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# A purified reconstituted bilayer matrix shows improved outcomes in treatment of non-healing diabetic foot ulcers when compared to the standard of care: Final results and analysis of a prospective, randomized, controlled, multi-centre clinical trial

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## Abstract

As the incidence of diabetic foot ulcers (DFU) increases, better treatments that improve healing should reduce complications of these ulcers including infections and amputations. We conducted a randomized controlled trial comparing outcomes between a novel purified reconstituted bilayer membrane (PRBM) to the standard of care (SOC) in the treatment of non-healing DFUs. This study included 105 patients who were randomized to either of two treatment groups ($n = 54$ PRBM; $n = 51$ SOC) in the intent to treat (ITT) group and 80 who completed the study per protocol (PP) ($n = 47$ PRBM; $n = 33$ SOC). The primary endpoint was the percentage of wounds closed after 12 weeks. Secondary outcomes included percent area reduction, time to healing, quality of life, and cost to closure. The DFUs that had been treated with PRBM healed at a higher rate than those treated with SOC (ITT: 83% vs. 45%, $p = 0.00004$, PP: 92% vs. 67%, $p = 0.005$). Wounds treated with PRBM also healed significantly faster than those treated with SOC with a mean of 42 versus 62 days for SOC

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($p = 0.00074$) and achieved a mean wound area reduction within 12 weeks of 94% versus 51% for SOC ($p = 0.0023$). There were no adverse events or serious adverse events that were related to either the PRBM or the SOC. In comparison to the SOC, DFUs healed faster when treated with PRBM. Thus, the use of this PRBM is an effective option for the treatment of chronic DFUs.

**KEYWORDS**

advanced wound care, advanced wound matrix, diabetic foot ulcers, standard of care, wound healing

**Key Messages**

- Patients with non-healing DFUs randomized to treatment with an advanced wound matrix, purified reconstituted bilayer membrane (PRBM), demonstrated a significantly improved healing rate over a 12-week period.
- PRBM demonstrated a statistically significant improvement in percent area reduction over 12 weeks versus standard of care (SOC)
- PRBM showed a statistically significant decreased time to healing versus SOC

## 1 | INTRODUCTION

It is estimated that 9% of the worldwide population is diabetic, and this is expected to affect over 600 million people in coming years.[1] A notable consequence of this is likely to be an increase in the incidence of diabetic foot ulcers (DFUs), which currently affect 19%–34% of diabetics.[2] As most clinicians can attest, the management of DFUs can be challenging, as the interruption of the normal healing cascade can result in 30% of these wounds becoming chronic.[3] Sadly, the 5-year mortality for DFUs is comparable to many cancers[4,5] and patients whose DFUs progress to amputation have a 5-year mortality of 55%.[6,7] With such a substantial burden on patients, and the healthcare system in general, there is an obvious demand for therapies that can ameliorate the healing process, thus leading to wound closure and preventing the complications that lead to the worst of the morbidities and subsequent premature mortality.

In light of the consequences that DFUs can have on patients' lives, as well as the prognosis for the increased cases, there is a clear need for methods of successfully treating these wounds. This is reflected in the current recognition, by the Centers for Medicare and Medicaid Services, of 76 skin substitutes of varied material properties for the treatment of DFUs.[8] The significance is further underscored by the ongoing clinical research, with Clinicaltrials.gov currently listing 838 studies for the treatment of DFUs.[9] Whether the grafts are derived from allogeneic, xenogeneic, or synthetic sources, they all have the same goals of providing a local environment that fosters the healing of the wound. While many devices purport to enhance healing, the results of the peer-reviewed publications point to evidence gaps and therefore there is a call for better information on wound healing and recurrence when using skin substitute products.[8]

With clinical necessity for modalities that will improve the chances of successful healing, the introduction of a Purified Reconstituted Bilayer Wound Matrix (PRBM, Geistlich Derma-Gide®, Geistlich Pharma AG, Switzerland) may offer clinicians another option. This bilayer matrix, which has been processed to remove cells, lipids, antigens and inactivate potential viruses, has a 3-dimensional structure that is similar to the human dermis. Importantly, the PRBM may impact the physiological processes and the wound environment. The growth factors TGF-β1, bFGF, and VEGF are essential constituents in wound healing,[10] while matrix metalloproteases (MMP) can impede healing.[11] The in vitro data for this PRBM has shown that the material allows the binding and proliferation of growth factors while also appearing to modulate MMP activity.[12]

With promising in vitro results, a pilot study in patients with chronic DFUs reported rapid healing in 90% of the patients.[13] While this was a small study ($n = 10$) it provided a reason to pursue further clinical research on this device. The recent interim results of this Randomized controlled trial (RCT), in which the PRBM was compared to a collagen alginate dressing (Fibracol; 3 M Maplewood, MN), showed that wounds treated with the PRBM healed significantly faster and were more likely to close.[14] Here we present the safety and efficacy results of the final completed RCT in which the targeted enrolment of over 100 subjects was met.

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## 2 | METHODS

A multi-centre, prospective, parallel-group, RCT was designed to evaluate the treatment of full-thickness, non-infected non-ischaemic (Wagner Grade 1/University of Texas 1A) DFUs with PRBM or standard of care (SOC). The study was registered on the German clinical trial register (DRKS) with a reference number of DRKS00016754 and approved by Western IRB (Protocol #20190130) and conducted in compliance with Good Clinical Practice standards and was in conformance with the ethical guidelines of the Declaration of Helsinki. Prior to any study activities, patients provided written, informed consent. The trial was performed at multiple specialty wound care centres between February 2019 and April 2023. The protocol schedule is shown in Table 1.

### 2.1 | TREATMENTS

#### 2.1.1 | Purified reconstituted bilayer membrane (PRBM)

Sterile, shelf-stable PRBM (Derma-Gide®; Geistlich Pharma AG, Wolhusen Switzerland) was provided in individual dry packaging in sizes from 1.1 to 12 $cm^2$. The PRBM was manually trimmed to match the size of each wound and placed dry with the porous lower layer facing down, directly onto the wound bed, thus allowing for uptake of wound fluids. If the PRBM was not completely hydrated by wound fluid, sterile saline was added in order to assure complete hydration, allowing the graft to conform to the wound bed before the application of a routine dressing.

#### 2.1.2 | Standard of care (SOC)

A moisture-retentive, conformable collagen alginate dressing (FIBRACOL Plus Dressing; 3 M Maplewood, MN) was the primary wound dressing in the SOC study arm. This dressing has been rigorously tested with favourable results and was chosen as a well-known, clinically accepted SOC product readily available in wound clinics.

#### 2.1.3 | Study endpoints

The primary study endpoint was a comparison of the proportion of index ulcers healed at 12 weeks. Secondary endpoints included comparisons of time to heal at 6 and 12 weeks, percentage area reduction (PAR) of the wound at 6 and 12 weeks, patient-reported quality of life outcomes, safety and cost to closure.

**TABLE 1** The study protocol schedule.

| |
|---|
| Weeks 1–2 screening phase |
| • Informed Consent, inclusion/exclusion criteria assessment<br>• Medical History & Physical, Vital Signs & Labs<br>• Assessment of diabetic wounds; DFU history<br>• Assessment of current wound therapies<br>• 10-point monofilament test<br>• X-ray<br>• ABI, SPP, TCOM TBI measurement or arterial Doppler study<br>• Patient completes Wound-QoL and pain assessment.<br>• Selection of index ulcer; measurement of surface area & digital imaging<br>• Index Ulcer Assessment of exudate and infection<br>• Treatment of index ulcer with SOC protocol<br>• Wound improvement over 14 days<br>• Confirm eligibility to continue enrolment into study |
| Weeks 3–14 treatment phase |
| • Medical history & Physical, Vital Signs & Blood Sugar<br>• Assessment of any adverse events<br>• Pain & Neuropathy Assessments<br>• Index Ulcer Assessment (exudate and infection), Cleaning & Debridement<br>• Measurement of surface area and digital imaging of index ulcer<br>• Assessment of Offloading<br>• Randomization (Week 3)<br>  ○ If randomized to SOC: Apply SOC therapy with Fibracol and outer dressing<br>  ○ If Randomized to PRBM: Apply SOC therapy with PRBM and outer dressing<br>• Weekly assessment of index ulcer, measurement, cleaning, debridement and repeat dressings<br>  ○ If Index ulcer is healed, no further treatment<br>  ○ After 6 treatment visits, if wound <50% healed, treatment phase ended; treatment failure |
| Week 15–16: End of study/confirmation visit |
| • Medical History and Physical Vital signs & Labs/Blood sugar<br>• Assessment of any adverse events<br>• Assessment of offloading<br>• Assessment of index ulcer (exudate and infection); complete epithelialization or if wound has re-opened<br>• Measurement of surface area and digital imaging of index ulcer<br>• Assessment of infection<br>• Cleaning, debridement and dressing of index ulcer if applicable<br>• Patient completes Wound-QoL and pain assessment |

#### 2.1.4 | Patient screening

After obtaining informed consent, participants were screened over a 14-day run-in period to determine eligibility according to inclusion and exclusion criteria (Table 2). The run-in preceded randomization to eliminate those patients in whom a short course of routine therapy would demonstrate effectiveness as measured by

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**TABLE 2** Inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|---|---|
| 1. At least 18 years old.<br>2. DFU, Wagner Grade 1<br>3. Index ulcer has been present ≥4 weeks prior to screening, but ≤1 year<br>4. Index ulcer ≥1.0 and ≤25 $cm^2$<br>5. The index ulcer will be the largest ulcer if two or more DFUs are present with the same Wagner grade and will be the only one evaluated in the study. If other ulcerations are present on the same foot, they must be more than 2 cm distant from the index ulcer.<br>6. Adequate circulation to the affected foot: TCOM or SPP ≥30 mmHg, or ABI between 0.7 and 1.3 within 3 months of screening or a biphasic Doppler ultrasound of dorsalis pedis and posterior tibial vessels at the level of the ankle or a TBI of >0.6.<br>7. Offloading of target ulcer ≥14 days before randomization.<br>8. Females of childbearing potential must be willing to use acceptable methods of contraception (birth control pills, barriers, or abstinence) during the course of the study and undergo pregnancy tests.<br>9. Subject understands and is willing to participate in the clinical study and can comply with weekly visits. | 1. Ulcer(s) deemed to be caused by condition other than diabetes.<br>2. Suspicion of malignancy in the index ulcer<br>3. Patients with ≥2 weeks of treatment with immune-suppressants, cytotoxic chemotherapy, or application of topical steroids ≤1-month prior to screening<br>4. Patients taking COX-2 inhibitors.<br>5. Patients on investigational drug(s) or therapeutic device(s) ≤30 days<br>6. History of radiation at the ulcer site<br>7. Index ulcer has been previously treated or will need to be treated with any prohibited therapies.<br>8. Osteomyelitis of the affected foot ≤30 days prior to randomization.<br>9. Subject is pregnant or breast-feeding.<br>10. Diabetes with poor metabolic control (HbA1c >12.0) within 90 days of randomization.<br>11. Patients with end stage renal disease, serum creatinine ≥3.0 mg/dL within 6 months<br>12. Ulcer has reduced in area by ≥20% 14 days of SOC from SV1 to the TV1/randomization visit.<br>13. Inability to complete this study or a history of poor adherence with medical treatment |

a >20% reduction in wound area. A review of each patient's medical history and a complete physical examination were performed including visual assessment of all foot ulcers with attention to signs of infection. The index ulcer was selected, imaged, and measured for area and depth. All wounds were managed during run-in using a standard protocol including cleaning, appropriate sharp debridement, infection management, dressing, and offloading using a diabetic offloading boot or when the patient's foot could not be accommodated with the offloading boot, a total contact cast was used. Subjects were instructed to keep the wound site dry and informed on the importance of offloading. They received education on infection indicators and asked to contact the clinic with concerns. Patients completed the wound quality-of life (Wound-QoL) questionnaire and scored their pain intensity on a scale of 0 to 10 using a visual analogue scale (VAS). After failing sufficient progress during the 2-week run-in period, subject eligibility was reconfirmed, and all eligible patients proceeded to randomization.

## 2.2 | Randomization and measures to minimize bias

Subjects were randomized to either the PRBM arm or SOC arm. To assure a balanced randomization, envelopes were created with a random allocation sequence in block sizes of 10. Wound assessment at conclusion of treatment was performed by a clinician, other than the investigator, who was blinded to the treatment. Additionally, confirmation of wound healing was overseen by an independent plastic surgeon adjudication committee.

## 2.3 | Treatment phase

Regardless of the study arm, wounds were managed with accepted routine SOC practices, including weekly sharp debridement as indicated. Patients randomized to PRBM arm were treated with a PRBM graft followed by a silicone non-adherent dressing (Adaptic Touch, 3 M Maplewood, MN) or equivalent, and those randomized to SOC arm were treated with calcium alginate dressing (FIBRACOL Plus 3 M Maplewood, MD). All wounds received an outer dressing comprised of a padded 3-layer dressing (Dynaflex, 3 M Maplewood, MN) or equivalent.

Study visits were performed weekly until either complete healing of the index ulcer or for 12 weeks, whichever came first. At each visit, the subject's overall health, glucose control, and offloading were assessed, and closure of the index ulcer was gauged by a blinded investigator. If the index ulcer was not completely reepithelialised, the wound was evaluated for signs of infection, cleaned, imaged, and measured. When the index ulcer was deemed to be 100% reepithelialised, further treatment ceased, and the patient was scheduled for a wound healing confirmation to visit 2 weeks later. Final wound area measurement and conclusive imaging were performed, and patients completed the Wound-QoL questionnaire at

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the final visit. Subjects whose index ulcer did not improve by 50% after 6 weeks were designated per protocol as a treatment failure and were allowed to receive alternative treatments outside of the study. Details specific to PRBM including trimmed size and handling characteristics were noted. Any adverse events (AEs), which were tracked using CTCAE v5, were identified, investigated, and managed as clinically appropriate. Suspected wound infections were diagnosed via wound swabs, and appropriate systemic antibiotics were prescribed. Topical antibiotics were contraindicated per protocol.

### 2.4 | Sample size and statistical analysis

According to the a priori power analysis, group sample sizes of 50 in each group would have achieved 88% power to detect a difference between the groups. Power calculations for the primary endpoint were based on a two-sided *Z*-test with pooled variance. Statistical testing between treatment groups at baseline was carried out to examine the success of randomization. For categorical variables, chi-square or Fisher exact tests were performed and for continuous variables independent *t*-tests or Mann–Whitney tests were used (depending on variable normality) to test for statistical differences.

The primary endpoint was evaluated by a Fisher exact test using an intent-to-treat (ITT) analysis of all randomized patients. Time to heal within 6 and 12 weeks was analysed using Kaplan–Meier log-rank test, while PAR at 6 and 12 weeks analysed using the Mann–Whitney test. The primary and secondary endpoints were tested hierarchically in a confirmatory manner. Other endpoints assessed in an exploratory manner included the Wound-QoL and pain score changes from baseline to 12 weeks. Statistical tests were two-sided and performed at a significance threshold of 0.05. The last observation carried forward (LOCF) principle was used in regard to missing data at study visits. Statistical Analysis was performed using SPSS Statistics 27 (IBM, Armonk, NY).

## 3 | RESULTS

A total of 123 patients were screened and 105 were randomized to treatment with either PRBM or SOC (Figure 1). In the PRBM group, 5 subjects were withdrawn or lost to follow-up: 1 subject withdrew consent and 1 patient's index ulcer had a PAR <50% at week 6. Although 3 people were removed due to an AE or SAE, these were not considered to be related to the PRBM. In the SOC group, 17 patients were withdrawn or lost to follow-up. A total of 14 patients were withdrawn due to the index ulcer presenting a PAR <50% at week 6 while 1 patient's ulcer was found to have reopened at the healing confirmation visit. In addition, two patients were removed due to AE or SAE, but as seen in the PRBM cohort, there were no instances of a causal relationship between the AE/SAE and the treatment.

The study cohort was representative of the diabetic population at the investigational sites, with a range of comorbidities that is typical to patients being treated for DFUs. Patient demographics and wound characteristics were well-balanced between the two arms, and the demographic characteristics at baseline (shown in Table 3) showed no significant differences between the groups. This was also seen for the wound characteristics at baseline (Table 4).

### 3.1 | Outcomes

Wound closure rate was significantly higher in the PRBM arm than the SOC arm. Using an ITT approach, at 12 weeks of treatment, 83% of the patients in the PRBM arm (45/54 DFUs) presented with healed wounds, compared to 45% in the SOC group (23/51 DFUs). This was significant at $p = 0.00004$. When this endpoint was evaluated per protocol (PP), 92% of patients (43/47) in the PRBM arm demonstrated wound closure compared to 67% (22/33) in the SOC arm ($p = 0.005$).

The secondary endpoints also showed that ulcers healed significantly faster for those patients in the PRBM group. For the ITT cohort the time to heal within 12 weeks was a mean of 42 days (95% CI: 35–49, median 42 days) among PRBM patients while in the SOC group this was a mean of 62 days (95% CI: 55–70, median 77 days), which was statistically significant ($p = 0.00074$). The Kaplan–Meier plot of healing, shown in Figure 2, illustrates this early divergence in the healing probability between the groups. This outcome was also statistically significant in the PP analysis, with PRBM showing a mean time to heal of 40 days (95% CI: 32.1–46.8) while SOC demonstrated a mean of 55 days (95% CI: 44.6–64.4), which was a significant difference ($p = 0.035$).

Analysis of time to heal within 6 weeks for the ITT cohort also showed a significant difference between study arms. The PRBM-treated wounds healed in a mean of 31 days (95% CI: 27–34) compared with a mean of 66.5 days (95% CI: 59–74) for wounds in the SOC arm ($p = 0.007$). The healing rate in the PRBM arm at the 6-week mid-study point was 56% compared with 29% in the SOC arm and was also significant ($p = 0.0098$).

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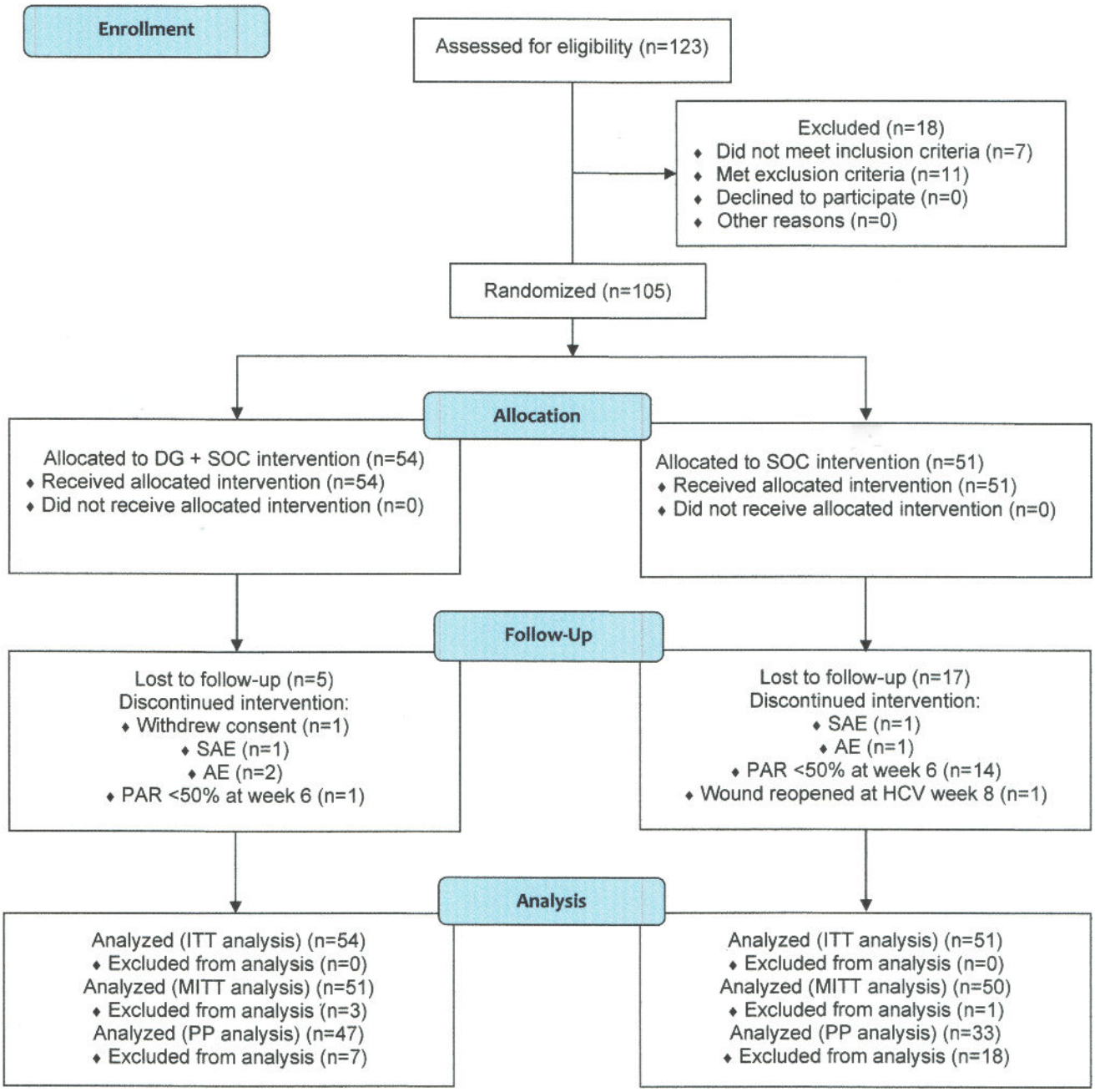


**FIGURE 1** CONSORT flow diagram.

The PAR values for the ITT cohort also showed a dramatic difference between the treatment arms. At week 6, the PRBM group had a mean PAR of 90.3 (SD:17.42) while in the SOC this was 52.6 (SD: 60.14) ($p = 0.00074$). Likewise, the week 12 mean values for PAR were 93.6 (SD:18.83) in the PRBM cohort compared to 50.5 (SD: 69.07) in the SOC cohort ($p = 0.0023$). The difference between groups in the PAR values is shown in Figure 3.

With regard to the exploratory measures of Wound QoL, for the ITT cohort the patients in the PRBM cohort had presented with a mean score of 1.2 (SD:0.90) at baseline, which decreased to 0.8 (SD:0.73) at the end of study, and this was similar to the SOC patients, with a change from 1.4 (SD:1.08) to 1.0 (SD:0.91), thus there was no significant difference ($p = 0.89$). Similarly, the change in the VAS for pain from baseline to end of treatment, were quite comparable for both groups: 1.2 (SD:2.26) at baseline and 0.8 (SD:1.67) at the end of study for PRBM versus 1.3 (SD:2.31) for SOC at baseline and 0.9 (SD:1.96) at the end of study.

Specific to safety, there were 13 SAEs, six in the PRBM group (five patients) and seven in the SOC group

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**TABLE 3** Key demographic variables at baseline.

| Variable | PRBM | SOC | *p* value |
|---|---|---|---|
| Age | 62.5 (12.01) | 63.8 (11.03) | 0.57 |
| BMI | 31.5 (6.73) | 33.2 (8.26) | 0.46 |
| Gender | | | |
| Male | 31 (57) | 32 (63) | 0.58 |
| Female | 23 (43) | 19 (37) | |
| Race/ethnicity | | | |
| Caucasian | 54 (94) | 50 (96) | 0.62 |
| African American | 3 (6) | 1 (2) | |
| Hispanic | 0 (0) | 1 (2) | 0.49 |
| Smoking | | | |
| Never | 23 (48) | 30 (59) | |
| Former | 19 (35) | 17 (33) | 0.33 |
| Current | 9 (17) | 4 (8) | |
| Patient age, 1st DFU | 54.7 (12.8) | 58.0 (10.8) | 0.16 |
| Significant foot deformities | 17 (32) | 11 (22) | 0.25 |
| Charcot | 15 (28) | 8 (16) | 0.13 |
| Hammer toes | 2 (4) | 3 (6) | 0.67 |
| Bunions | 2 (4) | 3 (6) | 0.67 |
| Other | 1 (2) | 2 (4) | 0.61 |
| Amputation | | | |
| Study foot, minor | 19 (35) | 10 (20) | 0.07 |
| Contralateral foot, minor | 8 (15) | 9 (18) | 0.69 |
| Contralateral foot, major | 4 (7) | 3 (6) | 1.0 |
| HbA1c screening | 7.3 (1.37)<br>Median 6.7 (IQR 1.5) | 7.0 (1.48)<br>Median 6.7 (IQR 1.5) | 0.14 |
| Creatinine | 1.2 (0.52)<br>Median 1.1 (IQR 0.5) | 1.1 (0.33)<br>Median 1.0 (IQR 0.4) | 0.13 |

(six patients). These are presented in Table 5. There were no instances of a causal relationship between the SAE and either the PRBM or the SOC. Not all SAEs required removal from the clinical trial.

Representative Cases document the healing with the use of PRBM in Figure 4.

## 4 | DISCUSSION

The increasing incidence of DFUs, with its attendant impact on clinical load and patient well-being, points to a need for effective treatment strategies. Our study, in which 105 patients were randomized to either PRBM or SOC, demonstrated a significantly shorter time to healing among those patients who had been treated with PRBM, as compared to SOC, and mirrored the interim results.[14] In addition to the proportion of wounds healed at 12 weeks in the PRBM cohort being close to twice that of SOC (83% vs. 45%), the time to heal within 12 weeks was remarkably shorter in the PRBM cohort, 42 days as opposed to 62 in SOC, while the PAR values at 12 weeks was a mean of 93.6 (SD: 18.83) for the PRBM patients in contrast to 50.5 (SD:69.07) for SOC. These remarkable differences in clinical outcomes support the use of PRBM as an effective and safe modality for the treatment of DFU.

With the impact that DFUs exert on patients, and the treatment difficulties as evidenced by both the risk of recurrence and the 5-year mortality,[4] there is an acute requirement for effective treatment. The plethora of products currently available and the amount of published data point to the awareness of this demand.[15–18] Despite this, the treatment of DFUs remains a challenge. These are difficult wounds to heal, often becoming chronic due to the healing process being interrupted or stalled.[19] Wound

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**TABLE 4** Wound related characteristics at baseline.

| Variable | PRBM | SOC | *p* value |
|---|---|---|---|
| Wound area ($cm^2$) | 2.8 (2.46) | 4.0 (3.59) | 0.059 |
| Wound age (weeks) | 14.7 (11.01) | 15.6 (11.39) | 0.68 |
| Prior offloading time at screening (weeks) | 12.9 (11.9) | 15.5 (16.8) | 0.59 |
| DFU side | | | |
| Left | 23 (43) | 22 (43) | 0.96 |
| Right | 31 (57) | 29 (57) | |
| DFU position | | | |
| Medial | 35 (65) | 33 (65) | 0.99 |
| Lateral | 19 (35) | 18 (35) | |
| DFU location | | | |
| Plantar | 40 (74) | 37 (73) | 0.86 |
| Dorsal | 14 (26) | 14 (27) | |
| DFU position | | | |
| Toe | 17 (32) | 8 (16) | |
| Forefoot | 10 (19) | 18 (35) | |
| Midfoot | 16 (30) | 13 (25) | 0.27 |
| Hindfoot | 3 (5) | 2 (4) | |
| Heel | 4 (7) | 6 (12) | |
| Ankle | 4 (7) | 4 (8) | |
| Index ulcer is a recurrence | 24 (44) | 21 (41) | 0.74 |
| Concurrent DFUs | 8 (15) | 13 (26) | 0.17 |
| Lifetime DFU count | 5.0 (4.92)<br>Median 3.5 (IQR 6) | 4.7 (4.72)<br>Median3 (IQR 4) | 0.81 |
| Offloading type (primary) | | | |
| Shoe | 11 (20) | 9 (18) | |
| Camboot/crowboot/walker | 39 (72) | 37 (72) | |
| TCC | 1 (2) | 3 (6) | 0.71 |
| Wheelchair | 1 (2) | 0 (0) | |
| None | 2 (4) | 2 (4) | |

healing itself is a complex physiological phenomenon requiring an interplay of physiologic process,[10,20] which may be why the healing of chronic wounds can be unpredictable. Therefore, the interaction of a graft's characteristics and its physiologic mechanisms are essential components of successful clinical outcomes.

In considering the overlapping phases of wound healing, from haemostasis to inflammation, proliferation, and finally remodelling, there is a complex and dynamic sequence of cellular and biochemical events that restore skin integrity and functionality after trauma.[21,22] Among the factors that have been noted to impact wound healing are transforming growth factor (TGF-β), vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF).[10] It has been reported that the PRBM that we used in the treatment of these subjects in the clinical trial has, in an in vitro experiment, bound the growth factors TGF-β1, bFGF and VEGF.[12] The notably improved healing that we observed is likely related to the grafts ability to bind these endogenous growth factors, which are essential components of wound healing.[23,24] Further with the in vitro data showing that the PRBM can bind and preserve the activity of TGF-β1, bFGF, and VEGF,[12] it seems logical that the retention of these endogenous growth factors would encourage wound healing, thus leading to the early closure that we have observed.

While growth factors are undoubtedly crucial in wound healing, the local environment can also be influenced in order to create more optimal conditions. One biochemical factor that can influence healing is the pH of

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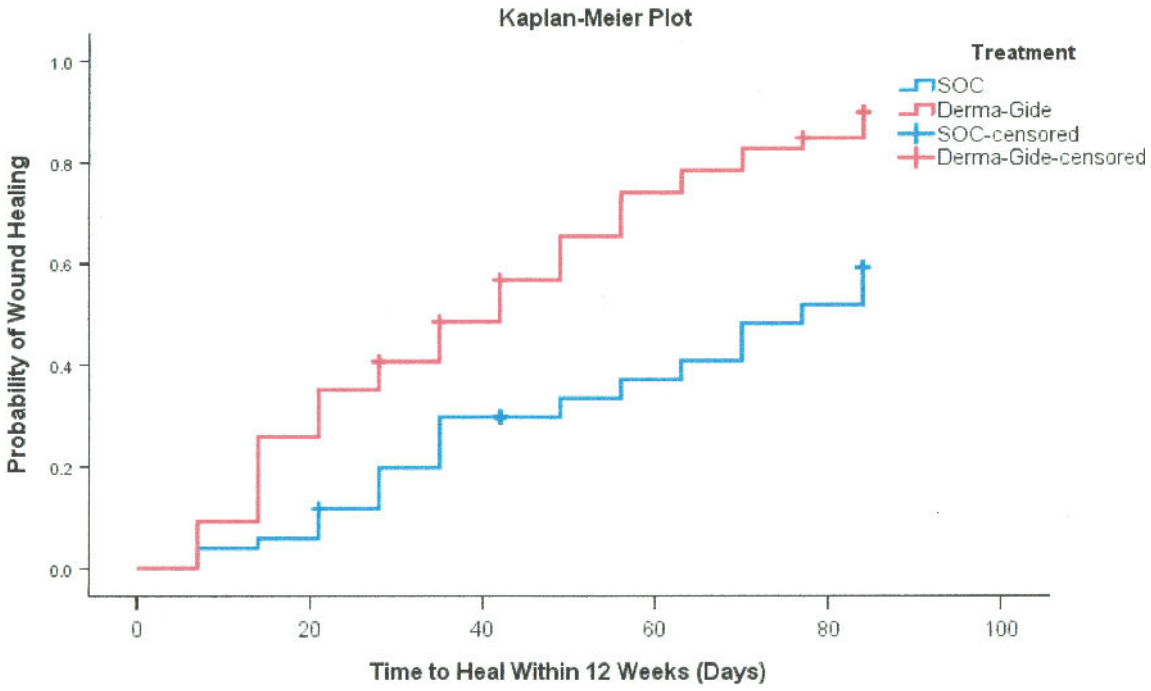
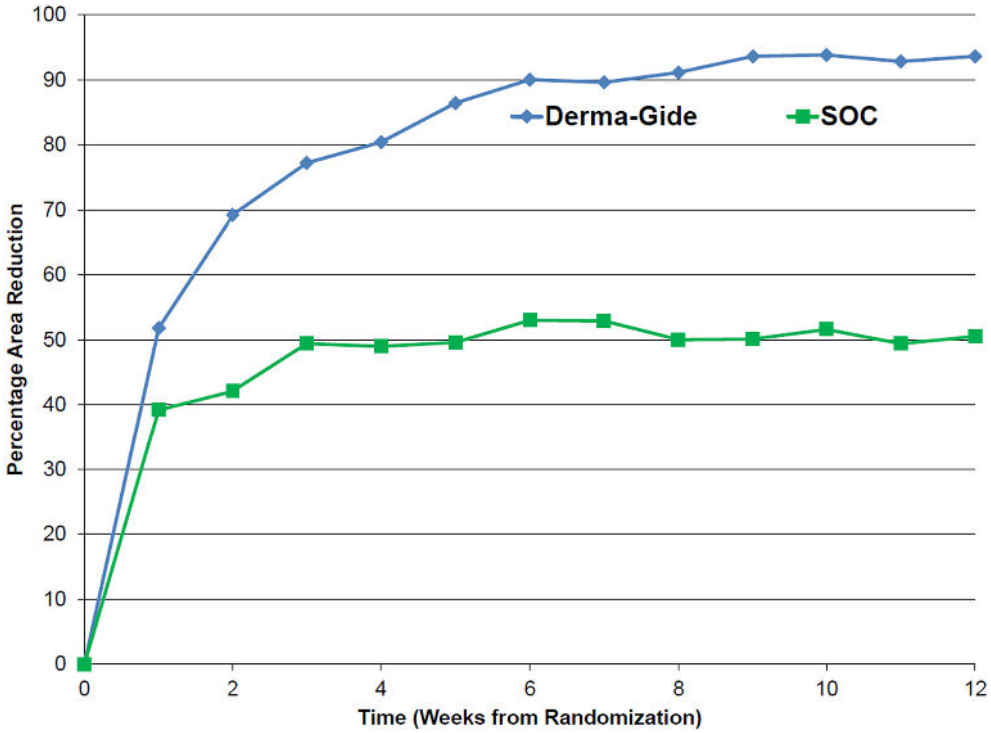



**FIGURE 2** Kaplan–Meier plot of probability of wound healing by treatment group. Unadjusted time depicted after randomization. Censor marks indicate subject exit prior to 12 weeks. A superior healing trajectory is demonstrated in the PRBM treatment group with a divergence apparent after about 1 week.

**FIGURE 3** Weekly PAR values for both treatment groups, based on the ITT data.




a wound, and it has been reported that chronic wounds have an alkaline pH whereas more normal tissue has a slightly acidic pH.[25] In this regard, the PRBM that we applied has also been documented to ameliorate the local environment with regard to pH.[12] This would certainly be consistent with publications stating that wound pH is a potent factor for the healing process, with different pH ranges being essential in the phases of wound healing.[26] Further a murine in vivo model had demonstrated that wound acidification promoted a number of cellular

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responses, such as an increased rate of re-epithelialization and collagen synthesis, thus inducing faster wound healing.[27]

**TABLE 5** A list of the SAE that were experienced by patients. None of these SAE were related to the PRBM or to the SOC.

| Derma-Gide | Standard of Care |
|---|---|
| • Necrotizing fasciitis to a leg venous ulcer, required hospitalization followed by septic shock (2 SAEs)<br>• Fracture in the left ankle that required surgery<br>• Cellulitis associated with a non-index ulcer<br>• Acute kidney failure<br>• Acute, non-infective gastroenteritis | • Deep vein thrombosis; cellulitis leukocytosis (2 SAEs)<br>• Pulmonary hypertension<br>• Plantar wound involving acute osteomyelitis (non-index ulcer), required extensive work-up including a bone biopsy and NPWT<br>• Acute osteomyelitis, index ulcer, required extensive work-up including a bone biopsy and NPWT<br>• Pneumonia, required extensive testing/ treatment<br>• Osteomyelitis, right hallux, required amputation. |

An additional consideration with regard to wound healing would relate to the role that matrix metalloproteinases (MMP) play in healing. It is known that these proteases play a key role in the degradation and remodelling of the extracellular matrix (ECM) during wound healing.[28] The wound healing process can thus be hindered by a number of biochemical or physiological processes, whether prolonged inflammation, imbalance in ECM synthesis and degradation, poor neovascularization, or impaired macrophage activity.[29] The MMPs are involved in all these processes and, unfortunately, high levels of MMP are associated with poor healing and dermal graft failure in DFU patients.[30] As a mechanism of healing, the PRBM that we applied has also been shown, in vitro, to modulate MMP activity.[12] In conjunction with the physiological mechanisms that we have previously noted (i.e., modified pH and the retention of growth factors), the modulation of MMP activity is likely another contributor to the improvement in healing that we observed in this study.

While our results showed an improved clinical outcome with shorter healing times, the cost-effectiveness of

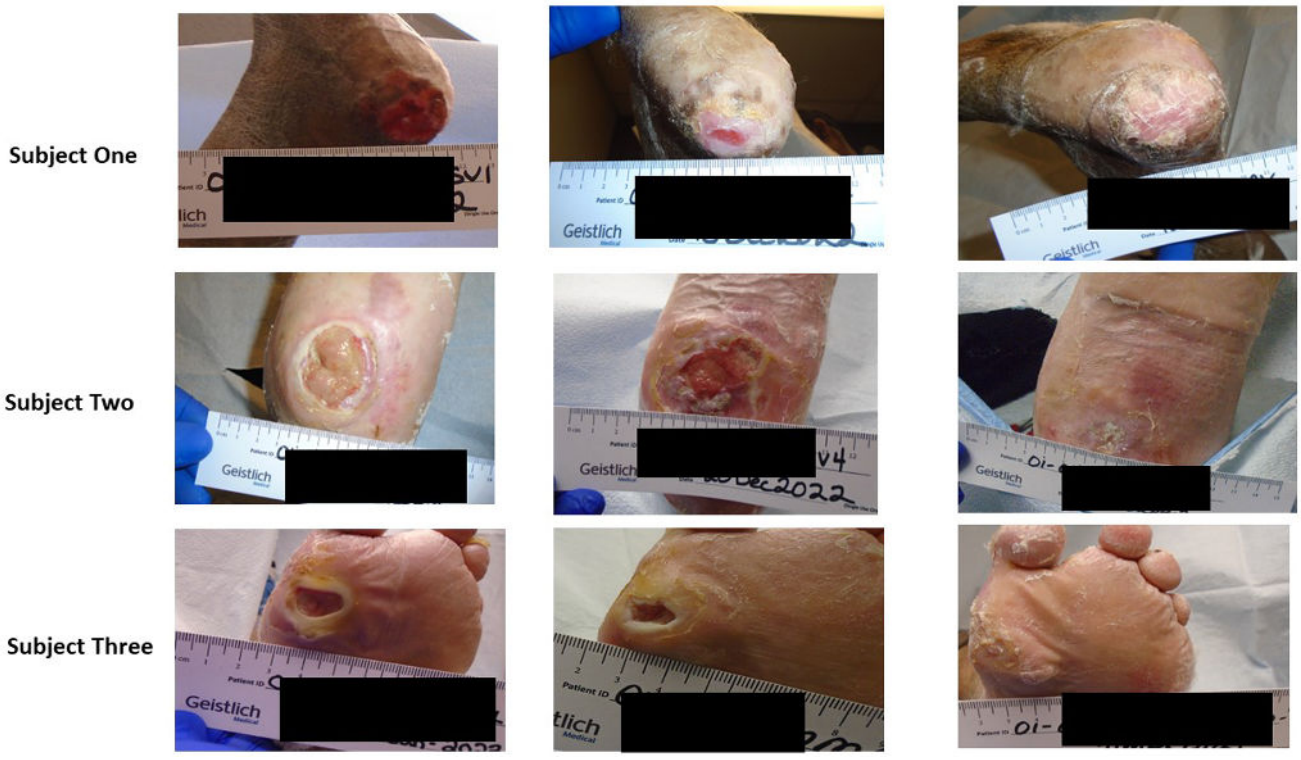


**FIGURE 4** Photos Depicting PRBM Treated Wounds During Their Study Participation. Representative cases depicting the time progression of wound healing following treatment with PRBM. Patient 1: Type 2 Diabetic Female with Ulcer x 15 weeks, HbA1c: 6.9%, Serum Creatinine 1.11 mg/dL and Wound Area of 7.5 $cm^2$. After 3 PRBM treatments the wound area decreased over 90%, and, following the fifth treatment, the ulcer was confirmed to be fully healed. Patient 2: Type 2 Diabetic Female with Ulcer x 5 weeks, HbA1c: 8.4%, Serum Creatinine 1.26 mg/dL and Would Area of 12.25 $cm^2$. After the 3 PRBM treatment the wound area had decreased by over 35% and after 12 treatments was confirmed to be fully healed. Patient 3: Type 2 Diabetic Female with ulcer x 4 weeks, HbA1c: 7.0%, Serum Creatinine 1.3 mg/dL and a Wound Area of 1.5 $cm^2$. After 5 PRBM treatments the wound area had decreased by over 50% and after nine treatments was confirmed to be fully healed.

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any treatment is a realistic concern in choosing a treatment modality. In fact, the mean cost of treating DFUs, per episode of care, has been reported from a healthcare public-payer perspective to be $31 024.[31]

With regard to the cost to closure specific to the use of PRBM in this study, there was a mean of 5.7 (SD:3.55) graft applications per patient, with a mean cost to closure of $1982 per patient (range $283–$14442). The costs we have calculated here are slightly higher than the mean graft cost to closure that had been reported, $1731.00, in the interim data of this study,[14] which is likely attributable to the variation of a much larger cohort. When we reviewed the individual data, we noticed that the patients with the highest costs, and who were statistical outliers, had either larger wounds or very high BMI, (e.g., patients with wounds of 10 $cm^2$ or BMI of 45). This cost is still comparable to the values for either amniotic/placental tissue costs of $1771 to $2252USD[16,32] while it is lower than the $3019 that has been reported for porcine grafts[33] or the $4089 for Fish-Skin grafts.[34] Considering the age of some of the publications that had provided cost information, the values are likely to be higher after adjusting for inflation.

The results of this RCT are promising, but we must acknowledge limitations. While the number of patients is larger than many other RCTs for this indication, it is still small by the standards of pre-market studies. Furthermore, the wounds were limited to Wagner one, non-infected and non-ischaemic wounds. Lastly, longer-term follow-up, in order to assess recurrence would benefit clinical decision making. Addressing these limitations would certainly be in line with the Agency for Healthcare Research and Quality, which has stated a need for studies that include patients with more comorbidities as well as more complex wounds.[8] Furthermore, longer-term studies are needed to evaluate recurrence, hospitalizations, amputations and mortality.

With a clear and pressing need for effective means of treating DFUs, the clinical outcomes that we have reported show that PRBM adds a viable option to the physician's armamentarium. The performance and the cost-effectiveness support its use, and we recommend further studies enrolling a more diverse patient population with heterogenous wound diagnoses.

## 5 | CONCLUSIONS

The application of the PRBM that we evaluated led to significantly shorter healing time of chronic DFUs, when compared to the Standard of Care and neither group had any SAEs that were related to treatment. In addition to the shorter healing time the costs were well below the mean cost of the standard of care for DFUs or the cost of alternative grafts used to treat DFUs. The outcomes in this study continue to build upon the data that had been presented, providing further validation of this PRBM as a first-line therapy in the treatment of DFU.

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## CONFLICT OF INTEREST STATEMENT

David Armstrong, DPM, MD, PhD received research funds from PERI to design and administrate the trial and also assist with the writing and review of the manuscript. Dennis Orgill, MD, PhD received research funds to serve as a validating/adjudicating plastic surgeon to review study photos and assist with the writing and review of the manuscript. Robert Galiano, MD received research funds to serve as a validating/adjudicating plastic surgeon to review study photos and assist with the writing and review of the manuscript. Paul Glat, MD received research funds to serve as a validating/adjudicating plastic surgeon to review study photos and assist with the writing and review of the manuscript. Jarrod Kauffman MD received research funds to assist in study design and in manuscript preparation. Marissa Carter, PhD received research funds to provide the statistical analysis plan, provide the statistical analysis for this trial and assist with writing the result section of the manuscript. Lawrence Didomenico, DPM is the medical director of the LEIRT and his company received research funds for enrollment in the clinical trial and write the paper for publication. Charles M Zelen, DPM is the medical director of the PERI and his company received research funds to administrate the clinical trial and write the paper for publication. There are no other conflict of interests with any of the authors in relation to this study, or with regard to Geistlich Pharma. IRB conflict of interest statements are on file with PERI.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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