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# A Prospective Study to Evaluate Efficacy and Safety of Autologous Platelet-Rich Fibrin Matrix for the Treatment of Chronic Diabetic Foot Ulcers

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## Abstract

Platelet-rich fibrin matrix (PRFM) acts as a scaffold to aid in the function of the cells contained within platelet rich plasma (PRP). In contrast to PRP, PRFM does not contain any coagulation additives, and it naturally forms a fibrin matrix clot which restricts the growth factor release to the clotting site. When the tissue begins to repair itself, it recruits fibroblasts to reorganize the fibrin matrix and starts building collagen. The trial being presented here studied the effects of PRFM on chronic DFUs older than 3 months. 25 patients were screened for the study, with 14 patients completing the trial. 8/14 (57.14%) had complete closure prior to or at the study endpoint of 12 weeks. This compares well with other cellular/tissue-based products available. Among the entire cohort, 13 patients (92.9%) had 50% or greater wound reduction by Week-12. No significant adverse events were determined to be related to PRFM. Based on our findings, PRFM is an effective way to treat challenging DFUs.

## Introduction

Diabetes mellitus has become a global epidemic, with approximately 422 million people affected worldwide [1] including 29 million people in the United States [2]. Foot ulcers are one of the main reasons for diabetes-related hospitalizations [3] while creating an economic burden on the healthcare system and considerably impairing quality of life [3,4]. Approximately one-third of diabetes-related costs have been linked to the treatment of foot ulcers [5]. Patients with diabetes have up to a 25% lifetime risk of developing a diabetic foot ulcer (DFU) [3]. A meta-analysis performed by Chen L, et al. found that the overall mortality of DFUs was high, with nearly 50% mortality within 5 years and cardiovascular disease and infection as the two leading causes of death [6].

**Diabetic foot ulcers can be generally divided into 2 types of wounds**: acute and chronic. Acute wounds heal through an organized, overlapping process of coagulation, inflammation, proliferation, and remodeling [7].

**Chronic wounds stall somewhere along the healing compendium which may be due to a variety of factors**: inadequate local blood supply, bioburden, infection, devitalized tissue, and lack of cellular signal activity among senescent cells [8]. These wounds tend to be more difficult to heal.

Currently, the standard of care (SOC) for initial treatment of DFUs is debridement, offloading, tight glycemic control and appropriate antimicrobial management and/or imaging when needed [9,10]. A meta-analysis of patients studied in controlled trials demonstrated, on average, healing rates of 31% at 20 weeks with SOC [11]. A substantial portion of these wounds will become infected over time, resulting in lower extremity minor and major amputation [12]. In cases where a wound fails to decrease in size by 50% within 4 weeks with SOC, advanced levels of care may be initiated to attempt to close the wound and limit these complications [13]. These may include topical platelet-derived growth factor (PDGF) [14], hyperbaric oxygen therapy (HBOT) [15], and cellular and/or tissue-based products (CTPs) [16-20]. Use of umbilical cord that has been cryopreserved and contains viable cells has also been shown to achieve full wound closure with less treatment and in less time [21].

Wound healing requires platelet activation, which subsequently releases cytokines and growth factors stored within the alpha granules of platelets. These cellular and humoral products stimulate mesenchymal stem cells to migrate and differentiate with the potential to regenerate tissue [22,23]. Some of these growth factors and their functions are listed in Table 1 [24]. Whole blood contains 45% cells, comprised of red blood cells, white blood cells and platelets. The remaining 55% is plasma, which contains mostly water, as well as electrolytes, metabolic wastes, and proteins [22]. The most notable protein in plasma is fibrinogen which is converted by fibrin into a binding scaffold after acute injury. This allows platelets and red blood cells to form a clot which is essential in wound healing and tissue growth [25].

Platelet-rich plasma (PRP), by definition, is plasma with an increased concentration of platelets above the normal levels found in blood [26]. Increased platelet concentration also increases growth factors, which through injury and clot formation, get released to tissues [22]. PRP has been used as a delivery medium to enhance tissue repair and its uses are widely reported in the literature [27-31]. However, given the poor mechanical and handling properties of liquid PRP, it is difficult to contain the PRP at its intended location [32]. Thrombin is commonly added to PRP to improve its handling properties, but this in turn causes an immediate release of the growth factors [33,34]. Growth factors have a relatively short half-life and when they are rapidly released after platelet activation, tissue receptor saturation may

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**Table 1**: List of some growth factors in PRP and their functions.

| Growth Factor | Function |
|---|---|
| **PDGF** | Enhances collagen synthesis, fibroblast activity, and macrophage activation |
| **TGF-B** | Promotes cell mitosis and increases type-I collagen production |
| **VEGF** | Stimulates angiogenesis and blood flow |

**Table 2**: Inclusion/Exclusion criteria.

| Study Characteristics | Inclusion Criteria | Exclusion Criteria |
|---|---|---|
| **Population** | Adults ages 18 years old or older at the time of treatment with a history of Type I or TypeII DM | |
| **DFU Characteristics** | **Size**: Greater than 1 $cm^2$ and less than or equal to 12 $cm^2$ atenrollment.<br>**History**: Present for at least 90days<br>**Presentation**: Full thickness,Distal to the malleolus | **Presentation**: DFUs with exposed capsule, tendon, or bone, tunneling, underminingor sinus tracts |
| **Perfusion** | **One of the following**: An ankle- brachial index ≥ 0.8, a transcutaneous oxygen pressure of 30 ≥ mmHg, or a toe pressure of 50 ≥ mmHg | |
| **DFU Healing Screening** | Less than 30% improvement with SOC alone at Day -14 andat Treatment Visit | Greater than 30% improvementwith SOC alone at Day -14 or at Treatment Visit |
| **Other health conditions/ treatments/ interventions** | | Treated with another biologic ortopical growth factor within four weeks of enrollment, Undergoing dialysis, active osteomyelitis, and another ulcerwithin 2 cm of study ulcer |

occur. This may prevent additional growth factors from binding to the receptors before all the growth factors have degraded [33,35], bearing short-term healing gains without long-term improvement [33,34].

Platelet-rich fibrin (PRF) consists of a matrix that acts as a scaffold to aid in the function of the cells contained within the PRP. In contrast to PRP, PRF does not contain any coagulation additives and it naturally forms a fibrin matrix clot which restricts the growth factor release to the clotting site. When the tissue begins to repair itself, it recruits fibroblasts to reorganize the fibrin matrix and starts building collagen [36]. PRF also maintains mesenchymal cells, which are necessary to regenerate tissue at the injured site [37]. PRFM has been demonstrated to have elevated levels of PDGF-AA, PDGF-AB, EGF, VEGF, bFGF and TGF-β1 which are all necessary growth factors to accelerate tissue repair [38,39].

Platelet-rich fibrin matrix, or PRFM (Fibrinet System, Royal Biologics, Hackensack, NJ), is produced through a series of centrifugation steps with the addition of calcium chloride and without the introduction of exogenous thrombin. When completed, it produces a membrane which is easily handled and can be mechanically affixed to a wound with sutures or sterile adhesive strips. The initial centrifugation isolates the PRP using a thixotropic gel separator at low speed (1069 g x 6 minutes) [32,38]. The PRP is then placed in a vial containing calcium chloride and centrifuged at 2217 g for 25 minutes. This yields a platelet fibrin matrix which can be manipulated in the surgical space, which is preferable to standard PRP or PRF [37,40].

## Materials and Methods

### Study design and administration

In this paper, we present the results of a single-arm, open-label, prospective trial evaluating the efficacy and safety of PRFM plus SOC in the treatment of chronic diabetic foot ulcers. The results will be compared to a historical control of SOC alone. Margolis [11] found the healing rate for DFUs with standard of care (sharp debridement, saline moistened gauze, and offloading) was 24.2% at 12 weeks. The percentage of patients in this study that are expected to have completed healing at 12 weeks is estimated to be at least 55% [41]. Based on this expected effect size, an a priori power analysis was conducted using RStudio to determine the minimum sample size required to test the study hypothesis. Results indicated the required sample size to achieve 80% power at a significance criterion of α=.05, was N=20 for two-tailed one-sample proportion test. To account for 20% dropout, the number of enrolled patients will be 25. The protocol was approved by the WCG Institutional Review Board (Protocol No. 20222836).

### Study Population

All patients were treated and assessed by the authors in an outpatient, single-center setting. Informed consent was obtained from all patients enrolled in the trial. The total length of the pilot study was 14 weeks. Patients were selected to receive PRFM as a treatment for their DFU, based on qualifications of the inclusion/exclusion criteria summarized in Table 2. Subjects had to be 18 years old or older at the time of treatment and have type I or II diabetes. The DFU had to be equal to or greater than 1 cm2 and less than or equal to 12 $cm^2$ at enrolment. Additionally, the ulcer must have been present for at least 90 days, full thickness and distal to the malleolus. The wound could not have exposed capsule, tendon, or bone, tunnelling, undermining or sinus tracts. Adequate vascular perfusion was demonstrated by having at least one of the following: an ankle-brachial index ≥ 0.8, a transcutaneous oxygen pressure of ≥ 30 mmHg, or a toe pressure of ≥ 50 mmHg. Subjects could not have had another biologic or topical growth factor within four weeks of enrolment. Main exclusion criteria included undergoing dialysis, active osteomyelitis, and another ulcer within 2 cm of study ulcer.

The DFU was assessed clinically, photographed, and measured via planimetric tracing at Screening Visit (Day -14 +/- 3 days). A 2-week run-in period was allotted, and all the wounds were treated with SOC alone. This included sharp surgical debridement (at screening, effectively Day -14), a saline-gel and gauze dressing, which was changed daily by the patient, and offloading with a removable cast walking boot, i.e., CAM boot. At Treatment Visit (Day 0 +/- 3 days), the wound was assessed again. If there was greater than 30% improvement with SOC alone, the patient would not qualify for the study, as this would be deemed not difficult to heal and would not typically require use of an advanced biologic. If there was less than a 30% improvement in wound size, the patient qualified for application

of PRFM. The ulcer was prepared prior to application of PRFM with sharp surgical debridement, until healthy, bleeding wound bed was created. The PRFM was prepared as discussed above in accordance with manufacturing recommendations and secured to the wound as per the surgeon's preference: sutures, surgical adhesive strips, or a combination of these methods. Saline gel was applied to the outside of the graft to prevent moisture loss, and a non-adherent contact layer and multilayer compression bandage above the graft, which was left intact until the next follow-up visit. Thereafter, weekly office assessments were made for a maximum of an additional 11 weeks (12 weeks in total). CAM boot compliance was assessed weekly via a questionnaire. If deemed appropriate by the investigator, additional applications of PRFM were allowed to be applied at subsequent visits. The reapplication of PRFM would follow the investigator's clinical judgement, as would be if the patient was not enrolled in a study. Some limited examples/reasons for re-application are improper graft adherence (slippage), seroma formation, stalled wound, or desire for enhanced tissue growth. At the end of a maximum of 12 study weeks, data on percentage complete closure and rate of closure was compiled. As a secondary endpoint, the mean-adjusted heal rate (Margolis percentage change-in-area method) at 4 weeks, with follow-up assessments at Week 8 and Week 12 following treatment with PRFM plus SOC as compared to patients receiving SOC alone [42]. Additionally, a report on adverse events (AEs) and/or serious adverse events (SAEs) for the duration of the study was also recorded. Patients were actively monitored during the trial for treatment-related adverse events (e.g., infections, cellulitis, dermatitis, osteomyelitis, etc.). All treatment-related adverse events were documented in the subjects' research record and classified based on the severity of the event (mild, moderate, severe, life-threatening or death related to adverse event) and whether the event is, in the opinion of the treating Investigator, related to PRFM.

## Study Device

Platelet-rich fibrin matrix, or PRFM, produced from blood draw and serial centrifugation as described by the Fibrinet System (Royal Biologics, Hackensack, NJ), is produced through a series of centrifugation steps with the addition of calcium chloride and without the introduction of exogenous thrombin. When completed, it produces a membrane which is easily handled and can be mechanically affixed to a wound with sutures or sterile adhesive strips. The initial centrifugation isolates the PRP using a thixotropic gel separator at low speed (1069 g x 6 minutes) [32,38]. The PRP is then placed in a vial containing calcium chloride and centrifuged at 2217 g for 25 minutes. This yields a platelet fibrin matrix which can be manipulated in the surgical space, which is preferable to standard PRP or PRF [38,40]. Figure 1 shows the centrifuge, blood draw kit, and PRFM graft.

## Results

Twenty-five patients were screened for the trial between September 2022 and December 2023. Of the 25 patients screened, 4 were female (16%) and 21 were male (84%). Seven patients failed screening due to size improvement >30% during screening period (four patients), infection at a different site requiring antibiotics (two patients), and loss to follow-up (one patient). Of the remaining 18 patients, 44.4% healed at 12-weeks (8/18 patients), 33.3% did not heal at 12-weeks (6/18 patients) and 22.2% (4/18 patients) discontinued or were removed from the trial. Reasons for removal from the active trial were infection at the ulcer site (2 patients) and medical admission not related to the ulcer (one patient). None of the adverse events were deemed to be directly linked to the PRFM. A summary of enrolment is presented in Table 3.

All patients had diabetic pedal ulcers. The wounds were planimetrically traced and photographed. They were analyzed using Image J (National Institutes of Health, Bethesda, MD, USA) for wound surface area over time by setting a scale based off a known object (i.e., ruler) in the image and tracing the outer edges of the wound. The mean ulcer size of the eligible patients at the screening visit was 1002.87 (SD=1773.35 mm2). A 2-week run-in phase was performed, where SOC alone was used to manage the DFUs. With comparison to the mean ulcer size at screening versus the first treatment visit at a level of significance of 0. 05, there were no differences within the healed group, the non-healed group or the entire cohort respectively (healed, P =1.00; non-healed, P=1.00; entire cohort, P=1.00). Comparisons of the means were done on R using three independent two-tailed Wilcoxon Rank Sum Test, with Bonferroni corrections made to the p-values. The analysis after the run-in phase indicates that all the wounds studied in the trial were stalled wounds that would otherwise not likely heal with SOC. The distribution of the ulcer size at the screening and the first treatment visits can be visualized in Figure 2.

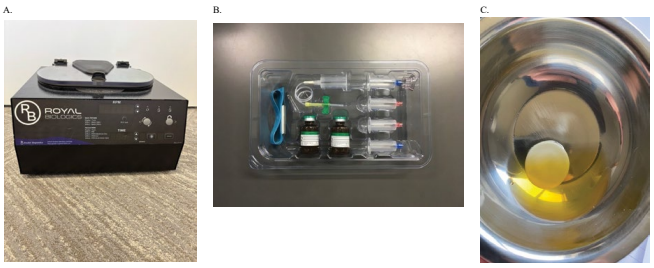


**Figure 1:** (A) Centrifuge (B) PRFM Kit (C) PRFM graft A.

**Table 3**: Summary of study enrollment.

| | |
|---|---|
| **Number of Patients Screened** | 25 |
| **Gender** | |
| Male | 21 (84%) |
| Female | 4 (16%) |
| **Number of Patients Failing Screening** | 7 |
| Wound >30% improvement after Screening Phase | 4 |
| Infection at different site within Screening Phase | 2 |
| Lost to follow-up within Screening Phase | 1 |
| **Number of Patients Passing Screening** | 18 |
| Healed | 8 |
| Did not Healed | 6 |
| Discontinued | 4 |
| **Total of Patients in Final Cohort** | 14 |

Of the 14 patients treated with PRFM, 3 patients (16.67%) were treated with a second application graft due to a stalled wound. The remaining 11 patients were treated with one application of the umbilical tissue graft.

Complete ulcer closure within 12 weeks was observed in 8 patients (57.1%), assessed by the treating physician based on the criteria outlined. In contrast, 6 patients (42.9%) had ulcers which failed to close by Week-12 of the trial. Of the 6 patients whose ulcers did not close by week-12, 5 patients (88.3%) had their ulcers close 50% or greater by week-12. The other patient did not have a 50% or greater reduction due to developing a blister at the ulcer site. Regarding the 50% closure by post-graft application Week-4 criterion, 9 patients had an ulcer size reduction greater than 50% (69.2%; 9/13) and 4 patients (30.8%; 4/13) had an ulcer-size reduction less than 50% (Table 4). One patient was excluded from this criterion due to missing the week 4 visitation.

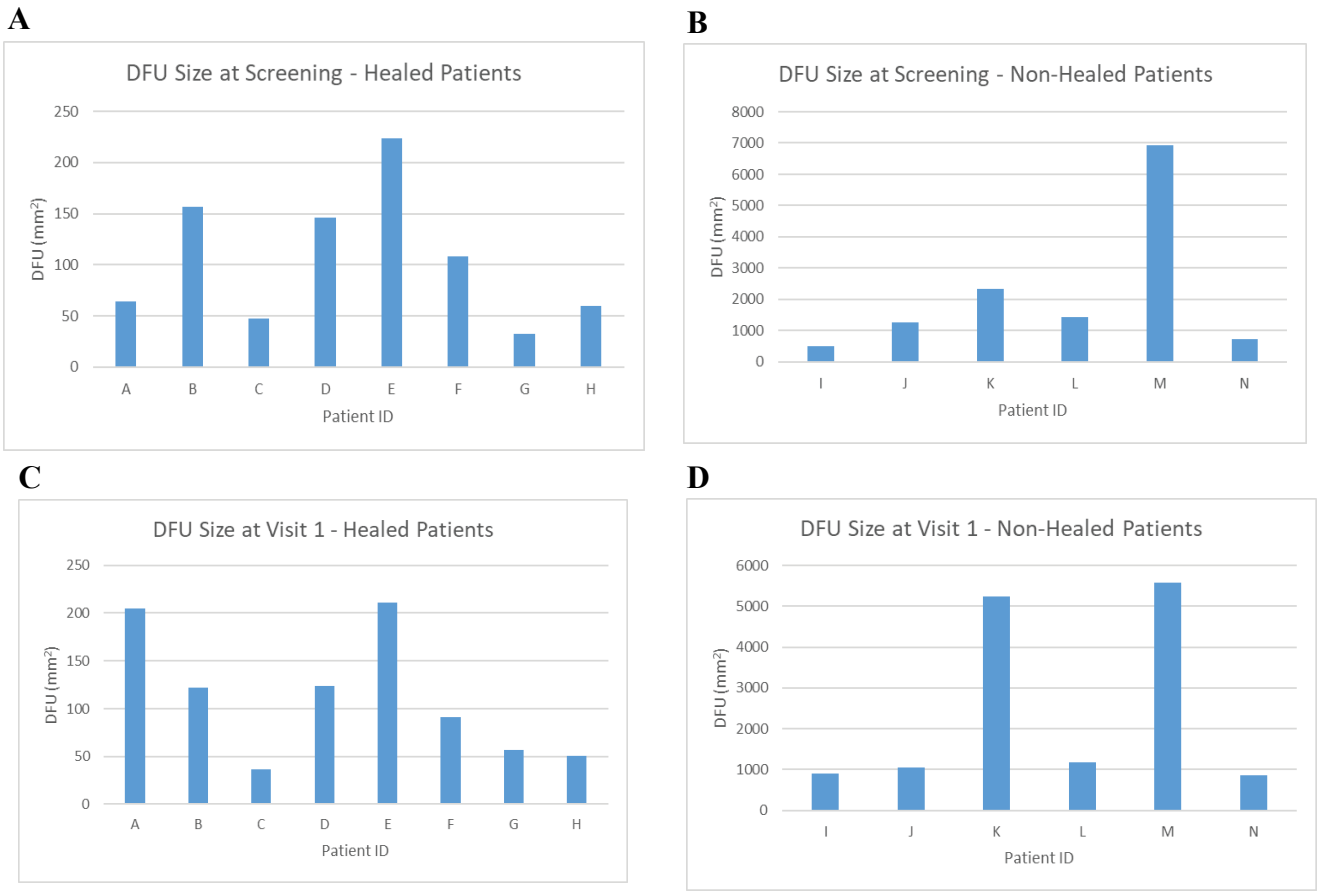


**Figure 2A-D**: DFU Sizes at Screening and Visit 1 for both Healed and Non-Healed Patients.

**Table 4**: Summary of DFU closure over time.

| | |
|---|---|
| **Patients with DFU Closing Within 12 Weeks** | 8 |
| **Patients with DFU Failing to Close Within 12 Weeks** | 6 |
| Ulcer size reduction greater than 50% by Week 12Ulcer size reduction less than 50% by Week 12 | 5 |
| | 1 |
| **Patients Included in Post-Graft Application Week-4 Criterion Analysis** | 13 |
| Ulcer size reduction greater than 50% by Week 4 | 9 |
| Ulcer completely healed by Week 12 Ulcer size reduction less than 50% by Week 4 | 7 |
| Ulcer completely healed by Week 12 | 4 |
| | 0 |

Among those patients with at least 50% ulcer-size reduction at Week-4, 53.8% (7 patients) of them were completely healed by Week-12. In comparison, among the 4 patients with less than 50% ulcer-size reduction by Week-4, 0 patients (0%) with the ulcer were healed within the same timeframe. Image 1, Image 2 and Image 3 show some clinical photos of the screening visit, PRFM application, treatment phase, and closure of the wounds.

For patients whose ulcer healed within the study period, the mean time to wound closure was 6.75 weeks (range, 3-11 weeks). The mean absolute weekly healing rate was 20.29 cm2/week, 170.87 cm2/week (-30.57 cm2/week including patient 'I' with the developed blister), and 78.20 cm2/week (-1.51 cm2/week including patient 'I' with the developed blister) for patients whose ulcers healed, not healed and the entire cohort, respectively. The difference in mean absolute weekly healing rates between patients whose ulcers healed and did not heal was 150.6 $cm^2$/week.

## Discussion

SOC has been shown to close an average of 24% of diabetic foot ulcers within 12 weeks, and 31% in 20 weeks [7]. Sheehan [9] demonstrated that the initial 4-weeks of DFU care have significant predictive value, in that if the percentage area reduction (PAR) is greater than 50%, the wound has a 60% chance of healing in 12 weeks. Conversely, if the PAR is less than 50% in 4 weeks, the wound has a 10% chance of healing in 12 weeks. It is imperative for DFUs to heal before infection develops, which may lead to lower extremity amputation [43].

Several advanced treatment modalities have been shown to augment and accelerate healing rates in DFUs. Weiman et.al showed that topically applied recombinant human platelet-derived growth factor (rh-PDGF-BB) significantly increased the incidence of complete wound closure by 43% and decreased the time to achieve complete wound closure by 32% as compared with placebo-controlled gel [44].


**Image 1**: 56-year-old male with ulcer submetatarsal left #1 for 4 months. (A) Screening Visit [SV1] (B) Application Visit, 2 weeks post-screening [TV1], (C) Treatment Visit #4 [TV4], (D) Ulcer closed on Treatment Visit #9 [TV9].

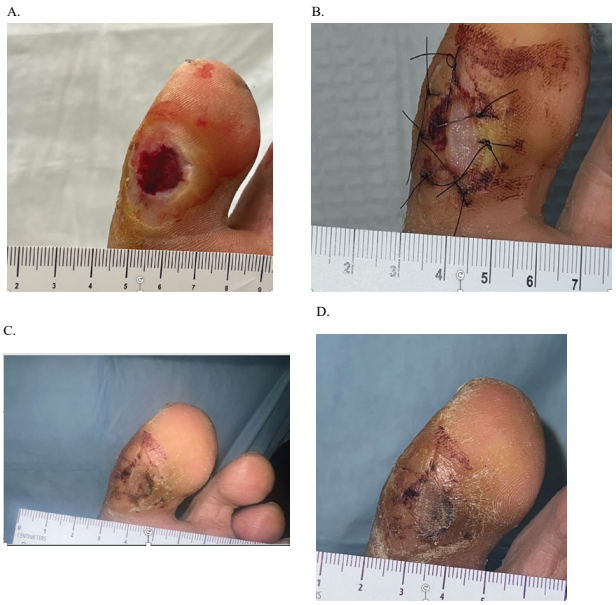


**Image 2**: 65 year old male with left hallux plantar interphalangeal joint ulcer for 6 months (A) Screening Visit [SV1] (B) Treatment Visit 2, One week post-PRFM application [TV2], (C) Treatment Visit #3 [TV3], (D) Ulcer closed on Treatment Visit #4 [TV4].

Veves A, et al. demonstrated 56% complete DFU closure at 12 weeks with Apligraf as compared with 38% in the control group [45]. Marston et.al studied the effects of Derma-graft on DFUs and reported 30% complete wound closure by week 12 as compared with 18.3% of control patients [46]. Driver VR, et al. studied the effects of Integra Dermal Regeneration Template (IDRT) on DFUs and found that complete

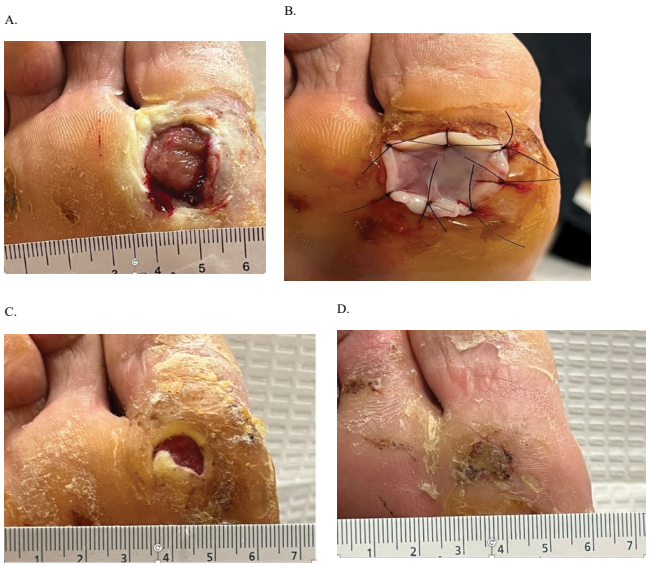


**Image 3:** 65 year old male with history of ulcer submetatarsal right #1 for 6 months A) Screening Visit [SV1] (B) Application Visit, 2 weeks post-screening [TV1], (C) Treatment Visit #3 [TV3], (D) Ulcer closed on Treatment Visit #5 [TV5].

DFU closure during the treatment phase was significantly greater with IDRT (51%) than control (32%). Additionally, the rate of wound size reduction was 7.2% per week for IDRT subjects vs. 4.8% per week for control subjects [18]. The benefits of using human placental tissues in covering non-healing wounds are now well documented [47,48]. Optimally preserved placental membranes are of particular interest as they contain a combination of growth factors and extracellular matrices as well as viable mesenchymal stem cells, fibroblasts, and epithelial cells). Fridman R, et al. [49] studied the effects of non-dimethyl sulfoxide viable umbilical cord in hard to heal DFUs and found that 40% had complete closure prior to or at the study endpoint at 12 weeks. Of the patients whose ulcers did not close by the endpoint, 89% had a 50% or greater improvement by Week-12 [49].

Toyoda T, et al. [50] studied the effects of calcium chloride on clotting platelets within PRP. They found that besides the well-known coagulation pathway, which activates platelets via thrombin conversion in a coagulation cascade, calcium chloride directly activates platelets, which then facilitate clot formation independently and in cooperation with the coagulation pathway.

Marinacci M, et al. [39] did a small study of seven patients with DFUs treated with PRFM and found that four patients achieved a total recovery of the ulcers, while three experienced a reduction of the diameter of the ulcers. O'Connell [32] researched PRFM in venous leg ulcers (VLU) and non-VLU lower extremity wound and demonstrated complete closure in 66.7% of the VLU patients (64.7% of treated ulcers) in 7.1 weeks and 44% percent complete closure was seen with non-VLU patients (31% of treated ulcers).

In this study, 57.1% (8/14) patients closed their chronic DFUs with PRFM plus SOC. Among the entire cohort, 10 patients (71.4%) had 50% or greater wound reduction by Week-4 and 13 patients (92.9%) by Week-12. This is comparable to other advanced treatment modalities listed above. Benefits of PRFM as compared to other cellular/tissue-based products include cost, availability, and although rare, cross-reactivity/allergy. Additionally, there are also patient preferences of using their own autologous blood rather than a commercially available allograft or xenograft.

## Conclusion

The trial being presented here studied the effects of PRFM on DFUs. PRFM does not need external activators, naturally contains growth factors and healing cells, and maintains a slower release of growth factors for prolonged healing. Based on our findings, PRFM is an effective way to treat challenging DFUs.

## Limitations

The study was limited by enrolment of a modest sample size. Although unfortunate with respect to the number of subjects completing the study per protocol, the rates were not inconsistent with this subject population (see e.g., Marston, 2004). Additionally, this was an open-label, non-blinded single-center study with no active control group, as we used historical data for SOC healing as a comparator. In the future, a larger study enrolment with a control group can be employed to further study this problematic population.

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