts seemed not to sufficiently increase the healing rate compared with the standard dressing treatment. It must be emphasized that in this study the fiberglass off-loading cast was utilized as the pressure relief apparatus in the treatment of all plantar ulcers. The results of this study confirm that an effectively off-loaded plantar ulcer heals with either the use of a traditional wound dressing or an autologous graft. It is clear, therefore, that what is fundamental to the healing of plantar diabetic foot ulcers is not the type of dressing, but rather correct debridement and off-loading of pressure to the foot.

Recently, two randomized and controlled studies of Apligraf and Dermagraft (13,16) have evaluated the efficacy of allogenic dermal substitutes, and one controlled randomized study of Regranex

Table 4—*Meta-analysis*

Study (reference no.)	Difference in wound healing proportion	95% CI	Treatment group % healing	Control group % healing
Dermagraft (13)	0.0678	−0.054423 to 0.190144	0.385	0.317
Apligraf (16)	0.1875	0.054011 to 0.320989	0.563	0.375
Regranex (17)	0.0809	−0.021649 to 0.183638	0.427	0.346
Hyalograft3D (plantar)	0.0454	−0.256722 to 0.347631	0.545	0.500
Hyalograft3D (dorsal)	0.3541	0.050469 to 0.657865	0.667	0.313
Pooled WD (29)	0.1164*	0.043244 to 0.189687		

*Estimate of between-study variance ($\tau^2 = 0.0011$) − test of difference in wound healing (WD) = 0 ($z = 3.12$, $P = 0.002$).

(17) assessed the topical use of recombinant human platelet-derived growth factor-BB in comparison with a traditional wound dressing. The off-loading techniques used in these studies were crutches, wheelchairs, or footwear with cushioning insoles; it has been demonstrated that these off-loading techniques do not permit total off-loading of the ulcer (10). The only substantial differences between the plantar ulcers treated in these studies and those in our study are the pressure off-loading technique and the study duration, which was 12 weeks in the three studies and 11 weeks in our study. It is important to highlight that only in our study, in which a gold standard total off-loading was applied to plantar ulcers, no statistically significant difference was detected between the control and the treatment groups.

The results of this clinical study clearly show that the use of total off-loading is so important to the tissue repair process in plantar ulcers that the efficacy of fibroblasts on Hyalograft3D and keratinocytes on Laserskin cannot be differentiated from control techniques. However, considering the favorable results in the previously mentioned studies, in which allogenic dermal substitutes and platelet-derived growth factor gel were used for treatment of neuropathic plantar ulcers without off-loading casts, and on the basis of the significantly positive results obtained on dorsal ulcers treated with autologous grafting, the use of this autologous graft system should also be considered in the management of neuropathic plantar ulcers in patients for whom the use of a total off-loading cast is not recommended, provided that adequate measures to ensure pressure relief are taken.

Acknowledgments— This study was supported by a research grant from Fidia Advanced Biopolymers (Abano Terme, Italy).

References

1. American Diabetes Association: Foot care in patients with diabetes mellitus (Position Statement). *Diabetes Care* 19 (Suppl. 1):S23–S24, 1996
2. Armstrong DG, Lavery LA, Bushman TR: Peak foot pressures influence the healing time of diabetic foot ulcers treated with total contact casts. *J Rehab Res Dev* 35:1–5, 1998
3. Armstrong DG, Nguyen HC, Lavery LA, van Schie CHM, Boulton AJM, Harkless LB: Off-loading the diabetic foot wound: a randomized clinical trial. *Diabetes Care* 24:1019–1022, 2001
4. Myerson M, Papa J, Eaton K: The total contact cast for the management of plantar ulceration of the foot. *J Bone Joint Surg* 74:261–264, 1992
5. Helm PA, Walker SC, Pulliam G: Total contact casting in diabetic patients with neuropathic foot ulcerations. *Arch Phys Med Rehab* 65:691–693, 1984
6. Mueller MJ, Diamond JE, Sinacore DR, Delitto A, Blair VP, Drury DA, Rose SJ: Total contact casting in treatment of diabetic plantar ulcers: controlled clinical trial. *Diabetes Care* 12:384–388, 1989
7. Sinacore DR, Mueller MJ, Diamond JE, Blair VP 3rd, Drury D, Rose SJ: Diabetic plantar ulcers treated by total contact casting: a clinical report. *Phys Ther* 67:1543–1549, 1987
8. Walker SC, Helm PA, Pulliam G: Total contact casting and chronic diabetic neuropathic ulcerations: healing rates by wound location. *Arch Phys Med Rehab* 68: 217–221, 1987
9. Sinacore DR: Total contact casting for diabetic neuropathic ulcers. *Phys Ther* 76: 296–301, 1996
10. Lavery LA, Vela SA, Lavery DC, Quebedeaux TL: Reducing dynamic foot pressures in high-risk diabetic patients with foot ulcerations: a comparison of treatments. *Diabetes Care* 19:818–821, 1996
11. Gentzkow GD, Iwasaki SD, Hershon KS, Mengel M, Prendergast JJ, Ricotta JJ, Steed DP, Lipkin S: Use of Dermagraft, a cultured human dermis, to treat diabetic foot ulcers. *Diabetes Care* 19:350–354, 1996
12. Grey JE, Lowe G, Bale S, Harding KG: The use of cultured dermis in the treatment of diabetic foot ulcers. *J Wound Care* 7:324–325, 1998
13. Pollak RA, Edington H, Jensen JL, Kroeker RO, Gentzkow GD, Dermagraft Diabetic Ulcer Study Group: A human dermal replacement for the treatment of diabetic foot ulcers. *Wounds* 9:175–183, 1997
14. Atillasoy E: Current and investigative uses of Graftskin (Apligraf) in wound care. *Wounds* 12 (Suppl. A):3A–11A, 2000
15. Milstone LM, Asgari MM, Schwartz PM, Hardin-Young J: Growth factor expression, healing, and structural characteristics of Graftskin (Apligraf). *Wounds* 12 (Suppl. A):12A–19A, 2000
16. Veves A, Falanga V, Armstrong DG, Sabolinski ML, Apligraf Diabetic Foot Ulcer Study: Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. *Diabetes Care* 24:290–295, 2001
17. Wieman TJ, Smiell JM, Su Y: Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (beclapermin) in patients with chronic neuropathic diabetic ulcers: a phase III randomized placebo-controlled double-blind study. *Diabetes Care* 21:822–827, 1998
18. Steed DL, Donohoe D, Webster MW, Lindsley L: Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. *J Am Coll Surg* 183:61–64, 1996
19. Andreassi L, Casini L, Trabucchi E, Diamantini S, Rastrelli A, Donati L, Tenchini ML, Malcovati M: Human keratinocytes cultured on membranes composed of benzyl ester of hyaluronic acid suitable for grafting. *Wounds* 3:116–125, 1991
20. Rastrelli A, Beccaro M, Biviano F, Calderini G, Pastorello A: Hyaluronate esters: a new class of semi-synthetic biopolymers: chemical and physico-chemical properties. In *Clinical Implant Materials: Proceedings of the Eighth European Conference on Biomaterials*. Vol. 9. Heimke G, Soltesz U, Lee AJC, Eds. New York, Elsevier Science, 1990, p. 230–253
21. Campoccia D, Doherty P, Radice M, Brun P, Abatangelo G, Williams DF: Semisynthetic resorbable materials from hyaluronan esterification. *Biomaterials* 19:2101–2127, 1998
22. Galassi G, Brun P, Radice M, Cortivo R, Zanon GF, Genovese P, Abatangelo G: In vitro reconstructed dermis implanted in human wounds: degradation studies of the HA-based supporting scaffold. *Biomaterials* 21:2183–2191, 2000
23. Caravaggi C, Faglia E, Dalla Paola L, De Giglio R, Cavaiani P, Mantero M, Gino M, Quarantiello A, Sommaria E, Tritelli C: Tissue engineering in the treatment of diabetic foot ulcers. In *New Frontiers in Medical Sciences Redefining Hyaluronan*. Abatangelo G, Weigel PH, Eds. New York, Elsevier Science, 2000, p. 313–320
24. Faglia E, Mantero M, Gino M, Quarantiello A, Signorini M: A combined conservative approach in the treatment of a severe Achilles tendon region ulcer in a diabetic patient: a case report. *Wounds* 11: 105–109, 1999
25. Hollander DA, Soranzo C, Falk S, Windolf J: Extensive traumatic soft tissue loss: reconstruction in severely injured patients using cultured hyaluronan-based three-dimensional dermal and epidermal autografts. *J Trauma* 50:1125–1135, 2000
26. Harris PA, di Francesco F, Barisoni D, Leigh IM, Navsaria HA: Use of hyaluronic acid and cultured autologous keratinocytes and fibroblasts in extensive burns. *Lancet* 353:35–36, 1999

27. Margolis DJ, Kantor J, Berlin JA: Healing of diabetic neuropathic foot ulcers receiving standard treatment: a meta-analysis. *Diabetes Care* 22:692–695, 1999
28. Caravaggi C, Faglia E, De Giglio R, Mantero M, Quarantiello A, Sommariva E, Gino M, Pritelli C, Morabito A: Effectiveness and safety of a non-removable fiberglass off-bearing cast versus a therapeutic shoe in the treatment of neuropathic foot ulcers: a randomized study. *Diabetes Care* 23:1746–1751, 2000
29. Der Simonian R, Laird N: Meta-analysis in clinical trials. *Controlled Clinical Trials* 7:177–188, 1986