Prospective study of cryopreserved placental tissue wound matrix in the management of chronic venous leg ulcers

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ABSTRACT

Objective: Chronic venous leg ulcers (VLUs) affect up to 2% of the general population, resulting in a significant socioeconomic burden. Placental tissue that contains mesenchymal stem cells and active growth factors has been shown to be beneficial in healing of chronic wounds. We compared the efficacy of a human viable wound matrix (hVWM) of cryopreserved placental tissue for the treatment of refractory VLUs with standard therapy.

Methods: This prospective single-center open-label single-arm study enrolled patients with Clinical, Etiology, Anatomy, and Pathophysiology clinical class C6 VLUs. The ulcers of all enrolled patients had failed to heal after a trial of standard therapy of at least 12 weeks, which included weekly multilayer compression therapy along with local wound care. The same patients subsequently received application of hVWM (Grafix; Osiris Therapeutics, Columbia, Md) every 1 to 2 weeks in addition to standard therapy. Healing with hVWM therapy was then compared with standard therapy, with each patient serving as his own control.

Results: There were 30 VLUs in 21 consecutive eligible patients who were enrolled in the study. All patients were men with an average age of 67 years (standard deviation [SD], ±10.8 years), and the average area of venous ulcers before hVWM initiation was 12.2 cm^2 (SD, ±14.6 cm^2; range, 3.3-12.3 cm^2). Duplex ultrasound confirmed superficial or deep system venous reflux in all patients. Complete ulcer healing was achieved in 53% (16/30) of VLUs refractory to standard therapy after application of hVWM. There was a mean reduction in wound surface area by 79% (SD, ±27.3%; $P < .001$ compared with standard therapy) after a mean treatment time of 10.9 weeks. Eighty percent of VLUs were reduced in size by half compared with 25% with standard therapy ($P < .001$). The mean rate of reduction in ulcer area after hVWM applications was 1.69% per day vs 0.73% per day with standard therapy ($P = .01$).

Conclusions: Cryopreserved placental tissue (hVWM) improves healing processes to achieve complete wound closure in a significant proportion of chronic VLUs refractory to standard therapy. Adjunctive therapy with hVWM provides superior healing rates in refractory VLUs. (J Vasc Surg: Venous and Lym Dis 2019;7:228-33.)

Keywords: Venous insufficiency; Venous ulcer; Wound matrix; Compression; Placental tissue

Chronic venous insufficiency is one of the most common diseases in the Western Hemisphere. It affects up to 40% of adult women and up to 17% of adult men, and 6 million Americans suffer from advanced venous disease.[1] Nearly 1% of adults in developed countries suffer from lower extremity venous ulcers at some time during their lifetime.[2] This presents with significant morbidity and has an impact on quality of life. The financial burden of treating lower extremity venous ulcers is estimated to be $2 billion per year in the United States.[3]

The standard therapy for patients with chronic venous leg ulcers (VLUs) has been graded or multilayered compressive bandaging to promote ulcer healing and to prevent recurrence. Clinical practice guidelines of the Society for Vascular Surgery and the American Venous Forum recommend compression therapy for the primary treatment of VLUs. Furthermore, ablation of incompetent superficial veins in addition to compression therapy is recommended to reduce ulcer recurrence[4] and more recently has been shown to accelerate ulcer healing rates.[5] Compression has been demonstrated to be effective in several randomized controlled trials.[6,7] Healing rates ranging from 30% to 60% at 24 weeks and 70% to 85% after 1 year have been reported with compression alone.[8]

A variety of approaches have been developed in an attempt to enhance the rate of closure of venous ulcers,

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such as negative pressure wound therapy, topical application of growth factors, and acellular matrix products (human and bovine dermis, porcine small bowel submucosa). Mesenchymal stem cells (MSCs) may be successful in the treatment of a variety of chronic wounds in animal models as well as in human studies. The human placental membrane is a convenient source for these MSCs that have demonstrated an ability to stimulate tissue regeneration and repair.[9] In this prospective single-center open-label single-arm crossover study, we compared the healing characteristics of chronic VLUs treated with cryopreserved human placental tissue in a human viable wound matrix (hVWM) plus standard compression therapy vs standard therapy alone.

ARTICLE HIGHLIGHTS

- **Type of Research:** Prospective open-label, single-arm cohort study
- **Key Findings:** Complete healing of venous leg ulcers (VLUs) refractory to standard therapy was achieved with cryopreserved placental tissue using human viable wound matrix in 53% of 30 VLUs, and 80% decreased in size by half compared with 25% with standard therapy ($P < .001$).
- **Take Home Message:** Human viable wound matrix improves healing of VLUs refractory to standard therapy and should be considered for adjunctive therapy for difficult-to-heal VLUs.

METHODS

This study reports our experience of a total of 21 consecutive patients with 30 VLUs (Clinical, Etiology, Anatomy, and Pathophysiology clinical class C6) from the Baltimore Veterans Administration Medical Center treated from September 2014 to July 2016. The study inclusion criteria were as follows:

- Duplex ultrasound confirmation of presence of superficial or deep venous reflux;
- Presence of active chronic VLU that had failed to heal with standard therapy (standard therapy was defined as weekly or biweekly local wound care, including surgical débridement, with multilayered compression therapy provided under the direction of a vascular specialist);
- All patients with evidence of superficial venous system reflux also underwent radiofrequency ablation as a part of standard therapy;
- No evidence of active or ongoing wound or systemic infections;
- No evidence of limb ischemia (ankle-brachial index<0.8); and
- Patients who were not immunosuppressed (ie, patients diagnosed with human immunodeficiency virus infection, patients who were organ transplant recipients, or patients receiving chronic steroid therapy).

The protocol was reviewed and approved by the Institutional Review Board. The protocol was granted exempt status for informed consent because this was a standard of care investigation of a Food and Drug Administration-approved product.

Once enrolled in the study, the patients were observed for 12 weeks, during which they received standard wound care and compression. Those who did not heal by 12 weeks immediately transitioned to hVWM therapy and were observed for an additional 12 weeks. This longitudinal observational crossover design of the study controls for patients' demographic and comorbidity variables because each patient serves as his own control. Patients were evaluated on a weekly basis at the clinic, where characteristics of the wound were evaluated and photographs of the wound were obtained to measure size. During each visit, all patients received local wound care and multilayered compression bandaging. The hVWM (Grafix; Osiris Therapeutics, Columbia, Md) was applied to the VLU up to a maximum of 12 times during the 12 weeks of the study (on visit days 0, 7, 14, and so on). If, by clinical observation, the percentage take of hVWM was <50%, another application to the ulcer site was performed. Ulcer sites with percentage take >50% did not undergo another application on that particular visit. No patient received more than 12 applications. No other biologics were used in the patients during the study period. Local wound care involved appropriate cleansing of the wound and surgical outpatient débridement of all nonviable tissue. The hVWM was applied to cover the entire surface of the wound and then covered with Mepitel (Medline Industries, Mundelein, Ill). Limbs were then wrapped with three-layered compression bandaging, which included a medicated Unna boot bandage covered with Kerlix gauze wrap followed by an elastic bandage wrap.

The primary end point of the study was complete closure of the index wound. Secondary end points were percentage change in total ulcer area during the follow-up period and reduction in wound area with application of hVWM. The data were analyzed as a self-controlled study with each patient serving as his own control. VLU healing rates were compared after completion of the hVWM treatment phase with healing rates during the standard therapy phase before initiation of hVWM in each patient.

Statistical analyses were performed using MedCalc (Ostend, Belgium). Patients' characteristics were compared using χ^2 test and Student *t*-test as appropriate. All *P* values were reported as two tailed, and significance was set at $P \leq .05$. Ulcer healing (closure and reduction in area) were computed and compared using Kaplan-Meier analysis.

Table I. Demographics and baseline characteristics of the patients (N = 21)

Variable	
VLUs	30
Age, years	66.6 ± 10.8
Male	21 (100)
BMI, kg/m^2	31.2 ± 4.4
Risk factors	
Diabetes mellitus	10 (47.6)
HbA_{1c}, %	7.6 ± 1.6
Lower extremity DVT	7 (33.3)
Obese[a]	12 (57)
Immunosuppressed[b]	0 (0)

BMI, Body mass index; *DVT*, deep venous thrombosis; *HbA_{1c}*, glycated hemoglobin; *VLUs*, venous leg ulcers.
Categorical variables are presented as number (%). Continuous variables are presented as mean ± standard deviation.
[a]BMI ≥30 kg/m^2.
[b]Patients with history of human immunodeficiency virus infection, patients who were organ transplant recipients, or patients receiving chronic steroid therapy.

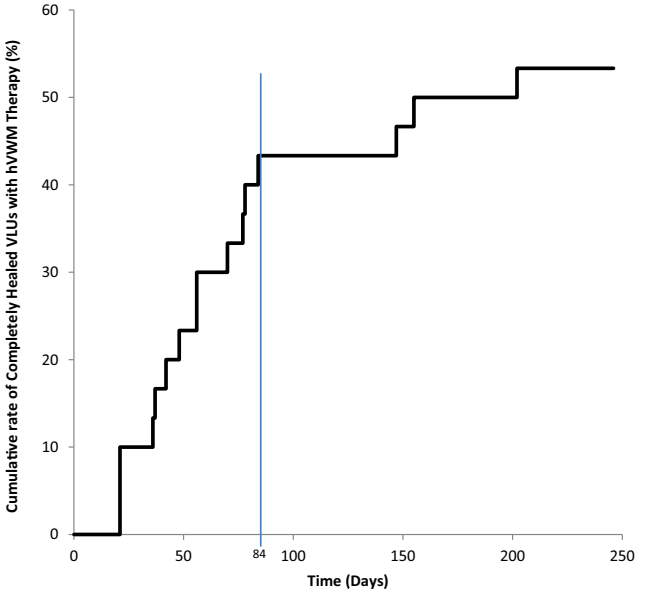

Fig 1. Kaplan-Meier analysis of probability of complete wound healing of venous leg ulcers (*VLUs*) with application of cryopreserved placental tissue in human viable wound matrix (*hVWM*) as an adjunctive therapy for VLUs that failed to completely heal after ≥12 weeks of standard therapy. The *reference line*, day 84 (12 weeks), is demarcated to reflect the reporting interval in this study (N = 30).

RESULTS

We observed 81 patients during 12 weeks to enroll 21 patients who had ulcers refractory to standard therapy and met all other inclusion and exclusion criteria. Table I outlines the demographics and baseline characteristics of patients in the study. A total of 30 limbs in 21 patients received hVWM for treatment of VLUs that failed to heal completely with standard therapy during weekly outpatient clinic visits. All patients were male, 52.9% were white, and the average age was 67 ± 10.8 years. The majority (57%) of patients were obese (body mass index >30 kg/m^2). Nearly half (48%) had diabetes mellitus and one-third had a prior history of lower extremity deep venous thrombosis. Radiofrequency ablation of the ipsilateral great saphenous vein was performed as part of the initial therapy in 14 of these patients. No perforators were identified that warranted individual ablation. Patients entered the study a mean of 4 ± 1 weeks after the ablation procedure. Of the VLUs that failed to heal with standard therapy, 53% (16/30) healed completely with hVWM therapy during a mean treatment time of 10.9 weeks. Fig 1 depicts the Kaplan-Meier plot of complete wound closure rates when hVWM was used as an adjunctive therapy in wounds that previously failed to heal with standard therapy. Of the remaining VLUs that did not achieve complete wound closure, 57% (8/14 limbs) had >50% wound area reduction. Fig 2 depicts Kaplan-Meier plots for patients who achieved at least 50% wound area reduction with hVWM therapy compared with standard therapy during the length of treatment follow-ups. Patients were observed for 12 weeks in the treatment arm. No ulcers recurred during this time.

Before initiation of hVWM therapy, mean VLU size measured 12.2 ± 14.6 cm^2 compared with 17.1 ± 15.9 cm^2 at initiation of standard therapy (P = .23). Mean treatment follow-up for duration of hVWM therapy compared with standard therapy did not differ significantly (10.9 vs 11.1 weeks; P = .93). After a mean follow-up of 10.9 weeks, mean wound size was reduced to 4 cm^2 with hVWM therapy (P = .002). On average, 79.2% wound surface area reduction was achieved in the hVWM group compared with 29.2% with standard therapy (P < .001). Patients received a mean of 7.2 applications of hVWM by the end of the 12-week study period.

A mean reduction of 1.7% in wound area was achieved per day using hVWM therapy compared with 0.7% with standard therapy alone (P = .01). However, the absolute area reduction per day did not differ significantly (0.15 vs 0.12 cm^2/d; P = .59). Table II summarizes the outcomes of wound healing with hVWM therapy compared with standard therapy. None of the patients enrolled in the study had an adverse event as a result of application of hVWM to the VLU during the treatment follow-up.

DISCUSSION

In addition to prolonged disability and significant psychosocial morbidity associated with VLUs, treatment of VLUs requires a considerable amount of resources. This is in large part due to chronicity of VLUs, necessitating long-term care. As a result, rapid complete ulcer

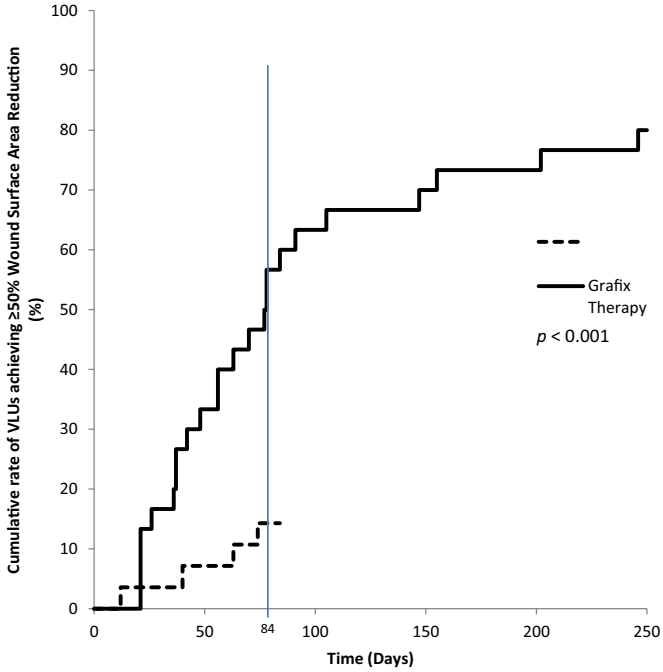

Fig 2. Kaplan-Meier analysis of probability of >50% wound surface area reduction in venous leg ulcers (*VLUs*) with application of cryopreserved placental tissue in human viable wound matrix (hVWM; Grafix) as an adjunctive therapy vs standard therapy alone. The *reference line*, day 84 (12 weeks), is demarcated to reflect the reporting interval in this study (n = 30).

healing and recurrence prevention are of paramount importance to lessen the socioeconomic impact of VLUs. There has been a substantial amount of interest in development of wound care products and therapies devoted principally to acceleration of wound healing.

Early wound healing rates have been demonstrated to predict later complete wound closure. Failure of wound size reduction by 30% to 40% at 4 weeks is highly predicative of nonhealing after an additional 8 weeks of therapy.[10] Therefore, adjunctive therapies to local wound care and compression therapy should be sought in an effort to aid wound closure. The aim of this study was to evaluate hVWM as an adjunctive therapy in closure of VLUs refractory to healing and wound size reduction after standard therapy.

The hVWM is an aseptic cryopreserved placental tissue engineered to maintain the integrity of placental tissue. This method of preservation is intended to protect placental tissue components consisting of MSCs, growth factors, and collagen-rich extracellular membranes against denaturation.[11] Published reports on use of amniotic membranes to accelerate wound healing date back more than a century.[12] The human placental membrane is a rich source of MSCs. MSCs are self-renewing pluripotent stem cells that have been demonstrated to promote healing of chronic wounds and to stimulate stalled healing processes.[9] In a multicenter randomized controlled trial, hVWM has been shown to significantly improve healing of chronic diabetic foot ulcers.[13]

Amniotic membrane grafting of chronic VLUs improves wound epithelialization and healing.[14] The results of this prospective study provide additional evidence in support of beneficial healing effects of placental tissue in healing of VLUs. All patients enrolled in this study failed to achieve significant wound healing with standard local wound care and compression therapy as demonstrated by reduction in wound size of 29.2% during a mean treatment time of 11.1 weeks. The same patients subsequently underwent a prospective trial with application of hVWM as an adjunct to standard therapy. The majority of VLUs healed completely with the application of hVWM after failing to heal with standard therapy (53% vs none; $P < .001$). The results of this prospective study are comparable to those of a retrospective study of VLU patients undergoing hVWM therapy demonstrating 68% complete wound closure at 12 weeks.[11] These findings compare favorably with porcine small intestine submucosa, which is the only bioengineered wound care product shown to achieve 55% healing rate (vs 34% to controls; $P = .02$) at 12 weeks for treatment of VLUs in a prospective randomized controlled trial.[15]

At mean follow-up of 10.9 weeks, 80% (24/30 limbs) achieved >50% reduction in wound surface area (compared with standard therapy; $P < .001$). Mean surface area of the VLUs was reduced by 79% when hVWM was used as an adjunctive therapy compared with 29% with standard therapy alone ($P < .001$). Most of the wounds refractory to standard therapy healed completely or the surface area was halved with hVWM therapy at about 4 to 14 weeks (Figs 1 and 2). Beyond 14 weeks of therapy, significant reduction in wound surface area was not observed.

Relative wound surface area reduction per day was statistically significant, whereas absolute surface area reduction per day was not. This perhaps is indicative of the fact that hVWM therapy may be more beneficial in closure of smaller wounds compared with larger ones even though baseline wound size at initiation of each therapy did not differ significantly. However, it is difficult to assess this for certain because of the sample size studied. Larger randomized trials are needed for further analysis.

Biologic products tend to be expensive; however, continued wound care and support for nonhealing ulcers is also expensive. We did not perform a comparative cost-benefit analysis in this small study; in the future, this information will facilitate decision-making on the overall benefit of this treatment modality. The study cannot rule out that some ulcers may have healed with standard therapy. Importantly, though, the wound healing rate during standard therapy was significantly slower than during hVWM therapy, and wounds with ≥50%

Table II. Summary of wound healing outcomes

	Standard treatment self-controls	hVWM	*P* value
Baseline wound size, cm^2			
Mean ± SD	17.1 ± 15.9	12.2 ± 14.6	.23
Median (IQR)	11.9 (5.4-23.6)	6.7 (3.3-12.3)	
Treatment duration, weeks			
Mean ± SD	12	10.9 ± 8.8	.93
Median (IQR)	12	8.5 (5.2-11.8)	
Final wound size, cm^2			
Mean ± SD	14.0 ± 14.7	4.0 ± 7.0	.002
Median (IQR)	7.5 (3.4-20.5)	0 (0-4.5)	
Completely healed wounds, No. (%)	0 (0)	16 (53)	<.001
Wound area reduction, %, mean ± SD	29.2 ± 28.8	79.2 ± 27.3	<.001
Wounds with ≥50% area reduction, No. (%)	7 (25)	24 (80)	<.001
Healing rate, %/d, mean ± SD	0.73 ± 1.44	1.69 ± 1.30	.01
Area reduction, cm^2/d, mean ± SD	0.12 ± 0.26	0.15 ± 0.19	.59

hVWM, Human viable wound matrix; *IQR*, interquartile range; *SD*, standard deviation.

wound area reduction were significantly higher with hVWM treatment too. These three end points together show the same directionality and indicate an overall better and more rapid wound healing response to hVWM. Certainly, larger studies are warranted to confirm these preliminary results. Saphenous vein ablation in patients with reflux results in faster ulcer healing, with 85.6% healed at 24 weeks.[5] Adjunctive hVWM in our patients with recalcitrant ulcers resulted in additional healing at 12 weeks. In the future, in addition to compression, the value of individual as well as combinations of adjunctive therapies (perhaps radiofrequency ablation plus hVWM) on healing of VLUs will need to be determined. Our population from the Veterans Administration Medical Center was all male. Although it is possible that there may be a sex-based difference in response to therapy of venous ulcers, this has not been reported.

CONCLUSIONS

VLUs continue to be associated with significant morbidity. Adjunctive wound care products remain an important tool to achieve accelerated complete closure of VLUs. Cryopreserved placental tissue in hVWM containing active growth factors and MSCs has the ability to improve healing processes. Application of hVWM after failure of standard therapy achieves complete wound closure in a significant majority of chronic refractory VLUs. Adjunctive therapy with hVWM provides superior healing rates with significant proportional surface area reduction in refractory VLUs.

AUTHOR CONTRIBUTIONS

Conception and design: BL
Analysis and interpretation: BF, ST, TM, JS, AU, RK, RS, BL
Data collection: BF, TM, AU, RK, BL
Writing the article: BF, ST, JS, BL
Critical revision of the article: BF, ST, TM, AU, RK, RS, BL
Final approval of the article: BF, ST, TM, JS, AU, RK, RS, BL
Statistical analysis: BF, JS, BL
Obtained funding: BL
Overall responsibility: BL

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