ORIGINAL RESEARCH–CLINICAL SCIENCE

Autologous platelet-rich fibrin matrix as cell therapy in the healing of chronic lower-extremity ulcers

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ABSTRACT

A novel autologous platelet-rich fibrin matrix membrane (PRFM) was assessed for the ability to facilitate healing in patients with chronic lower-extremity ulcers. Preliminary data are presented from a prospective trial ($n=21$). Twelve patients were identified with 17 venous leg ulcers (VLU) and nine bearing 13 nonvenous lower-extremity ulcers. Before enrollment, the patients were evaluated for vascular status and received appropriate surgical intervention to optimize arterial and venous circulatory status. None of the ulcers had responded to a variety of standard treatments from 4 months to 53 years. Initial ulcer size ranged from 0.7 to 65 cm^2 (mean, 11.2 cm^2). Each PRFM-treated patient received up to three applications of either a 35 or 50 mm fenestrated membrane, depending on initial ulcer size. The primary endpoints were percent and rate of complete closure as measured by digital photography, computerized planimetery, and clinical examination. Patients were followed weekly for 12 weeks with a follow-up visit at 16 weeks. At each 4-week interval, the extent of healing was assessed, and those patients with $>50\%$ reduction in wound area were allowed to continue to complete closure. Patients with $<50\%$ closure received repeated applications. Complete closure was achieved in 66.7% of the VLU patients (64.7% of treated ulcers) in 7.1 weeks (median, 6 weeks) with an average of two applications per patient. Forty-four percent complete closure was seen with non-VLU patients (31% of treated ulcers). From the results of this small-scale pilot study, PRFM shows significant potential for closing of chronic leg ulcers.

Acute wounds heal by progression through a complex but orderly and sequential series of physiologic and molecular processes. These processes include coagulation, inflammation, cell recruitment, migration, proliferation, and connective-tissue production followed by matrix remodeling and maturation.[1] In contrast, chronic wounds are characterized as having stalled somewhere in this progression to healing due to a variety of systemic and local factors including inadequate blood supply, high microbial burden, excess devitalized tissue, chronic venous insufficiency; senescent epithelial cells that are poorly responsive to cell signaling,[2] and decreased growth-factor production and response.

Optimal wound-bed preparation of chronic ulcers consists of debridement, control of infection, and establishment of a balanced moist healing environment. However, when these measures fail (30% area reduction by week 4), advanced therapies should be considered,[3] such as cellular therapies to replace deficient components (autologous epidermis, allografts, and living skin equivalents), complimentary therapies (hyperbaric oxygen, negative pressure, ultrasound, and electrical stimulation), dermal matrix equivalents, and exogenous growth factors (purified single growth factors, autologous growth factor, and growth factor/fibrin preparations).[2]

The platelet-rich fibrin matrix (PRFM) process yields over a $60\times$ concentration of platelets and fibrin

(E. Lucarelli, personal communication) from whole blood in the form of a dense, easy-to-handle, suturable membrane. Studies on the composition of the PRFM membrane are ongoing and will be detailed in a separate publication. The process does not require exogenous thrombin for platelet activation and clot formation. This pilot study was designed to investigate the clinical safety and potential efficacy of autologous PRFM membrane in the treatment of severe nonhealing lower-extremity ulcers in conjunction with standard wound-care regimens.

MATERIALS AND METHODS

Materials

Preparation of PRFM membrane

Whole blood is drawn from the patient in nine 9 mL increments by standard venipuncture using a sterile vacuum

PRFM	Platelet-rich fibrin matrix
PRP	Platelet-rich plasma
VLU	Venous leg ulcer

tube containing trisodium citrate and a thixotrophic polyester separator gel and a 21 g "butterfly" blood collection kit (Becton-Dickinson, Franklin Lakes, NJ). The red blood cells and platelet-rich plasma (PRP) are separated by spinning the tube for 6 minutes in a standard centrifuge at 1,100 RCF. The supernatant PRP is transferred from the first tube into a 35 mm Wheaton bottle, containing calcium chloride (1.0 M), using a 20 mL syringe and 19 G needle. The Wheaton bottle is placed back into the centrifuge equipped with a flat carrier-container and spun at a higher g-force (4,500 RCF) for 25 minutes. A flat, circular membrane of PRFM is formed at the bottom of the container as it is spun using radial centrifugation. Eighteen milliliters of blood will produce 7–8 mL of PRP, which in turn yields a 35 mm membrane of 0.03 cm thickness; 36 mL of blood will produce a 50 mm membrane of 0.03 cm thickness. All membranes were produced from blood drawn from the patient immediately before application.

Study design

This study was designed as a prospective, pilot trial of patients with lower-extremity ulcers. Eligible patients aged 18–85 who were enrolled in the study were treated with PRFM membrane together with therapeutic compression therapy when appropriate. The study duration was 12 weeks with 1-month follow-up. Patients with venous leg ulcers (VLU) (12 patients with 17 ulcers) who had failed to improve after 4 weeks of conventional compression therapy and who were free of infection at the time of treatment were eligible for inclusion. In addition, nine patients with 13 wounds of nonvenous origin, refractory to standard therapy, were also treated with PRFM. One week before treatment, each patient underwent aggressive, surgical debridement to remove excess fibrin, callous, and necrotic tissue. Digital photographs with computerized planimetry of the target ulcer were taken before and during treatment.

Human ethical considerations

This study was approved by the Englewood Institutional Review Board, protocol F03-VLU-001. Eligible patients were enrolled after informed consent was obtained and the protocol conformed to ethical guidelines of the 1975 Declaration of Helsinki.

Study population

All patients were evaluated for vascular status and underwent surgical intervention to optimize arterial and venous circulatory status. Vascular evaluations consisted of pulse volume recording for determination of arterial status as well as arterial and venous duplex sonography when indicated. Ulcers secondary to venous insufficiency were characterized and diagnosed as: absence of significant arterial insufficiency, ankle-brachial index > 0.7 and evidence of venous stasis and incompetence showed by venous duplex ultrasonography. Exclusion criteria included cellulitis, vasculitis, osteomyelitis, pregnancy, uncontrolled diabetes, active deep venous thrombosis, and other clinical conditions that would impair wound healing such as renal, hepatic, hematologic, neurologic, or immunologic disease. Patents receiving corticosteroids, immunosuppressive

agents, radiation therapy, chemotherapy, topical antibiotics, or growth factors at the target site within 1 month of study enrollment were excluded. In addition, patients treated with PRFM, Apligraf, OrCel, Dermagraft, Oasis or other advanced therapy at the target site during the 6-month period before enrollment were also excluded. Patients accepted into the study underwent a 1-week pre-screening period and were then were treated with one to three applications of PRFM membrane.

Treatment protocol and follow-up

Wound preparation and screening period

One week before PRFM treatment, all ulcers were debrided by sharp mechanical methods.[4] The wound beds were then irrigated with sterile saline. Baseline photographs and computer planimetry were obtained. Principles for standard wound care were followed including application of a nonadherent contact layer secured with gauze wrap and followed by a class 3 multilayer compression dressing.
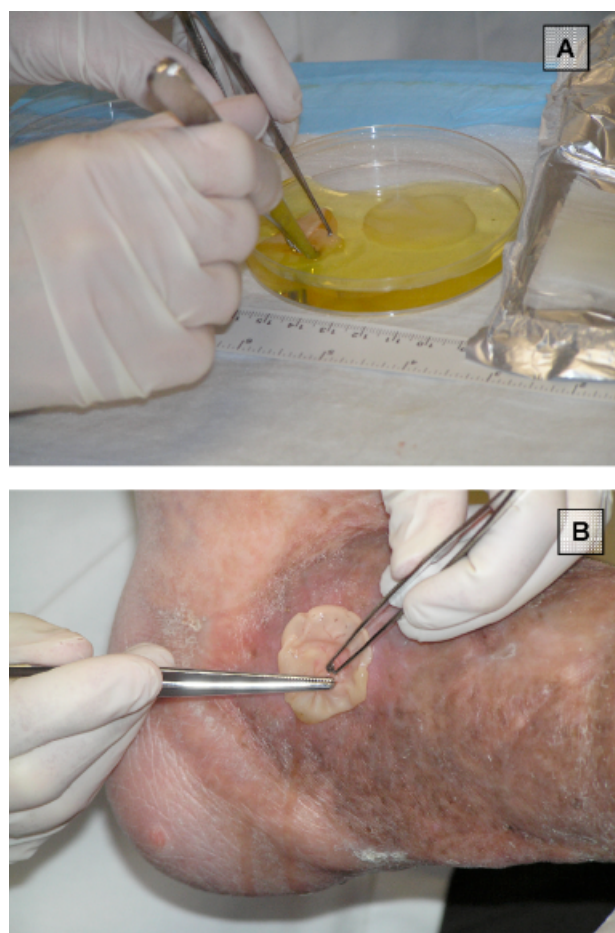
PRFM membrane application

Blood collection and PRFM membrane preparation were carried out immediately before application. The freshly prepared membrane was fenestrated using sterile forceps and scissors to introduce uniform slits, approximately 3 mm long, to allow drainage of wound exudate, increase moisture and air exchange, and improve intimate application to the wound bed (Figure 1A). The membrane was then trimmed to conform to the wound outline and applied with a 0.5 cm overlap of the wound margin onto the peri-wound area (Figure 1B).

Dressings and follow-up

A nonadherent contact layer (such as Adaptic, Ethicon, Somerville, NJ, Xeroform, Covidien, Mansfield, MA, or Wound Veil by Smith and Nephew, St. Petersburg, FL) was secured directly over the fenestrated PRFM with gauze wrap followed by a multilayer compression wrap. Nonvenous ulcers were dressed in a similar fashion but without the multilayer compression. The primary dressing was left in place for 7 days and was changed only by site personnel at weekly intervals. Care was taken not to disturb the wound bed (including the PRFM) unless there was concern about possible infection.

Study evaluations

All patients were evaluated weekly for up to 12 weeks or to the time of wound closure. Follow-up visits were also scheduled at 16 or 4 weeks after closure. At each visit, ulcer healing was evaluated and recorded by digital photography. The primary efficacy endpoints were the incidence and time to complete closure in the absence of drainage. Secondary endpoints were the incidence and time to 75% closure.

750

Figure 1. Fenestration, trimming (A), and application (B) of platelet-rich fibrin matrix membrane to the prepared lower-extremity (venous) ulcer.

Digital photography and computer planimetry

Digital photographs of the target ulcer site were taken at a fixed focal length of 8 in with automatic, flash-adjusted white balancing. All ulcer photographs were framed with a 5–10 cm scale calibration sticker affixed just outside the wound margin. The resolution of the digital photographs was at least 300×300 dpi (> 900 kb).

Wound area determination

Healing rate, defined as the change in wound area over time, was calculated based on the method of Margolis,[5] without factoring in linear perimeter advance.[6]

RESULTS

Patient demographics and ulcer characteristics are presented in Table 1. In total, 12 patients with 17 venous ulcers and nine patients with 13 nonvenous lower-extremity nonhealing wounds were treated with PRFM together with appropriate standard wound care. Of the patients

Table 1. Patient demographics and ulcer characteristics

N	Venous	Nonvenous
Gender		
Male	6 (50%)	7 (77.8%)
Female	6 (50%)	2 (22.2%)
Age-mean (SD)	62.3 (13.9)	68.6 (12.0)
Race		
Black	3 (25%)	0 (0)
Hispanic	1 (8.3%)	2 (22.2%)
White	8 (66.6%)	7 (77.8%)
Ulcer size (cm^2)	11.2	16.1
Mean (range)	(0.7–58.0)	(1.0–63.0)
Ulcer duration (years)	1.0	ND
Median (range)	(0.5–27.0)	
Atherosclerosis	3 (25%)	7 (77.8%)
Hypertension	3 (25%)	9 (100%)
Diabetes	1 (8.3%)	7 (78%)
Coronary artery disease	3 (25%)	5 (55.6%)
Cholesterolemia	3 (25%)	4 (44.4%)
COPD/smoking	4 (33.3%)	3 (33.3%)
Obesity	4 (33.3%)	1 (8.3%)

COPD, chronic obstructive pulmonary disease; ND, not determined.

with venous ulcers, three had prior peripheral arterial bypass surgery and nine had previous venous surgery (subfascial endoscopic perforating vein surgery and radio-frequency ablation). Of the nine patients with nonvenous ulcers, six had prior peripheral vascular surgery.

Clinical efficacy

The overall efficacy outcomes were measured by complete wound closure and time to closure. Results are depicted in Table 2.

Complete closure

For the primary endpoint of complete closure as defined by complete epithelialization in the absence of drainage: 64.7% of venous ulcers (66.7% of patients) treated with PRFM closed within the study period of 16 weeks and an additional two ulcers reached 75% closure (secondary endpoint) during the same period. In the nonvenous ulcer group 44% of the treated patients (31% of treated ulcers) achieved complete closure. No ulcer that achieved complete closure during the 16-week study reopened during the evaluation period.

Time to closure

The mean time to complete closure in patients with venous ulcers was 7.1 weeks (median 6 weeks). The percentage and time to complete closure for each patient and treated ulcer is given in Table 3.

Table 2. Efficacy: Frequency and time to wound closure

Number of patients treated	Number of ulcers treated	Percent ulcers achieving 75% closure	Percent ulcers achieving 100% closure (patients)	Median time to 75% closure (mean)	Median time to 100% closure (mean)	Mean number of applications
			VLU patients			
12 total	17 total	76.5	64.7 (66.7)	5 weeks (6.1)	6 weeks (7.1)	1.8
9 responders	13 responders	100	84.6	NA	NA	1.6
3 nonresponders	4 nonresponders	0	0	0	0	2.5
			Non-VLU patients			
9 total	13 total	NM	31.0 (44.4)	NM	NM	2.3
4 responders	4 responders	NM	100	NM	NM	1.7
5 nonresponders	9 nonresponders	NM	0	NM	NM	2.6

NA, not applicable; NM, not measured.

The cumulative progression to complete closure for venous ulcers treated with PRFM is depicted in Figure 2. Percent closure at each visit ($\pm$ standard error of the mean) was calculated from the decrease (or increase) in wound area as measured by digital photography and computerized planimetry normalized to the initial wound area. The two curves show the progress to 100% closure for *responders* to the PRFM treatment as defined by ulcers that achieved 75% or greater reduction in wound area by week 16 vs. *nonresponders*, PRFM-treated ulcers that did not achieve at least 75% wound closure. These data indicate ulcers responding to the PRFM diverge rapidly (by week 3) in their progress to closure compared with ulcers that do not respond. This divergence occurred, in general, between the first and second applications of PRFM.

Rate of healing

In the VLU cohort, the average wound area closure rate for the responders was $-0.63 \pm 0.19 \, mm^2$/day for the *responder* group vs. $0.20 \pm 0.26 \, mm^2$/day for the *nonresponders* during the 12-week course of treatment. Figure 3 shows the mean healing rate for each group. In these graphs, the mean daily change in area from initial baseline is plotted for each visit. Hence, a negative rate is indicative of a positive healing response and progression toward closure. Healing rates for the *responder* group (Figure 3A) uniformly displayed progress toward closure (decrease in area per day) while the healing rates for the *nonresponders* (Figure 3B) were neutral or negative (increase in wound area per day). For example, the mean healing rate for the *responder group* for week 3 was $-1.75 \, mm^2$/day, or stated simply, for the period between study weeks 2 and 3, ulcers in the *responder group* decreased in area by an average of $-1.75 \, mm^2$ each day. The maximal healing rate for the *responder* group was reached by week 6, after the second application.

Clinical safety

No treatment-associated complications or adverse reactions were observed during the course of the 16-week study.

DISCUSSION

The ability of living tissues to repair themselves after injury is fundamental for all surgical interventions. Wound healing is essentially the same process for most tissues and involves a multitude of cellular and humoral components, including local influx and activation of platelets.[7] The subsequent release of cytokines and growth factors provide the initial stimulus for the wound-healing process. Platelets release a multitude of growth factors including platelet-derived growth factor (PDGF), a potent chemotactic agent, and transforming growth factor-β, which stimulates the deposition of extracellular matrix.[7] Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues. Other healing-associated growth factors produced by platelets include: basic fibroblast growth factor, insulin-like growth factor-1 (IGF-1), platelet-derived epidermal growth factor, and vascular endothelial growth factor. Local application of these factors in increased concentrations through PRP has been used as an adjunct to wound healing for several decades.[8–12]

Preparation of autologous PRP requires collection of peripheral whole blood, separation of platelets and plasma from other blood cellular elements, and polymerization of the plasma fibrin to concentrate the platelets into a platelet-rich gel, with enough stability for surgical implantation.[13] Currently, several commercial methods of PRP preparation use calcium and bovine thrombin[14] or prepared autologous thrombin[13] to create the platelet–fibrin matrix. The preparation of autologous thrombin requires additional steps and greater blood volume, whereas the use of bovine thrombin has been associated with the development of antibodies to clotting factors V and XI and thrombin, increasing the risk of coagulation abnormalities.[15–17] In addition, to ensure complete platelet degranulation and stable clot formation, high levels of thrombin are used, which may cause an immediate release of these growth factors.[14]

Our current method avoids using thrombin as an activator.[18–20] Our system uses only calcium and centrifugation to activate the polymerization of fibrin to form a PRFM. PRFM, in the form of a gel or a dense pliable

Table 3. Patient data summary by treated ulcer

| Patient ID	Ulcer etiology	Initial area	Duration	App. number	Closure	Time to closure
1	Venous	1.5 cm^2	6 months	1	Yes	2 weeks
2	Venous	2.87	7 months	2	Yes	4
3	Venous	11.4	12 months	3	Yes	16
4a	Venous	4.04	5 years	2	No	—
4b	Venous	5.15	5 years	2	No	—
5a	Venous	2.73	12 months	1	Yes	5
5b	Venous	0.45	12 months	1	Yes	4
6a	Venous	0.71	12 months	1	Yes	3
6b	Venous	0.65	12 months	1	Yes	3
7	Venous	8.45	4 years	1	No	—
8	Arterial–venous	58.4	18 years	2	No	14 (75%)
9	Venous	5.5	2 years	2	Yes	6
10a	Venous	3.5	4 years	2	Yes	6
10b	Venous	1.9	4 years	2	Yes	13
11	Venous	6.37	12 months	2	No	5 (75%)
12a	Venous	3.7	8 months	3	Yes	15
12b	Venous	1.3	8 months	3	No	—
13	Trauma	15.6 cm^2	4 months	1	Yes	ND
14a	Arterial-diabetic	5.1	7 years	3	No	ND
14b	Arterial-diabetic	ND	7 years	3	No	ND
15	Arterial-diabetic	2	2 years	2	Yes	ND
16	Arterial-diabetic	28.5	4.5 months	2	Yes	ND
17	Raynaud's	5.8	2 years	3	No	ND
18	Arterial-diabetic	1	6 months	3	No	ND
19	Arterial-diabetic	50	3 months	2	Yes	ND
20a	Arterial- diabetic	7.2	27 years	2	No	ND
20b	Arterial- diabetic	7.6	27 years	2	No	ND
20c	Arterial- diabetic	5.2	27 years	2	No	ND
20d	Arterial- diabetic	2.4	27 years	2	No	ND
21	Trauma	63	53 years	3	No	ND

ND, not determined.

membrane, can then be applied to the wound, and the release of PDGFs is triggered by autologous activators present at the wound site. This method may allow a gradual timely release of growth factors at the wound site, which in turn may signal various cell types to respond at appropriate times. In vitro studies indicate that the PRFM exhibits a gradual steady-state release of platelet growth factors for as long as 7 days.[20] We think that the PRFM provides a reservoir of growth factors to the wound during the healing process and potentially provides a fibrin scaffold to facilitate the tissue repair process. This would also allow a more convenient dosing schedule (2-week intervals) when compared with PRP preparations that use thrombin, with the associated complete release of the platelet growth factor complement and attendant need for frequent (3–4-day interval) PRP applications.[12]

VLU, one of the most common (estimated US prevalence > 1,600,000 in 2003) and perhaps the most costly of all vascular disorders,[21,22] is a significant cause of morbidity, disability, and financial burden.[23–25] The pathophysi-

ology of venous ulcers is based on calf blood pump failure, which results in edema, rupture of venules, trapping of white blood cells and growth factors, and deposition of pericapillary fibrin. Fibrin cuffs prevent nutrient diffusion as the white blood cells release toxic oxygen metabolites and proteolytic enzymes that damage capillaries, leading ultimately to tissue breakdown and ulceration.[26–28]

Since the beginning of the previous century, the main treatment for these ulcers was compression of the ulcer-bearing limb,[29–31] but this pathologic process is often refractory to such treatment. In recent years, minimally invasive techniques employing radiofrequency and laser energy sources have been used to ablate veins that are pathologic.[32,33] Additionally, advances in cellular therapy,[34,35] purified growth factors,[36,37] negative pressure therapy,[38,39] and PRP[11,12] have provided the clinician with useful options for adjuvant therapy in the treatment of venous ulcers and other nonhealing wounds.
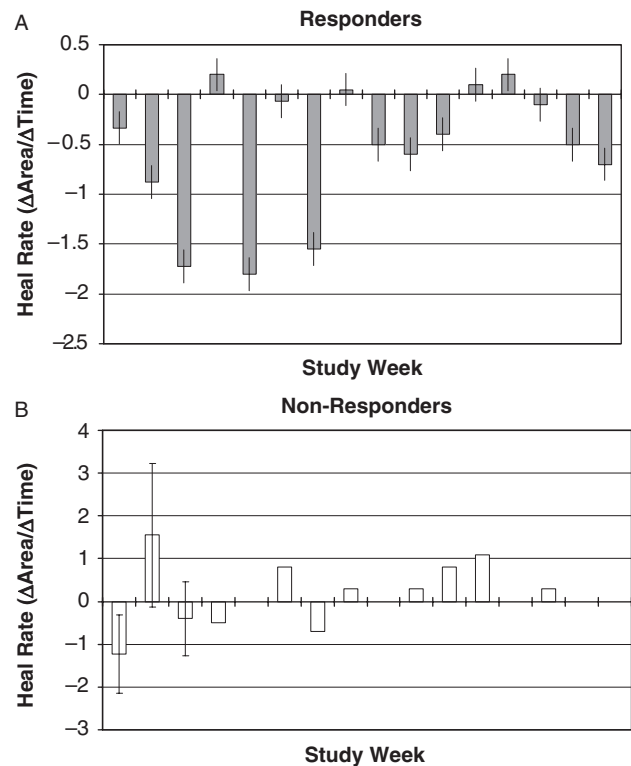
In this prospective, auto-controlled, small-scale 16-week pilot study, complete healing was achieved in a majority of
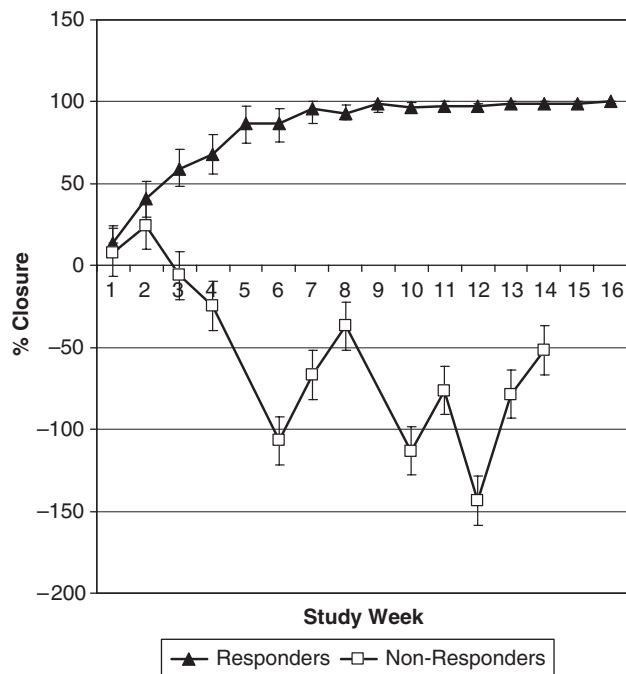
Figure 2. Cumulative closure for venous ulcer healing for wounds responding to platelet-rich fibrin matrix treatment ($n=13$; $>75\%$ closure by week 16) and nonresponding ($n=4$; $<75\%$ closure by week 16). Percent closure was calculated from ulcer area reduction normalized to initial baseline area. Values are given as mean percent closure $\pm$ standard error of the mean for each visit.

Figure 3. Healing rate analysis. This figure shows the mean daily healing rate, the change in wound area relative to the initial baseline area, for each group calculated on a weekly basis. A negative rate is indicative of a positive healing response and progression toward closure (A). In contrast, no consistent change in the healing rate was observed in the nonresponders (B). Data represent mean change in area over time (mm^2/day) $\pm$ standard error of the mean for each time point.

patients with severe nonhealing venous ulcers of long duration who were treated with autologous PRFM. Standard compression therapy had failed previously for all patients. In addition, PRFM treatment resulted in complete closure in 44% of patients (31% of ulcers) with hard-to-heal ulcers of nonvenous origin. More than 76% of the treated venous ulcers achieved the secondary endpoint of 75% or greater closure at 16 weeks (*responders*). In contrast, three patients bearing four ulcers did not achieve the minimum 75% closure by week 16 (*nonresponders*) after a least two PRFM applications (Table 3). Looking at the progression to complete closure in Figure 2, it is interesting to note that the *responder* group diverges rapidly from the *nonresponder* group with the divergence occurring between weeks 2 and 3. This time corresponds to the period immediately after the second application for most of the treated ulcers. Looking further at the *responder* group, 84.6% achieved complete closure.

This difference in response to the PRFM treatment is also evident in the analysis of healing rates (Figure 3A and B). The reason for the *nonresponse* was not immediately obvious, but could be attributed to one or more factors not assessed in the patients' history, pretreatment evaluation, or continuing evaluation, including possible increased proteolytic activity at the wound site. Alternatively, given the severity and long duration of these ulcers, it is possible that these ulcers failed to respond to the concentrated growth factors because of unresponsive, senescent cells within the wound bed and margins.[40] Further studies on the clinical response to autologous PRFM, and in-depth character-

ization of the PRFM itself are needed to address this question. It should be noted that while the number of patients and ulcers treated is too small for clinical significance, a closure rate of two out of three venous ulcers treated and two out of five nonvenous ulcers is indicative of the potential of PRFM therapy. This is especially true considering the severity of the venous ulcers (average 1 year duration) and the marked severity of the nonvenous ulcers treated in the study, none of which responded to prolonged standard therapy and, in most cases, vascular intervention.

In conclusion, autologous PRFM represents a safe, convenient easy-to-use adjuvant therapy that shows significant potential for closing of chronic leg ulcers. Current studies are underway at an independent second center to confirm these results in a randomized, placebo-controlled trial of patients with VLU of long duration. In addition, studies are underway to determine the efficacy and utility of PRFM in the treatment of neuropathic diabetic foot ulcers.[19]

ACKNOWLEDGMENTS

REFERENCES

1. Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet* 2005; 366: 1736–43.
2. Woo K, Ayello EA, Sibbald RG. The edge effect: current therapeutic options to advance the wound edge. *Adv Skin Wound Care* 2007; 20: 99–117.
3. Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin JA. The accuracy of venous leg ulcer prognostic models in a wound care system. *Wound Rep Regen* 2004; 12: 163–8.
4. Robson MC, Cooper DM, Aslam R, Gould LJ, Harding KG, Margolis DJ, Ochs DE, Serena TE, Snyder RJ, Steed DL, Thomas DR, Wiersma-Bryant L. Guidelines for the treatment of venous ulcers. *Wound Rep Regen* 2006; 14: 649–62.
5. Margolis DJ, Gross EA, Wood CR, Lazarus GS. Planimetric rate of healing in venous ulcers of the leg treated with pressure bandage and hydrocolloid dressing. *J Am Acad Dermatol* 1993; 28: 418–21.
6. Kantor J, Margolis DJ. A multicentre study of percentage change in venous ulcer area as a prognostic index of healing at 24 weeks. *Br J Dermatol* 2000; 142: 960–4.
7. Singer AJ, Clark RAF. Cutaneous wound healing. *N Engl J Med* 1999; 341: 738–46.
8. Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler ELL. Classification and treatment of chronic nonhealing wounds: successful treatment with autologous platelet-derived wound healing factors (PDWHF). *Ann Surg* 1986; 204: 322–30.
9. Knighton DR, Ciresi K, Fiegel VD, Schumerth S, Butler E, Cerra F. Stimulation of repair in chronic, nonhealing, cutaneous ulcers using platelet-derived wound healing formula. *Surg Gynecol Obstet* 1990; 170: 56–60.
10. Celotti F, Colciago A, Negri-Cesi P, Pravettoni A, Zaninetti R, Sacchi MC. Effect of platelet-rich plasma on migration and proliferation of SaOS-2 osteoblasts: role of platelet-derived growth factor and transforming growth factor-β. *Wound Rep Regen* 2006; 14: 195–202.
11. McAleer JP, Sharma S, Kaplan EM, Perisch G. Use of autologous platelet concentrate in a nonhealing lower extremity wound. *Adv Skin Wound Care* 2006; 19: 354–63.
12. Driver VR, Hanft J, Fylling CP, Beriou JM. A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. *Ostomy Wound Manage* 2006; 52: 68–87.
13. Mazzucco L, Medici D, Serra M, Panizza R, Rivara G, Orecchia S, Libener R, Cattana E, Levis A, Betta PG, Borzini P. The use of autologous platelet gel to treat difficult to heal wounds: a pilot study. *Transfusion* 2004; 44: 1013–8.
14. Kevy SV, Jacobson MS. Comparison of methods for point of care preparation of autologous platelet gel. *JECT* 2004; 36: 28–35.
15. Bänninger H, Hardegger T, Tobler A, Barth A, Schüpbach P, Reinhart W, Lämmle B, Furlan M. Fibrin glue in surgery: frequent development of inhibitors of bovine thrombin and human factor V. *Br J Haematol* 1993; 85: 528–32.
16. Ortel TL, Mercer MC, Thames EH, Moore KD, Lawson JH. Immunologic impact and clinical outcomes after surgical exposure to bovine thrombin. *Ann Surg* 2001; 233: 88–96.
17. Schoenecker JG, Johnson RK, Lesher AP, Day JD, Love SD, Hoffman MR, Ortel TL, Parker W, Lawson JH. Exposure of mice to topical bovine thrombin induces systemic autoimmunity. *Am J Pathol* 2001; 159: 1957–69.
18. Vercellino V, Carbone V, Griffa A. The use of autologous materials for treatment of large maxillary cysts: filling of the residual post-cystectomy cavity. 5th Congress, Society Odontostonatologica Italiano, Turin, Italy, December 1, 2000.
19. Gosch C, Zeichner A, Carroll R, Bois J. Evaluation of an autologous platelet rich fibrin matrix technology for diabetic foot ulcer treatment. *Wound Rep Regen* 2007; 15: A38.
20. O'Connell SM, Impeduglia T, Hessler K, Wang X-J, Carroll RJ, Dardik H. Autologous platelet-rich fibrin matrix as a stimulator of healing of chronic lower extremity ulcers. *Wound Rep Regen* 2006; 14: A76.
21. Black SB. Venous stasis ulcers: a review. *Ostomy Wound Manage* 1995; 4120–32.
22. Trent JT, Falabella A, Eaglstein WH, Kirsner RS. Venous ulcers: pathophysiology and treatment options. *Ostomy Wound Manage* 2005; 51: 38–54.
23. Phillips T, Stanton B, Provan A, Lew R. A study of the impact of leg ulcers on quality of life: financial, social, and psychologic implications. *J Am Acad Dermatol* 1994; 31: 49–53.
24. Olin JW, Beusterian KM, Childs MB, Seavey C, McHugh L, Griffiths RI. Medical costs of treating venous stasis ulcers: evidence from a retrospective cohort study. *Vasc Med* 1999; 4: 1–7.
25. Phillips TJ, Dover JS. Leg ulcers. *J Am Acad Dermatol* 1991; 25: 965–87.
26. Falanga V. Venous ulceration. *Wounds* 1996; 8: 102–8.
27. Angle N, Bergan JJ. Chronic venous ulcer. *Br Med J* 1997; 314: 1019–23.
28. Bergan JJ, Schmid-Schonbein GW, Smith PD, Nicolaides AN, Boisseau MR, Eklof B. Chronic venous disease. *N Engl J Med* 2006; 355: 488–98.
29. Partsch H. Compression therapy of the legs. *J Dermatol Surg Oncol* 1991; 17: 799–805.
30. Kramer SA. Compression wraps for venous ulcer healing: a review. *J Vasc Nurs* 1999; 17: 89–97.
31. Cullum N, Nelson EA, Fletcher AW, Sheldon TA. Compression for venous leg ulcers (Cochrane Review). *Cochrane Database Syst Rev* 2000; 3:CD000265.
32. Elias SM, Frasier KL. Minimally invasive vein surgery: its role in the treatment of venous stasis ulceration. *Am J Surg* 2004; 188 (Suppl. to July 2004): 26–30S.
33. Elias SM. Minimally invasive vein surgery for varicose vein. *J Cardiovasc Surg* 2005; 46: 35–9.
34. Falanga V, Margolis D, Alvarez O, Auletta M, Maggiacomo F, Altman J, Jensen J, Sabolinski M, Hardin-Young J. The Human Skin Equivalent Investigator Group. Rapid healing of venous ulcers and lack of a clinical rejection with an

allogeneic cultured human skin equivalent. *Arch Dermatol* 1998; 134: 293–300.

35. Omar AA, Mavor AI, Jones AM, Homer-Vanniasinkam S. Treatment of venous leg ulcers with dermagraft. *Eur J Vasc Endo Vasc Surg* 2004; 27: 666–72.

36. Steed DL. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. The Diabetic Ulcer Study Group. *J Vasc Surg* 1995; 21: 71–8; discussion 79–81.

37. Steed DL. Clinical evaluation of recombinant platelet-derived growth factor for the treatment of lower extremity ulcers. *Plast Reconstr Surg* 2006; 117 (Suppl.): 143–51S.

38. Joseph E, Hamori CA, Bergman S, Roaf E, Swann NF, Anastasi GW. A prospective randomized trial of vacuum assisted closure versus standard therapy of chronic nonhealing wounds. *Wounds* 2000; 12: 60–7.

39. Evans D, Land L. Topical negative pressure for treating chronic wounds: a systematic review. *Br J Plast Surg* 2001; 54: 238–42.

40. Hasan A, Murata H, Falabella A, Ochoa S, Zhou L, Badiavas E, Falanga V. Dermal fibroblasts from venous ulcers are unresponsive to the action of transforming growth factor-β1. *J Dermatol Sci* 1997; 16: 59–66.