

A Prospective, Randomized, Controlled Trial of Autologous Platelet-Rich Plasma Gel for the Treatment of Diabetic Foot Ulcers

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Nonhealing diabetic foot ulcers are a common cause of amputation. Emerging cellular therapies such as platelet-rich plasma gel provide ulcer management options to avoid loss of limb. The purpose of this prospective, randomized, controlled, blinded, multicenter clinical study was to evaluate the safety and efficacy of autologous platelet-rich plasma gel for the treatment of nonhealing diabetic foot ulcers. One hundred, twenty-nine (129) patients were screened; 72 completed a 7-day screening period and met the study inclusion criteria. Patients were randomized into two groups – the standard care with platelet-rich plasma gel or control (saline gel) dressing group – and evaluated biweekly for 12 weeks or until healing. Healing was confirmed 1 week following closure and monitored for another 11 weeks. An independent audit led to the exclusion of 32 patients from the final per-protocol analysis because of protocol violations and failure to complete treatment. In the 40 wounds per-protocol group, 13 out of 19 (68.4%) of the platelet-rich plasma gel and nine out of 21 (42.9%) of the control wounds healed. After adjusting for wound size outliers (n = 5), significantly more platelet-rich plasma gel (13 out of 16, 81.3%) than control gel (eight out of 19, 42.1%) treated wounds healed (P = 0.036, Fisher's exact test). Kaplan-Meier time-to-healing also was significantly different between groups (log-rank, P = 0.0177). No treatment-related serious adverse events were reported and bovine thrombin used in the preparation of PRP did not cause Factor V inhibition. When used with good standards of care, the majority of nonhealing diabetic foot ulcers treated with autologous platelet-rich plasma gel can be expected to heal.

KEYWORDS: platelet-derived growth factors; platelet releasate; platelet-rich plasma; PRP; platelet gel; diabetic foot ulcers; Factor V; bovine thrombin, autologous growth factors

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More than 20.8 million persons in the US have diabetes mellitus; 2002 data estimates from the Centers for Disease Control and Prevention indicate that 82,000 lower limb amputations

were performed in persons with diabetes.¹ Characteristic pathological changes attributed to autonomic and sensory neuropathy, often combined with vascular disease, lead to a high-risk situation for the person with diabetes.^{2,3} Persons

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who have had such pathology and experience trauma or infection are at high risk for developing ulceration of the foot or ankle. Pecoraro et al⁴ documented the causal pathway of an amputation and found that in 81% of cases faulty wound healing contributed to amputation. Healthcare practitioners should utilize wound treatments that can reduce the rate of faulty wound healing; thus, preventing amputations.

According to the American Diabetes Association, more than 60% of nontraumatic lower-limb amputations occur in people with diabetes; the rate of amputation for people with diabetes is 10 times higher than for people without diabetes; Mexican Americans are 1.8 times as likely, non-Hispanic Blacks are 2.7 times as likely, and American Indians are three to four times as likely to experience lower-limb amputations. Amputation rates are 1.4 to 2.7 times higher in men than women with diabetes.⁵ Frykberg et al⁶ cites a 1998 study of 67,000 diabetes-related lower extremity amputation (LEA) and a similar study that resulted in a total of 984,000 hospital days, each length of stay averaging 15 days. Nonhealing diabetic foot ulcers and the resulting potential amputations present significant costs to the healthcare system and reduce patient quality of life.

The goal of diabetic foot ulcer treatment is to obtain wound closure as expeditiously as possible. Accepted therapeutic objectives and standards of care for diabetic foot ulcers include wound debridement, pressure relief in the wound area, appropriate wound management (eg, moist wound healing), infection management, ischemia management, medical management of comorbidities, and surgical management as needed.⁶ Emerging cellular therapies such as platelet-rich plasma (PRP) can have an adjunctive role in a standardized, quality treatment plan.

Platelet releasates, including multiple growth factors, have been used to treat wounds since 1985. *In vivo* prospective controlled studies as well as retrospective and cost effectiveness studies documenting the effect of this therapy have been

published.⁷⁻²¹ *In vitro* research has shown that platelets contain components and properties for wound healing²²; likewise, plasma contains fibrin matrix.²³

In 2001, Margolis²⁴ published a retrospective study analyzing the treatment results of 26,599 patients with diabetic neuropathic foot ulcers who had been treated with an autologous platelet releasate. The results suggest that platelet releasate provided with standardized care was more effective than standard care alone.

The purpose of the current study was to determine the safety and effectiveness of treating diabetic foot ulcers with PRP gel versus a control treatment (normal saline gel). The primary objective of the 12-week study was to compare the safety and incidence of complete wound closure between PRP gel- and control-treated wounds at the end of the study. Secondary objectives included comparing the rate of wound healing during the 12-week study and incidence of wound recidivism among healed ulcers during a 3-month follow-up period. Safety variables included adverse events, serious adverse events, and clinical laboratory tests.

Study Design and Methods

This prospective, randomized, controlled, double-blinded, multicenter trial was conducted under the US Food and Drug Administration (FDA) Investigational Device Exemption (IDE) regulations. Constella Clinical

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KEY POINTS

- While some foot ulcers in persons with diabetes mellitus will heal within a reasonable period of time when optimal care is provided, others will not; thus, increasing the risk of complications such as amputation.
- To test the safety and efficacy of autologous platelet-rich plasma (PRP) gel in the treatment of chronic nonhealing diabetic foot ulcers, researchers randomly assigned persons with nonhealing diabetic foot ulcers to a 12-week treatment period of offloading and control or offloading and PRP gel.
- No short-term or long-term (24-week) safety concerns were observed but a variety of protocol violations reduced the power of the study to detect statistically significant differences.
- In the total group, 68% of PRP gel and 43% of control gel treated wounds healed. When standardized for baseline size, the proportion of wounds healed was significantly different (81% versus 42%).
- The results of this controlled study suggest that, as part of an overall program of optimal care, PRP gel is more effective than non-PRP gel. The results also confirm the appropriateness of using moisture-retentive control dressings in all chronic wound studies.

Informatics (Durham, NC) served as the Clinical Research Organization (CRO) to implement and monitor the trial, gather the data into a central database, and audit the data. A data safety monitoring board provided evaluations of the safety-related events throughout the treatment phase of the study. Independent statisticians were contracted to initially power the study, develop the statistical plan, and analyze the safety and effectiveness data.

Fourteen (14) investigative sites from across the country participated in the study. Sites included wound care physicians' and podiatrists' offices, outpatient wound care centers, a university-based college of podiatric medicine clinic, Veteran's Administration wound care clinics, and an Army hospital limb preservation program. Each site obtained IRB approval to conduct the study.

Study eligibility. Persons with type 1 or type 2 diabetes between the ages of 18 and 95 with an ulcer of at least 4-weeks' duration were eligible for the study if they met additional inclusion/exclusion criteria: hemoglobin A1C <12; index foot ulcer located on the plantar, medial, or lateral aspect of the foot (including all toe surfaces); and wound area (length x width) measurement between 0.5 cm² and 20 cm², inclusive. Wounds located under a Charcot deformity had to be free of acute changes and must have undergone appropriate structural consolidation. The index ulcer had to be clinically noninfected (although a culture was obtained, infection was diagnosed through clinical signs and symptoms rather than culture results²⁵) and full-thickness without exposure of bone, muscle, ligaments, or tendons (University of Texas Treatment-Based Diabetic Foot Classification System: Grade 1A²⁶).

The protocol required that post debridement the ulcer would be free of necrotic debris, foreign bodies, sinus tracts, tunneling, and undermining; comprised of healthy vascularized tissue; and at least 4 cm from any additional wound. Additionally, the limb had to have adequate perfusion as shown by examination and non-invasive vascular testing ankle brachial index (ABI) and toe brachial index (TBI). Women of childbearing age could not be pregnant or lactating; both men and women had to be willing to use a medically accepted form of birth control throughout the trial and for 6 months following. Patient history, physical examination (including a Semmes-Weinstein monofilament test for neuropathy), and blood for baseline laboratory studies were obtained.

Approved, informed, signed consent stipulating that the patient was able to comply with all specified care and visit requirements was secured from the patient, caregiver, or legal representative before study enrollment. The Investigator documented reasonable expectation that the patient was medically stable and capable of completing the study. Study exclusion criteria are listed in Table 1.

After meeting all initial inclusion criteria and signing the informed consent, all patients completed a 7-day screening-period. This included initial excision/debridement, baseline wound measurements and evaluation, and application of the control saline gel to the wound. For standardization, sharp debridement guidelines were provided as part of the protocol. Patients, a family member, or other designated parties were provided supplies and instructed to change the dressings once midway through the screening period. The patient also was required to use a fixed ankle-foot orthoses that could be removed for the dressing change and at night. Crutches or a walker were used for added safety. Screening data were captured on case report forms (CRFs) for data analysis. Patients whose wounds reduced in area by >50% during the screening period were not randomized to treatment and discontinued from any further study participation because they appeared to be able to heal without more advanced intervention.

Randomization and blinding procedures. The randomization schedule was electronically generated, blocked per investigational center, and provided to the site by the contract research organization (CRO). Each eligible study participant was assigned to one of two treatment groups, PRP or control, and received the next available consecutive randomization number. Each site had one designated "unblinded" person to treat the patient (also blinded) and maintain documents in a secure private area to maintain blinding of the investigator, investigative site staff, patient, sponsor, and CRO staff and monitor. This person did not participate in any other aspect of the patient's care. The blinded investigators and staff measured the wounds; performed all tests, assessments, and debridement; and determined wound closure. A strategically placed drape prohibited the patient from seeing which treatment was applied to the wound. Blood was drawn from both the treatment and control patients to maintain blinding.

PRP preparation process. The PRP separation system utilized in the study is a newer generation, point-of-care

TABLE 1 STUDY EXCLUSION CRITERIA

- Patient currently enrolled in another investigational device or drug trial or previously enrolled (within last 30 days) in investigative research of a device or pharmaceutical agent
- Ulcer decreased $\geq 50\%$ in area during 7-day screening period
- Ulcer is due to non-diabetic etiology
- Patient's blood vessels are non-compressible for ABI testing
- Evidence of gangrene in ulcer or on any part of the foot
- Patient has radiographic evidence consistent with diagnosis of acute Charcot foot
- Patient is currently receiving or has received radiation or chemotherapy within 3 months of randomization
- Topical, oral, or IV antibiotic/antimicrobial agents or medications have been used within 2 days (48 hours) of randomization
- Patient has received growth factor therapy (eg, autologous platelet-rich plasma gel, becaplermin, bilayered cell therapy, dermal substitute, extracellular matrix) within 7 days of randomization.
- Screening serum albumin level < 2.5 g/dL
- Screening hemoglobin < 10.5 mg/dL
- Screening platelet count $< 100 \times 10^9/L$
- Patient is undergoing renal dialysis, has known immune insufficiency, known abnormal platelet activation disorders – ie, gray platelet syndrome, liver disease, active cancer (except remote basal cell of the skin), eating/nutritional, hematologic, collagen vascular disease, rheumatic disease, or bleeding disorders
- History of peripheral vascular repair within the 30 days of randomization
- Patient has known or suspected osteomyelitis
- Surgical correction (other than debridement) required for ulcer to heal
- Index ulcer has exposed tendons, ligaments, muscle, or bone
- Patient is known to have a psychological, developmental, physical, emotional, or social disorder, or any other situation that may interfere with compliance with study requirements and/or healing of the ulcer
- History of alcohol or drug abuse within the last year prior to randomization
- Patient has inadequate venous access for blood draw
- Patient has a religious or cultural conflict with the use of platelet gel treatment

system for processing autologous platelets and plasma to be used for the treatment of nonhealing wounds. This system is comprised of two components: a small, portable centrifuge to separate whole blood into PRP and a convenience kit that includes items for the blood draw, processing and PRP gel application. The platelet-rich plasma gel (AutoloGel™, Cytomedix, Inc, Rockville, Md) was used to treat patients in the treatment group. Wounds in the control group were treated with a saline gel (NormlGel®, Mölnlycke Health Care, Norcross, Ga). Either PRP gel or saline gel was applied to the prepared wound bed.

The first step of the PRP separation process included performing a venipuncture to draw ≤ 20 mL of blood, depending on the wound size, from the patient. The blood was spun in a small, portable centrifuge for 1.5 minutes to separate the PRP from the whole blood. The PRP was extracted into a syringe where reagents were added to activate the platelets and plasma as well as to achieve proper gel consistency (gel consistency was usually attained within 15 to 30 seconds); the gel then was immediately applied to the wound. A contact layer dressing was applied over the gel. A foam dressing (non-absorbent side) was placed over the contact dressing layer so the PRP gel was not absorbed. This was covered with the absorbent side of a foam dressing (to absorb any leaking wound exudates) and secured. For protection, barrier cream was placed on intact skin surrounding the wound.

For patients randomized to the control group, normal saline gel was applied to the wound following wound bed preparation. Similar to PRP gel application, a contact layer dressing was applied over the saline gel, followed by the non-absorbent side of a foam dressing and covered with the absorbent side of a foam dressing before being secured.

Clinical evaluations and procedures. Wounds were assessed and measured (length, width, and depth using a metric tape measure) at each visit. The measurements and other wound variables including undermining or tunneling, characteristics of wound exudates (ie, presence, color, amount, and odor), necrotic tissue, and granulation tissue were documented.²⁷ Results relevant to additional variables/findings will be published in a separate paper.

Care and management efforts provided at each treatment visit included cleansing and assessing the wound and obtaining vital signs and an interim wound history, including information regarding adverse events, concomitant medications, nutrition and weight-bearing status, and

**TABLE 2
LABORATORY STUDIES' SCHEDULE**

Laboratory Parameter	Baseline/ Screening	q2 Weeks During Treatment	6 Weeks	12 Weeks	24 Weeks
Complete blood count	X	X	X	X	
Chemistry 7 panel, serum albumin	X	As needed	X	X	
HgbA1C	X				X
Partial thromboplastin time, prothrombin time, thrombin time	X	X	X (if during treatment)	X (if during treatment)	
Antibody for Factor V Fibrinogen	X		X	X	X
Antigenic fibrinogen	PRN positive fibrinogen				

week later but asked to continue wearing the offloading orthosis walker. At this visit, if the wound had reopened, the patient was re-entered into the study at the same timeline (coinciding with the return visit for continued care) and continued until the wound either healed or until week 13, visit 1 without healing. If the wound stayed healed after the 1-week interval, the patient entered the follow-up phase and returned after 3, 7, and 11 weeks.

other aspects of care since the last visit. A facility designee performed phlebotomy; the unblinded person performed all subsequent gel processing. The principal investigator, who did not observe treatment procedures, directed wound care provision during the care visit.

The need for consistency in product application and maintenance of the blinding process dictated that dressings were applied only at the Investigator's site except for the provision of a one-time dressing change at home should circumstances prevent clinic attendance. Patients returned twice weekly at 3- or 4-day intervals; procedures and processes described were performed at each visit for a maximum of 12 weeks. Treatment continued until the wound healed, the 12-week treatment phase was complete, or patient study participation was terminated by the Investigator, sponsor, or because the patient withdrew consent or failed to return for visits.

To evaluate safety, clinical laboratory tests were conducted throughout the study to determine the impact of treatment interventions (see Table 2). Expected or unexpected adverse events (AE) that occurred during the course of the study, whether observed by the Investigator or by the patient, were reported in detail. The Investigator monitored the patient for AEs or lab abnormalities until the parameter returned to normal or it was determined that follow-up was no longer necessary.

End-of-treatment visit procedures. When the Investigator pronounced the wound closed—ie, 100% epithelialized—the patient was scheduled for a visit 1

During this 3-month follow-up phase, the healed wound was evaluated for breakdown and the patient was queried regarding adverse events.

At the end of the 12-week treatment period, unhealed wounds were treated per physician protocol. The patient was discharged from the site for follow-up at a facility of his/her choice. All participants were asked to return for final Factor V testing at week 24 post-randomization date. End-of-study occurred at completion of the week 24 clinical lab evaluation, withdrawal of patient consent, or death of the patient. Patient procedures/instructions were repeated, including a complete history and physical examination with testing of pedal pulses, vascular testing (ABI and TBI), Semmes-Weinstein monofilament testing, laboratory tests, dressing change as per the Investigator's order, and education with a discussion of healthcare options (see Figure 1).

Statistical Analysis

Healing rate. The power of the study was determined based on two data sources. The PRP gel healing rate was determined from the healing rate in an unpublished diabetic foot ulcer retrospective study²⁸ and feedback from clinicians who had used the PRP gel.²⁹ The control group healing rate was based on a meta-analysis of healing rates in the control groups of 10 prospective studies.³⁰

Sample size. To determine the sample size, the expected proportion of patients with completely closed wounds was determined to be 0.60 (π_1) in the PRP gel arm and 0.20 (π_2) in the control arm. To calculate the sample size,

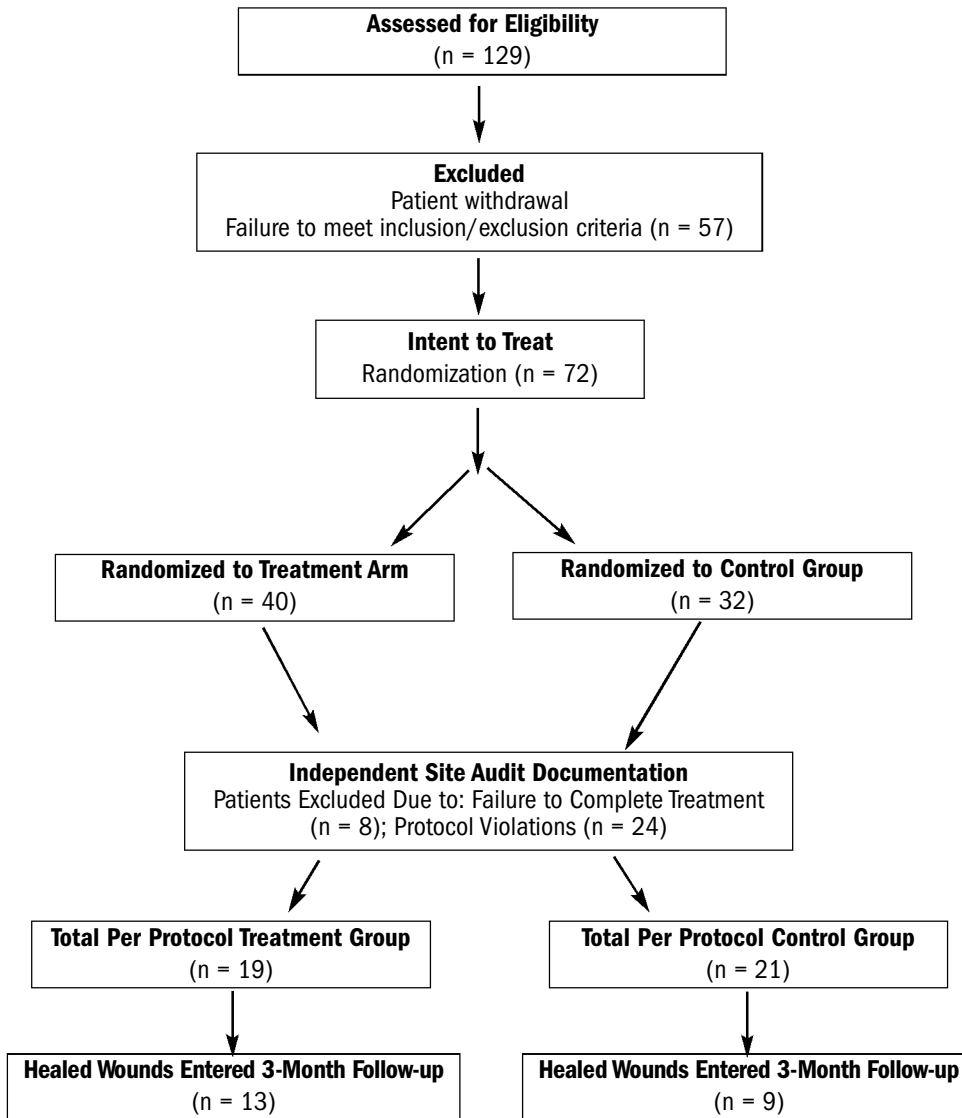


Figure 1. Trial profile.

the following null hypothesis (H_0) and the alternative hypothesis (H_A) were formulated:

$$H_0: \pi_t = \pi_c \text{ versus } H_A: \pi_t \neq \pi_c .$$

A Fisher's exact test with a 0.050, two-sided significance level would have 80% power to detect the difference between PRP gel proportion, π_t , of 0.60 and control proportion, π_c , of 0.20 when the sample size in each arm of treatment is 27 (ie, a total of 54 patients). The sample size was increased from 54 to 72 patients to accommodate drop-outs.

The primary efficacy variable was the proportion of patients with a healed wound. Fisher's exact test was applied to compare the two treatments for proportions

healed within each group of investigative sites and all groups combined.

Because of varying enrollment at each site (between one and 14 patients), sites were grouped for analysis purposes according to provider setting and demographics (Groups I to V). Due to the varying nature of investigative sites and their ability to enroll patients, sites were grouped into five categories: teaching facilities, army facility, physicians in private practice (two sites), and ambulatory care clinics. The goal was to enroll eight to 21 patients in each group. Results from the independent audit eliminated all patients in one group, leaving four groups for per protocol (PP) analysis.

Odds ratio/confidence level. The odds ratio and 95% confidence interval for each group were calculated for the proportion of patients with healed wounds. The Mantel-

Haenszel (M-H) combined odds ratio (combining over groups) along with the 95% confidence interval was calculated. In addition, the M-H test for homogeneity of odds ratios of groups was performed. The M-H method was used to test the hypothesis whether the M-H combined odds ratio was one.

Additional variables. Other efficacy variables were 1) percent change in wound area at end-of-study visit (EOSV) from baseline (BL); 2) percent change in wound volume at EOSV from BL; 3) area closure rate per day at EOSV; and 4) volume closure rate per day at EOSV. These efficacy variables were of continuous type. For each of the variables, the two treatments were compared using

Student's *t*-test. The statistical tests were performed using software package STATA, Release 8.2 (Stata Corporation, College Station, Tex).

Kaplan-Meier. In addition, the Kaplan-Meier^{31,32} product-limit method was used to analyze time to healing of the PP wounds and the majority of wound sizes dataset. Kaplan-Meier functions or curves of the PRP gel and control groups were obtained for each dataset. The log-rank test was used to test the hypothesis that the Kaplan-Meier healing functions are the same across the two treatments.

Laboratory safety. To evaluate clinical laboratory safety, observed values at each visit and changes from baseline at post-baseline visits and endpoint were summarized descriptively (number (n), mean, standard deviation (SD), minimum, median, maximum) for each treatment group. Results between treatments at the endpoint were compared using non-parametric analysis of variance techniques (Wilcoxon Rank Sum Test, two-sided).

Shift analyses. The number and percent of patients from each treatment group that shifted in and out of normal range from baseline to endpoint were calculated for each laboratory variable. To compare treatments for important shift changes, the number of patients whose laboratory results shifted from NORMAL or LOW at baseline to HIGH at endpoint and those shifting from NORMAL or HIGH at baseline to LOW at endpoint were compared in separate 2 x 2 tables and differences were tested for statistical significance using two-sided Fisher's exact tests. Patients whose laboratory results did not shift from baseline range or who were NORMAL at endpoint were analyzed together with those that had out of range shifts via exact tests to compare treatment groups (Exact test for row [R] x column [C] tables). With these three separate analyses, variables can be evaluated either for single direction shifts of interest or shifts in either direction, when both HIGH and LOW deviations may be of significance.

Results

Initially, 129 patients provided Informed Consent forms and participated in active screening (see Figure 1). Of these patients, 57 (44%) were dropped from the study due to reduction in the wound size of $\geq 50\%$ during the 7-day screening period or for failure to meet the inclusion/exclusion criteria. Ultimately, 72 patients were enrolled, each patient having one wound (index ulcer) designated for study inclusion.

**TABLE 3
INTENT TO TREAT GROUP: PATIENT DEMOGRAPHIC AND BASELINE WOUND VARIABLES**

Variable	PRP Gel (n = 19)					Control (n= 21)					P value
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	
Patient											
Age (years)	40	56.4	10.2	31.0	75.0	32	57.5	9.1	45.0	86.0	NS*
Hgb A ₁ C	37	8.1	1.8	5.5	13.1	30	8.0	1.8	5.0	11.5	NS
Wound											
Area (cm ²)	40	4.0	5.3	0.4	24.0	32	3.2	3.5	0.5	15.8	NS
Volume (cm ³)	40	1.7	4.1	0.1	24.8	32	0.9	1.2	0.1	5.4	NS

Characteristics	Treatment				Total	
	PRP Gel (n = 40) [†]		Control (n= 32) [†]		No. of patients	Percent
	No. of patients	Percent	No. of patients	Percent		
Sex						
Male	32	80.00	27	84.38	59	81.94
Female	8	20.00	5	15.63	13	18.06
Race						
Caucasian	26	65.00	18	56.25	44	61.11
Hispanic	8	20.00	9	28.13	17	23.61
Black	5	12.50	3	9.38	8	11.11
Other, specify	1	2.50	2	6.25	3	4.17
Foot						
Right	23	57.50	18	56.25	41	56.94
Left	17	42.50	14	43.75	31	43.06
Location						
Toe	13	32.50	14	43.75	27	37.50
Heel	18	45.00	10	31.25	28	38.89

* NS = not significant ($P > 0.05$)

[†] Demographic / ulcer location variables not statistically significantly different between groups

In the intent-to-treat (ITT) population, the mean and standard deviations (SD) for age, HgbA₁C, wound area, and volume in the two treatments were not significantly different, but the wound volume in the PRP gel group was significantly more variable than in the control group (SDs 4.1 versus 1.2, $P < 0.0001$) (see Table 3). Ulcer location information was missing for nine patients (three in PRP gel group and six in the control group). No significant differences in patient demographics, wound distribution, or ulcer location were observed between the two treatment groups (additional data to be analyzed in a future publication). For purposes of the ITT analyses, the ITT population comprised all active patients who completed the study as well as those who were lost to follow-up, failed to complete the treatment, or had protocol

violations. In the ITT group, 13 out of 40 patients (32.5%) in the PRP gel and nine out of 32 patients (28.1%) in the control group had completely healed wounds after 12 weeks ($P = 0.79$). Because the results of the ITT analyses did not seem to reflect previous clinical outcomes, the study sponsor commissioned an independent audit to ensure study compliance with Good Clinical Practices (GCP) at the investigative sites.

During the audit, patient source documents, Case Report Forms (CRF), and other study source documents were reviewed. Five objective criteria were developed against which all audited patient records were evaluated. The protocol violations that caused exclusion of patients included use of the wrong centrifuge (causing the patient not to receive the right treatment — ie, PRP gel); lack of

source documentation to support case report form entries; and inclusion of patients and/or wounds that did not meet the inclusion/exclusion criteria. The predetermined statistical plan identified that analysis would be performed on patients who completed treatment; thus, patients with early termination due to reasons unrelated to the index wound and patients lost to follow-up also were excluded from the PP analysis.

Site audits revealed that 32 out of 72 patients (44%) had protocol violations or did not meet the criteria for participation throughout the course of the study. Of the 32 excluded patients, 24 (75%) had protocol violations and eight (25%) failed to complete treatment. The protocol violations appeared to affect outcomes; thus, the PP dataset, at audit completion, became the primary dataset for analysis — 19 patients were in the PRP gel group and 21 patients were in the control group. These patient outcomes reflect patients/wounds treated PP.

In the PP group, only the proportion of Caucasian versus non-Caucasian participants was significantly different ($P = 0.02$). The proportion of Caucasians was significantly higher in the PRP gel group. No statistically significant difference between the PRP gel group and the control group related to age, HgbA1C, wound area, wound volume, sex, or wound location were observed. This is the same as the ITT group (see Table 4). (Additional differences between baseline findings for other wound variables will be addressed in a later publication.)

Efficacy outcomes. In the PP dataset, 13 of 19 (68.4%) patients in PRP gel and nine out of 21 (42.9%) patients in the control group healed ($P = 0.125$, two-sided Fisher's exact test). The 95% CI for the percent proportion of completely healed wounds was 47.5% to 89.3% and 21.7% to 64.0% for PRP gel and control groups, respectively. The Kaplan-Meier median time to complete closure was 45 days for PRP gel compared to 85 days for control (log-rank test, $P = 0.126$) (see Figure 2).

Although the inclusion/exclusion criteria included wounds with an area range at least 0.5 cm² to no larger than 20 cm², size frequency distributions showed that the majority (35 out of 40, 88%) of wound sizes were in the range of both ≤ 7.0 cm² in area and ≤ 2.0 cm³ in volume and five (three patients/wounds in the PRP and two in the control group) were outliers. The mean area of the outliers was 10.53 cm² (SD 8.9) and 14.63 cm² (SD 1.6) for the PRP and control group, respectively. Because the size range of this group correlates with the average wound size in multiple published diabetic foot ulcer studies,³³ the analyses were repeated using ulcers in this size range only. This subset of the PP dataset will be referred to as the majority wounds group.

When standardized for size, (mean area PRP = 2.01 cm² (SD 1.3) and control 2.43 cm² (SD 1.6), the proportion of completely healed wounds was 13 out of 16 (81.3%) and eight out of 19 (42.1%) in PRP gel and control treatment groups, respectively ($P = 0.036$, Fisher's exact test). The 95% CI for the percent proportions of completely healed wounds was 62.1% to 100% for the PRP and 19.9% to 64.3% for the control group.

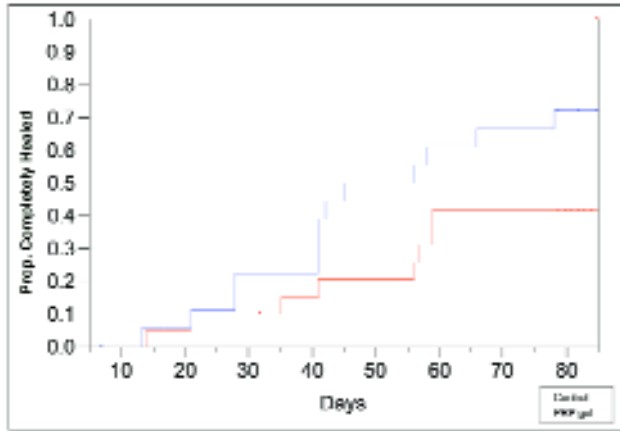


Figure 2. Per protocol patient group (n = 40) Kaplan-Meier time-to-healing curves.
 Test = log-rank; Chi square = 2.34;
 Degrees of freedom = 1; P value = 0.126

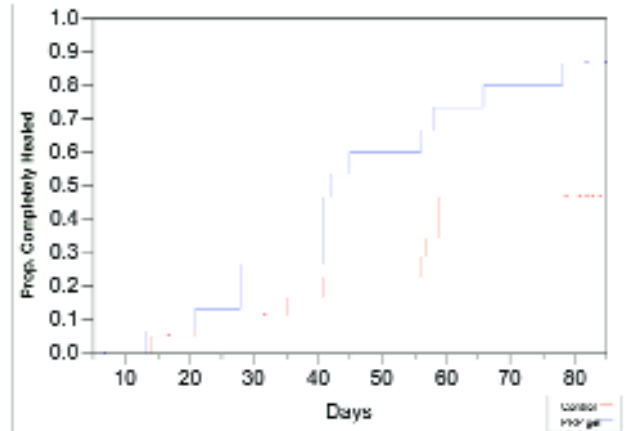


Figure 3. Majority wound patient group (n = 35) Kaplan-Meier time-to-healing curves.
 Test = log-rank; Chi square = 5.62
 Degrees of freedom = 1; P value = 0.0177

TABLE 4
PER PROTOCOL PATIENT DEMOGRAPHICS AND WOUND CHARACTERISTICS AT BASELINE

Variable	PRP Gel (n = 19)					Control (n= 21)					P value
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	
Patient											
Age (years)	19	58.3	9.7	43.0	75.0	21	55.9	8.1	45.0	78.0	NS*
Hgb A ₁ C	18	7.8	1.5	5.5	11.1	20	8.1	1.8	5.0	11.4	NS
Wound											
Area (cm ²)	19	3.4	4.5	0.8	20.0	21	3.6	4.0	0.5	15.8	NS
Volume (cm ³)	19	0.9	1.3	0.1	4.7	21	1.0	1.4	0.1	5.4	NS

Characteristics	Treatment				Total	
	PRP Gel (n = 19)		Control (n= 21)		No. of patients	Percent
	No. of patients	Percent	No. of patients	Percent		
Sex						
Male	16	84.21	16	76.19	32	80.00
Female	3	15.79	5	23.81	8	20.00
Race						
Caucasian	15	78.95 [†]	9	42.86 [†]	24	60.00
Hispanic	4	21.05	8	38.10	12	30.00
Black			3	14.29	3	7.50
Other, specify			1	4.76	1	2.50
Foot						
Right	14	73.68	10	47.62	24	60.00
Left	5	26.32	11	52.38	16	40.00
Location						
Toe	6	31.58	9	42.86	15	37.50
Heel	10	52.63	6	28.57	16	40.00

* NS = not significant (P > 0.05)

[†] P = 0.02, two-sided Fisher's Exact Test

The Kaplan-Meier curves of proportion for completely healed wounds over time for the majority wound dataset showed that the two curves started separating from each other on about day 28 (see Figure 3), log-rank test, P value = 0.018.

When evaluating the range of healing outcomes in the four investigative site groups groupings, wide variations in healing outcomes between the site groups were observed. For the PP dataset, the site group percent of complete healing proportion varied from 50% to 100% for PRP gel-treated wounds and from 25% to 67% for control-treated wounds. For the majority wound dataset, the site group percent of complete healing proportion varied from 60% to 100% and from 25% to 60% in the PRP gel- and control-treated wounds, respectively. A trend for increased wound healing in the PRP group compared to the control group also was observed. Despite the between-site group variations, healing outcomes in each treatment group were consistent and similar in both the total dataset of the per protocol and majority wound groups.

Rate of healing. In the PP dataset, the average wound area closure rate per day was 0.051 cm² for the PRP gel group versus 0.054 cm² for the control group. In the majority wound dataset, the wound area closure per day was 0.042 cm² for the PRP gel group and 0.043 cm² for the control group; these differences were not statistically significant.

In the PP dataset, wounds in the PRP gel group healed after a mean of 42.9 days (SD 18.3) compared to 47.4 days (SD 22.0) for wounds in the control group. While the number of days to healing was the same in the majority wound group (mean 42.9 and 42.8 days), 81.3% of PRP gel-treated wounds and 42.1% of control gel-treated wounds healed during that time.

Follow-up. Of the 40 patients in the PP dataset, 22 with healed wounds participated in the 12-week follow-up phase; of those, one in the PRP gel group had a wound that reopened. None of the control-treated patients' wounds re-opened; this difference was not statistically significant.

Safety outcomes: adverse events (AE). An AE in a clinical study patient who has been administered an investigational agent is any unusual medical occurrence that has appeared or worsened after the start of study whether or not the occurrence was related to the use of the investigational product. Adverse events were captured for any clinical abnormalities that appeared or worsened between the patient's start of the 7-day screening period and 30 days after receiving the last dose of study treatment.

Of the 127 adverse events, five occurred in two patients before randomization during the 7-day screening period. Of the remaining 122 adverse events occurring after randomization, 60 (49%) were in the PRP gel group and 62 (51%) in the control group. Of these, two were identified as definitely related to the treatment: one case of contact dermatitis occurred in a PRP gel treated wound and one instance of maceration occurred in a control treated wound.

Safety outcomes: serious adverse events (SAE). An SAE is an adverse event that meets any of the following outcome criteria:

TABLE 5
REPORTED SERIOUS ADVERSE EVENTS (N = 23 EVENTS)

PRP Gel Group (6 events; 5 patients)					
Medra Term	During Treatment	Post Treatment	Event	Severity as determined by Investigator	Relationship to device
Myocardial infarction		X	Death	Severe	Unrelated
Congestive heart failure	X		Hospitalization	Mild	Unlikely
Pneumonia	X		Hospitalization	Moderate	Unlikely
Pneumonia	X		Hospitalization	Mild	Unrelated
Pneumonia; osteomyelitis	X		Hospitalization	Mild	Unrelated
Control Group (17 events; 7 patients)					
Localized infection	X		Hospitalization	Severe	Unlikely
Chest pain (gallstones)	X		Hospitalization	Mild	Unlikely
Infected left foot; left foot ulcer	X		Hospitalization	Mild	Unlikely
Bacterial arthritis/encephalopathy	X		Hospitalization	Moderate/severe	Unlikely
Gangrene, anemia, renal failure, cardio-respiratory arrest		X	Death	Severe	Unlikely
Diabetic foot, cellulitis, osteomyelitis	X		Hospitalization	Severe	Unlikely
Cellulitis, arthritis bacterial, atrioventricular block, elevated blood glucose	X		Hospitalization	Mild/severe	Unrelated

- is fatal
- is life-threatening — ie, the patient was, in the view of the Investigator, at immediate risk of death from the reaction as it occurred; however, it does not include a reaction that, had it occurred in a more serious form, might have caused death
- requires or prolongs inpatient hospitalization
- results in significant or persistent disability/incapacity
- is a congenital anomaly or birth defect
- is an important medical event, based on appropriate medical judgment, that may jeopardize the patient or require the patient to seek medical or surgical intervention to prevent one of the other outcomes above.

Of the 122 adverse events after randomization, 23 were classified as serious adverse events; six occurred in the PRP gel group and 17 in the control group. All serious adverse events were unlikely or unrelated to device usage as defined by the investigators (see Table 5).

Clinical laboratory results. To analyze the safety of treating patients with the PRP gel, laboratory tests were conducted according to the study's predetermined time-frame. Because a prospective trial on the use of PRP gel in patients with diabetes had not been conducted before, questions were raised whether the blood draws, use of bovine thrombin, or the treatment itself would have a systemic effect on persons with diabetes. These concerns determined the clinical laboratory tests that were conducted during the trial (see Table 6).

Of the 72 participating patients, 56 (78%) returned for the day 168 laboratory tests. No statistically or clinically significant differences were noted between the PRP gel and control from baseline to endpoint laboratory shifts in hematology, clotting factors, and Factor V tests. Although no statistically significant difference was noted between the PRP gel and control in relation to shifts of clotting factors, a shift (increase) was noted in PT and PTT results in both treatment groups. No clinically important changes in clotting factors that would cause concern about the

effect of the PRP gel or control on Factor V activity were found during an independent monitor review of the medical records, including concomitant medications.

No clinical or statistically significant differences were noted in chemistry test results between the PRP gel and control from baseline to endpoint for sodium, potassium, chloride, bicarbonate, creatinine, or albumin. A statistically significant difference was observed between treatments in the change from baseline for BUN; the BUN of the PRP gel-treated patients decreased while the BUN of control patients increased (no explanation was determined).

Serum glucose or HbA1C results showed that more patients shifted to high at endpoint in the PRP gel compared to the control group. These differences were not statistically significant or clinically meaningful; they suggested that more patients in the PRP gel group had uncontrolled diabetes.

These safety results document the minimal occurrence of adverse events. No serious adverse events were attributable to PRP gel and minimal were attributable to laboratory shifts. These effects were comparable with the control group.

Discussion

This is the first reported prospective, randomized, blinded, controlled trial in the US on the use of PRP for the treatment of diabetic foot ulcers. In this FDA-approved study, some of their requirements (certain inclusion/exclusion criteria, blinding system, choice of control treatment, extensive laboratory tests, and documentation) added to the rigor and complexity of the study design, which in turn caused some difficulty enrolling patients.

This study comprised two levels of screening: pre- and active screening. Initially, an investigator evaluated whether a patient was a potential candidate for the study. Approximately 650 patients were

**TABLE 6
CHANGES IN CLINICAL LABORATORY RESULTS**

Laboratory Test	PRP Gel (n = 40)		Control (n = 32)		P value*
	Baseline (mean)	Endpoint (mean)	Baseline (mean)	Endpoint (mean)	
Hematology					
Hemoglobin (G/DL)	13.7	13.4	13.1	12.8	0.740
Hematocrit (%)	40.6	40.1	39.2	38.5	0.644
Platelet count (10 ³ /UL)	264	280	263	262	0.076
White blood cells (10 ³ /UL)	8.0	7.9	7.8	8.0	0.877
Chemistry					
Albumin (G/DL)	3.8	3.7	3.7	3.6	0.4693
Bicarbonate (MEQ/L)	23.9	23.7	23.9	23.4	0.6674
Blood urea nitrogen (MG/DL)	21.1	19.6	20.7	23.1	0.0405
Chloride (MEQ/L)	101.5	102.2	102.1	102.4	0.5721
Creatinine (MG/DL)	1.1	1.1	1.1	1.1	0.4143
Glucose, serum (MG/DL)	187.2	202.7	175.6	211.5	0.4045
Potassium (MEQ/L)	4.6	4.5	4.4	4.5	0.2343
Sodium (MEQ/L)	138.0	137.9	137.8	137.3	0.6172
Hemoglobin A1C (%HB)	8.0	8.5	8.0	8.0	0.1232
Factor V activity (%)	105.2	104.8	101.0	103.1	0.6113
Clotting Factors					
PT (seconds)	12.8	13.4	13.0	13.2	0.8545
PTT (seconds)	29.7	31.9	30.2	30.3	0.3738
TT (seconds)	12.4	12.9	12.8	12.4	0.4315
TT Human (seconds)	11.3	11.7	11.3	11.1	0.2196

* Wilcoxon rank sum test, two-sided

pre-screened — ie, reviewed by the investigator for possible inclusion — to secure the 129 active screening patients. Active screening comprised baseline wound assessment, physical exam, laboratory tests, wound culture, vascular tests, wound excision/debridement, and the 7-day screening period for patients meeting the requirements. From the 129 actively screened patients, 72 were randomized and 40 met study protocol requirements (the clinical data audit prompted exclusion of 32 patients, dropping the PP number to 40 patients). Thus, ultimately, only 6% of patients who participated in the pre-screening process were enrolled.

During the analysis of the PP group, frequency distribution demonstrated that the majority of the wounds (35 out of 40) randomized into the study met the criteria of wound area ≤ 7.0 cm² and volume ≤ 2.0 cm³. The remaining five larger wounds had areas of 9 cm² to 20 cm² and results of various studies suggest that a wound size of < 7.0 cm² is most common.³⁰⁻³²

Efficacy variables also were analyzed for the subset of “majority wound.”

Average baseline area in the majority wound group was similar to that reported in a tissue-engineered product study (n = 208: mean wound area 2.97 – 3.1 cm²),³⁴ another tissue-engineered product (n = 15 wounds; efficacy noted in wounds < 6 cm²),³⁵ two recombinant growth factor studies (n = 118 patients and 132 wounds respectively; mean area 5.5 cm² and 2.6 cm²),^{36,37} and one retrospective study of diabetic foot ulcers (n = 26,599 patients of which 5,320 wounds averaged 1.53 cm², 5,320 wounds averaged 1.84 cm², and 5,319 wounds averaged 4.41 cm² in area²⁴). In the largest study published to date,²⁴ 60% of patients had wounds that matched the majority group in this PRP gel study, increasing the potential external validity of the current study results.

In the majority wound group, PRP gel-treated wounds were significantly more likely to heal than control-treated wounds even though healing rates in the control group

were higher (42% healed after 12 weeks) than most control group healing rates reported in other studies³⁰ (see Figure 4). Specifically, results of a meta-analysis of healing outcomes in the control arm of other diabetic foot prospective studies suggest that 24% can be expected to heal after 12 weeks of providing good care.³⁰ Most studies use wet-to-moist gauze saline gauze dressings, the recognized standard treatment by the medical community⁵ as a control dressing. However, wet-to-moist dressings require dressing changes three to four times daily by

the patient or caregiver and may not provide an optimal moist environment for healing. Some studies have shown that hydrocolloid dressings may be more effective than wet-to-moist for the treatment of certain types of ulcers.³⁸

The power calculations were based on reported results of control treatments in other studies and a sample size of 27 in each treatment arm. The better-than-expected control healing rates and large number of protocol violations caused underpowering of the PP and the majority wound groups yet some statistically significant differences were observed, suggesting that larger between-group differences could be expected in studies using a larger sample size.

The FDA's concern about the effects of frequent though small amount of blood collection (30 mL or less each visit) on health and safety as patients underwent periodic hematology and other laboratory tests throughout the trial was addressed. Test results documented that these frequent blood draws did not reduce the hemoglobin, hematocrit, or platelet count. Because bovine thrombin was used in the processing to activate the PRP and evidence exists of Factor V leading to bleeding disorders in patients exposed to large doses of bovine thrombin,³³ concerns were raised as to whether patients would similarly develop Factor V antibodies to the bovine thrombin with

% Diabetic Foot Ulcers Healed In The Control Arm of Prospective Studies

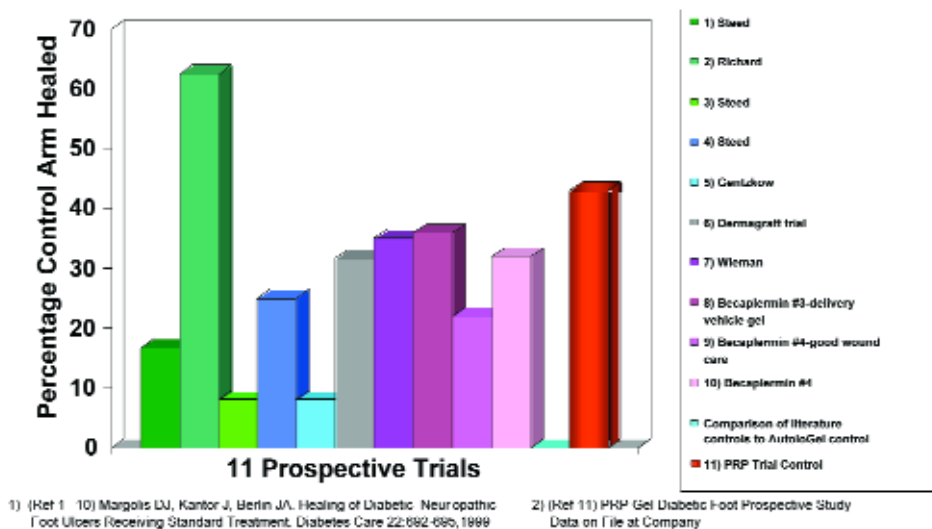


Figure 4. Comparison of diabetic foot ulcer prospective trial control outcomes.³⁰

small frequent exposure to it. To evaluate this potential impact, Factor V tests occurred at 6-week intervals for all patients regardless of randomization arm and at 24 weeks post randomization. None of the patients demonstrated any Factor V inhibition throughout the study and into the follow-up. This is the first time that this has been documented in a prospective study.

Laboratory tests indicated that the majority of patients had elevated blood glucose throughout the study period. Elevated glucose levels have been documented to reduce healing^{40,41}; however, in this study, the majority of the wounds healed.

Numerous articles have been published regarding the use of PRP in the surgical setting; notably, the orthopedic, plastic surgery, and dental field.⁴²⁻⁴⁴ Some of the PRP separation systems used require specialized technicians to perform the necessary procedures. This is the first published study of an autologous PRP separation system to heal wounds that can be used by health professionals within a traditional healthcare setting. A small, compact, point-of-care system such as the one described in the study makes this technology available to multiple care providers, including physician office, hospital unit, outpatient clinic, long-term care facility, and home health care staff.

Conclusion

The results of this study show that PRP gel is safe for use in the treatment of nonhealing diabetic foot ulcers. In the most common size of diabetic foot ulcers (≤ 7.0 cm² in area and ≤ 2.0 cm³ in volume), PRP gel-treated wounds are also significantly more likely to heal than control gel treated wounds. Treating wounds with PRP or saline gel resulted in healing in approximately 6 weeks, but in the most common wound sizes, almost twice as many PRP treated wounds healed in that time-frame. The number of adverse events was minimal; no adverse events were serious. Further, the study demonstrated that bovine thrombin used in the preparation of PRP does not cause Factor V inhibition; thus, it does not cause coagulopathy. In addition, withdrawal of a small amount of blood twice weekly did not affect patient hemoglobin, hematocrit, or platelet count. Clinically meaningful shifts in laboratory values studied from baseline to endpoint were not observed. This type of PRP system could be utilized by healthcare providers to treat diabetic foot ulcers in multiple settings. Using PRP gel to treat diabetic foot ulcers may not only enhance healing, but it also may prevent lower extremity amputations caused by nonhealing wounds.

Implications for future research include implementing a trial design that would permit greater subject enrollment on a larger sample size to validate these results. This trial would not need to re-evaluate some of the major questions answered in this trial, such as Factor V inhibition, impact on the patient's hematology and other clinical laboratory outcomes, and impact of a control that had not performed in a prospective trial previously. Diabetic foot ulcers with challenging presentations (ie, mild to moderate vascular disease, exposed tendon or bone, patient hyperglycemia, and/or inadequate nutritional status) could be studied to determine whether PRP gel could assist in healing in these compromised scenarios. In addition, studies to determine whether this novel therapy is synergistic with other advanced wound care modalities could be conducted. - OWM

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