A multicenter, randomized, single-blind trial comparing the efficacy of viable cryopreserved placental membrane to human fibroblast-derived dermal substitute for the treatment of chronic diabetic foot ulcers

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ABSTRACT
Randomized controlled clinical trials, the gold standard to determine treatment efficacy against control, have demonstrated advantages of skin substitutes for the treatment of chronic diabetic foot ulcers in comparison to standard of care. However, randomized controlled clinical trials comparing efficacy between two or more skin substitutes are very limited. With growing numbers of new skin substitutes, such studies are essential for treatment and policy-making decisions by wound care providers and payers. In this study, we analyzed clinical outcomes and product cost between a viable cryopreserved placental membrane (vCPM) and a human fibroblast-derived dermal substitute (hFDS) for the treatment of chronic diabetic foot ulcers in a prospective, multicenter, single-blind study. The outcomes of 62 patients were analyzed: 31 patients in the vCPM treatment group and 31 patients in the hFDS treatment group. Utilizing a non-inferiority trial design and the established treatment regimen of 8 applications for hFDS, we demonstrated that vCPM was not inferior to hFDS for the proportion of patients achieving complete wound closure (9.68, 90% CI: [10.67, 28.94]). However, preliminary findings show that vCPM may have better outcomes for wounds ≤ 5 cm²: 81.3% (13/16) of wounds in the vCPM group vs. 37.5% (6/16) of wounds in the hFDS group reached complete closure at the end of treatment (p = 0.0118). A preliminary product cost analysis for wounds ≤ 5 cm² may show significant savings for patients treated with vCPM. Average per-patient costs during the course of treatment were $3,846 and $7,968 (p < 0.0001) for vCPM and hFDS patients, respectively. These results may be used as guidance to wound care providers and payers.

According to the National Diabetes Statistics Report, 2017, 30.3 million people in the United States had diabetes in 2015, with an estimated 1.5 million new cases diagnosed annually in adults.¹ Up to 25% of diabetics are estimated to develop a foot ulcer in their lifetime.² Due to underlying comorbidities associated with diabetes—including peripheral neuropathy, ischemia, and peripheral arterial disease (PAD)—many of these diabetic foot ulcers (DFUs) are unable to proceed through the normal phases of wound healing in a timely manner and are deemed chronic.³,⁴ Chronic ulcers can lead to infections, prolonged hospitalizations, and amputations. In patients with diabetes, approximately 80% of nontraumatic lower-limb amputations are preceded by foot ulcers.⁵ Chronic
DFUs and associated complications significantly decrease patient quality of life and increase overall cost of care.\cite{6,8}

The current standard of care (SOC) treatment for chronic DFUs consists of wound-bed cleansing and sharp debridement of surrounding devitalized tissue. Dressings are applied to protect and cover the wound, maintain a clean and moist environment, and absorb heavy exudate. SOC also includes appropriate offloading of the wound, establishment of adequate circulation, management of wound infection, and nutritional support (e.g., blood glucose control).\cite{9} Many DFUs do not respond to SOC and require adjunctive wound care therapies.\cite{10} Increasingly, advanced biological therapies, known as skin substitutes, are employed after failure of first-line SOC treatment. Examples include a bilayered skin substitute (Apligraf\textsuperscript{8}; Organogenesis, Canton, MA), a cryopreserved split-thickness allograft (TheraSkin\textsuperscript{9}; Soluble Systems, Newport News, VA), and a cryopreserved human fibroblast-derived dermal substitute (Dermagraft\textsuperscript{10}; Organogenesis, Canton, MA).\cite{11,12,13} More recent developments in tissue preservation techniques have led to the rapid commercialization of various placental membrane products, representing a new type of skin substitute. One such product currently on the market is Grafix\textsuperscript{PRIME}\textsuperscript{14} (Osiris Therapeutics, Inc., Columbia, MD), a viable cryopreserved human placental membrane (vCPM) that can be stored at \(-80\)\textdegree{}C for up to 3 years.\cite{15}

The objective of the comparative clinical study presented in this paper was to investigate the clinical outcomes of viable cryopreserved human placental membrane (vCPM) and human fibroblast-derived dermal substitute (hFDS), two commonly used advanced skin substitutes. The benefits of placental membrane, specifically amnion, have been reported in the literature for more than 100 years.\cite{16} vCPM retains the extracellular matrix, growth factors, and viable cells native to the placental membrane and can be used in the treatment of acute and chronic wounds of different etiologies and locations.\cite{17,18,19} In contrast, hFDS (Dermagraft\textsuperscript{10}), a product that has been on the U.S. market since 2001, is a bioengineered skin substitute.\cite{20} Fibroblasts are seeded onto a polyglactin-mesh scaffolding, creating a 3-dimensional dermal substitute containing collagen, matrix proteins, growth factors, and cytokines.\cite{21}

Prior clinical studies have demonstrated the safety and efficacy of both vCPM and hFDS for chronic DFU treatment in their respective trials. In a multicenter, randomized, controlled clinical trial, vCPM application adjunct to SOC resulted in superior clinical outcomes and lowered cost of care compared to SOC alone.\cite{22,23} Ninety-seven patients were enrolled, of which 50 received vCPM plus SOC and 47 received SOC treatment alone. In the vCPM arm, the proportion of patients that achieved complete wound closure by 12 weeks was significantly higher (62% vs. 21%), with median time to closure significantly shorter (42 days vs. 69.5 days), and with significantly fewer wound-related infections (18% vs. 36.2%) compared to SOC alone.\cite{22}

In a similar RCT conducted in 2003, Marston et al. investigated the efficacy of hFDS as an adjunct to SOC compared to SOC alone for the treatment of chronic DFUs. A total of 314 patients were enrolled and the primary clinical endpoint was complete wound closure by 12 weeks. Patients with ulcers greater than 6 weeks duration achieved wound closure at a higher rate when treated with hFDS and SOC compared to SOC alone (30% vs. 18.3%).\cite{13}

Though prior studies have compared clinical outcomes between vCPM and SOC, there remains the need to evaluate performance against other skin substitutes in prospective RCTs. Both vCPM and hFDS are cryopreserved products containing viable cells, making clinical outcomes in the current study a valuable comparison. In this paper, we present the results of a prospective, randomized, single-blind study comparing vCPM to hFDS for the treatment of chronic DFUs. This head-to-head study fills a gap in the literature regarding skin substitute comparisons.

**MATERIALS AND METHODS**

**Study design and treatment regimen**

This clinical trial was a prospective, randomized, single-blind study of up to 9-weeks duration, comparing the efficacy of vCPM and hFDS for chronic DFU treatment at 7 different centers across the United States. The trial was registered at www.clinicaltrials.gov (NCT02675855).

A third-party telephone system randomized patients in a 1:1 ratio to receive weekly treatment of either vCPM or hFDS for up to 8 applications or until complete wound closure was achieved, whichever occurred first. Eight applications was determined by recommended dosing for hFDS use.\cite{24} Two vCPM sizes were offered during the trial: 5 cm \(	imes\) 5 cm and 2 cm \(	imes\) 3 cm. hFDS is only available in a 5 cm \(	imes\) 7.5 cm size. Prior to product application, SOC consisted of cleaning and debriding wounds at the discretion of the investigator. Following application of the study product, a non-adherent dressing and a secondary dressing were applied, both of which remained intact until the following treatment visit. All patients with plantar wounds were required to offload any weight-bearing on their wound with the use of a standardized fixed ankle walker. Patients with dorsal wounds were required to wear a standard post-operative shoe. Alternative offloading devices were permitted with approval from the sponsor (e.g., wedge shoes, knee caddies). Outcome and safety evaluations were performed at each treatment visit until wound closure was achieved or until the week 9 visit, whichever occurred first.

The primary endpoint was the proportion of patients who achieved complete closure of the index wound (defined as 100% reepithelialization as determined by the investigator) by the end of treatment. Additional endpoints included the proportion of patients who achieved complete wound closure for wounds \(\leq 5\text{ cm}^2\) and \(> 5\text{ cm}^2\), time to closure, number of grafts used to achieve wound closure, proportion of patients who achieved a 50% or greater reduction in wound size by day 28, percent area reduction (PAR) of nonclosed wounds at day 56, and per-patient product cost during the course of treatment. Safety endpoints included number and types of adverse events (AEs).

**Study population**

Seventy-five patients, enrolled between January 2016 and May 2017, were prospectively treated with weekly applications of either vCPM or hFDS. Patients eligible for this trial

**SAS**

Statistical Analysis System

**SOC**

standard of care

**TBI**

toe brachial index

**vCPM**

viable cryopreserved placental membrane

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were between 18 and 80 years of age, diagnosed with type 1 or type 2 diabetes, and had a chronic foot ulcer (present between 4 and 52 weeks) between 1 cm² and 15 cm² in size that extended through the dermis with no exposed muscle, tendon, bone, or joint capsule. Vascular inclusion criteria required that each patient have an ankle brachial index (ABI) between 0.7 and 1.3, a toe brachial index (TBI) of ≥0.5, or a Doppler waveform demonstrating biphasic or triphasic flow in the foot. Patients with index ulcers that decreased ≥20% in size during the 1-week screening period were excluded.

**Statistical analysis**

This was a non-inferiority (NI) trial designed and powered to show that vCPM is not inferior to hFDS for wound closure. A treatment effect difference of 20% for the NI test was used in this analysis based on published clinical outcomes of vCPM and hFDS. A conservative NI margin of 15% was used per U.S. Food and Drug Administration (FDA) Guidance for Industry: Non-Inferiority Clinical Trials to Establish Effectiveness. Using these parameters, 74 patients was determined to be the sample size needed to meet the primary endpoint. In this analysis, non-inferiority of vCPM compared to hFDS could be proven only if the lower bound of the Newcombe 90% confidence interval for the difference in the two proportions (difference = vCPM – hFDS) was greater than −15%.

The intent-to-treat (ITT) population was used for patient demographics, baseline wound characteristics, and safety analyses. The per protocol (PP) population was used for the NI, clinical outcomes, and cost analyses.

### Table 1. Patient demographics (ITT population)

|                        | vCPM (n = 38) | hFDS (n = 37) | p value |
|------------------------|---------------|---------------|---------|
| Mean Age (Years) (SD)  | 55.3 (12.09)  | 58.1 (11.89)  | 0.317   |
| Age ≥ 65 Years (n, %)  | 9 (23.7%)     | 11 (29.7%)    | 0.554   |
| Gender (n, %)          |               |               | 0.166   |
| Male                   | 28 (73.7%)    | 32 (86.5%)    |         |
| Female                 | 10 (26.3%)    | 5 (13.5%)     |         |
| Race (n, %)            |               |               | 0.751   |
| White or Caucasian     | 32 (84.2%)    | 34 (91.9%)    |         |
| Black or African American | 3 (7.9%)   | 1 (2.7%)      |         |
| American Indian or Alaska Native | 1 (2.6%) | 0 |         |
| Other                  | 2 (5.3%)      | 2 (5.4%)      |         |
| Ethnicity (n, %)       |               |               | 0.921   |
| Hispanic or Latino     | 22 (57.9%)    | 21 (56.8%)    |         |
| Not Hispanic or Latino | 16 (42.1%)    | 16 (43.2%)    |         |
| Mean BMI (SD)          | 33.73 (8.904) | 31.05 (7.885) | 0.171   |
| BMI ≥ 30 (n, %)        | 26 (68.4%)    | 20 (54.1%)    | 0.201   |
| Type of diabetes (n, %)|               |               | 0.200   |
| Type 1                 | 5 (13.2%)     | 1 (2.7%)      |         |
| Type 2                 | 33 (86.8%)    | 36 (97.3%)    |         |
| Mean Years of DM (SD)  | 14.84 (11.366)| 18.14 (10.292)| 0.192   |
| Smoking History (n, %) |               |               | 0.768   |
| Never Smoked           | 23 (60.5%)    | 25 (67.6%)    |         |
| Current Smoker         | 4 (10.5%)     | 2 (5.4%)      |         |
| Former Smoker          | 11 (28.9%)    | 10 (27.0%)    |         |
| Heart Disease (n, %)   | 35 (92.1%)    | 35 (94.6%)    | 1.000   |
| Prior Amputation (n, %)| 21 (55.3%)    | 20 (54.1%)    | 0.916   |
| Mean Glycated Hemoglobin (SD) | 8.70 (1.447) | 8.51 (1.549) | 0.601   |
| Glycated Hemoglobin > 9% (n, %) | 12 (31.6%) | 13 (35.1%) | 0.669   |
| Mean Albumin (g/dl) (SD)| 3.24 (0.294) | 3.09 (0.304) | 0.334   |
| Ankle Brachial Index (ABI) (n, %) | | | 0.484 |
| ABI 0.7 – 0.90         | 12 (31.6%)    | 9 (24.3%)     |         |
| ABI > 0.90             | 26 (68.4%)    | 28 (75.7%)    |         |

*p values are from a 2-sample t-test for continuous variables and chi-squared test or Fisher’s exact test for categorical variables. ABI: ankle brachial index; BMI: body mass index; DM: diabetes mellitus; g/dl: grams per deciliter; hFDS: human fibroblast-derived dermal substitute; ITT: intent to treat; n: number; p: calculated probability; SD: standard deviation; vCPM: viable cryopreserved placental membrane.*

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Multicenter RCT comparing the clinical outcomes

Ananian et al.

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A third-party vendor performed statistical analyses. The analyses were performed using Statistical Analysis System (SAS) version 9.4. For continuous variables, descriptive statistics included the mean, standard deviation, median, and subject counts. For categorical variables statistics included frequencies and percentages. The Shapiro-Wilk test was used for data distribution analysis. Statistical analysis approaches are specified in the footnotes of Tables 1–4. A p value of ≤ 0.05 was considered significant.

Product cost analysis

A cost analysis was performed on wounds ≤ 5 cm². For logistical purposes, only one size of vCPM (2 cm × 3 cm) was available for use during this trial for wounds ≤ 5 cm². However, to present a more realistic cost analysis of the vCPM patients, wound area at each visit was matched with a corresponding graft size that is available on the market. Currently, vCPM is available in multiple sizes ranging from 16 mm to 5 cm × 5 cm, which allows for smaller grafts to be used as wounds progress toward closure, thus reducing wastage and costs. For this analysis, 3 vCPM sizes were used to calculate cost: 2 cm × 3 cm (currently priced at $1,100), 1.5 cm × 2 cm ($880), and a 16 mm disc ($495), as these sizes would have accommodated wounds ≤ 5 cm². hFDS is manufactured in only one size (5 cm × 7.5 cm, priced at $1,250 at the time of this publication) so there were no adjustments made in this analysis with respect to graft size and cost for the hFDS patients.

Ethical considerations

This trial was conducted in compliance with good clinical practice (GCP) and the ethical rules outlined in the Declaration of Helsinki. Institutional Review Board (IRB) approval was acquired at each of the seven sites participating in this trial, and written informed consent was obtained from each patient before any study procedures were performed.

Table 2. Baseline wound characteristics (ITT population)

|                        | vCPM     | hFDS     | p value |
|------------------------|----------|----------|---------|
| Wound Size (cm²)       |          |          |         |
| Mean                   | 7.15     | 5.70     | 0.732   |
| Median                 | 5.0      | 5.0      |         |
| Wound Duration (days)  |          |          |         |
| Mean                   | 199.32   | 146.32   | 0.022   |
| Median                 | 191      | 125      |         |
| Wound Location (n, %)  |          |          |         |
| Dorsal                 | 4 (10.53%) | 3 (8.11%) | 1.000   |
| Medial                 | 2 (5.26%) | 4 (10.81%) | 0.430   |
| Lateral                | 1 (2.63%) | 8 (21.62%) | 0.014   |
| Posterior              | 2 (5.26%) | 3 (8.11%) | 0.675   |
| Toe                    | 5 (13.16%) | 7 (18.92%) | 0.496   |
| Ankle                  | 1 (2.63%) | 0        | 1.000   |
| Plantar                | 23 (60.53%) | 12 (32.43%) | 0.015   |
| Patient with 1 or More Prior Advanced Therapies (n, %) | 13 (34.2%) | 6 (16.2%) | 0.073   |

*p values were determined from Wilcoxon rank-sum test. cm: centimeters; hFDS: human fibroblast-derived dermal substitute; ITT: intent to treat; n: number; p: calculated probability; vCPM: viable cryopreserved placental membrane.

Table 3. Non-inferiority test for complete closure (PP population)

| Statistics                  | vCPM (n = 31) | hFDS (n = 31) | Estimated Difference in Percentages | 90% CI[II] |
|-----------------------------|---------------|---------------|-------------------------------------|------------|
| Patients with complete closure | 15 (48.39%)   | 12 (38.71%)   | 9.68%                               | −10.67%, 28.94% |

[II]The Confidence Interval (CI) for the difference is calculated using the Newcombe method. The sponsor will conclude non-inferiority if the lower limit of the 90% CI (corresponding to a 95% 1-sided lower bound) is greater than −15%. CI: confidence interval; PP: per protocol; hFDS: human fibroblast-derived dermal substitute; n: number; vCPM: viable cryopreserved placental membrane.
Table 4. Clinical and cost outcomes for wounds ≤ 5cm² (PP population)

| Metric                                             | vCPM (n = 16) | hFDS (n = 16) | p value<sup>II,III</sup> |
|----------------------------------------------------|---------------|---------------|--------------------------|
| Wound closure (n, %)                               | 13 (81.3%)    | 6 (37.5%)     | 0.0118                   |
| Mean Time to Wound Closure (days)                  | 38            | 31            | 0.2016                   |
| Mean Number of Applications                        | 5.3           | 4.0           | 0.1478                   |
| 50% or Greater Wound Area Reduction at Day 28 (n, %) | 14 (87.5%)    | 11 (68.8%)    | 0.3944                   |
| Mean PAR at Day 56 for Nonclosed Wounds            | 77.9 (n = 3)  | 63.7 (n = 10) | 0.9340                   |
| Mean Cost of Product Per-Patient                   | $3,846.25     | $7,968.75     | < 0.0001                 |

<sup>i</sup>p values for wound closure and 50% or greater wound reduction at day 28 were determined from a chi-squared test.

<sup>ii</sup>p values for mean time to wound closure, mean percent area reduction at 8 weeks and mean number of applications were determined from a Wilcoxon rank-sum test.

<sup>iii</sup>p value for mean cost of product per-patient was determined from a Whitney-Mann test for non-normal data distribution. cm: centimeters; hFDS: human fibroblast-derived dermal substitute; n: number; p: calculated probability; PAR: percent area reduction; PP: per protocol; vCPM: viable cryopreserved placental membrane.

Figure 1. Schematic of study population
RESULTS

Patient demographics and wound characteristics

Figure 1 illustrates the flow chart of patient disposition. In total, 64 patients completed the entire study protocol, and 62 of these patients were evaluable in the PP population, including 31 patients in each treatment arm. The PP population was defined as any enrolled patient who did not terminate from the study early or have any major noncompliance deviations with the offloading protocol. Major noncompliance was defined as any patient not using the offloading device at all during the treatment period. Patient demographics and baseline-wound characteristics are presented in Tables 1 and 2. Twenty-eight men and 10 women, with a mean age of 55.3 years and an average wound size of 7.15 cm² were randomized to the vCPM treatment group. Thirty-two men and 5 women, with a mean age of 58.1 years and an average wound size of 5.70 cm² were randomized to receive hFDS. Both patient cohorts had noteworthy comorbidities, such as heart disease (92.1% of vCPM patients and 94.6% of hFDS patients) and prior amputations (55.5% of vCPM patients and 54.1% of hFDS patients). There were no statistical differences in baseline patient characteristics. However, there were significant differences in wound characteristics with longer wound chronicity and more planter wounds in the vCPM group, and more lateral wounds in the hFDS group.

Offloading devices offered in this trial included walking boots, walking boots with peg insoles, offloading walkers, and postoperative shoes. In both treatment groups, walking boots were used more than any other device (94.7% in vCPM arm and 81.1% in hFDS arm). There were no significant differences regarding offloading use between treatment groups.

Clinical outcomes and cost analysis

The primary efficacy outcome in this study was the percentage of patients in each treatment group who achieved 100% reepithelialization of the study wound by the end of study visit. At the end of treatment, 48.4% (15 patients) of vCPM patients and 38.7% (12 patients) of hFDS patients had achieved 100% reepithelialization. The result of the NI test is presented in Table 3. This analysis shows that vCPM met its primary endpoint of non-inferiority to hFDS for wound closure using the 8-application treatment regimen established for hFDS. At day 28 post-initial application of study product, 70.8% (22 patients) of vCPM patients and 67.7% (21 patients) of hFDS patients had achieved 50% or greater wound area reduction. The average PAR of study wounds were analyzed at day 28 and day 56. At day 28, the average PAR of study wounds was 68.4% for vCPM patients compared to 58.6% for hFDS patients. At the end of the study (day 56), vCPM patients had achieved an average of 86.3 PAR compared to 78.1 PAR for hFDS patients. vCPM patients required an average of 5.4 applications to achieve 100% reepithelialization compared to 4.4 applications for hFDS patients. There were no significant differences between treatment groups for outcomes described above.

Clinical outcomes and cost analysis for wounds ≤ 5 cm² are presented in Table 4. Mean baseline wound size was 3.09 cm² for vCPM patients and 2.67 cm² for hFDS patients. There was a significant difference in efficacy outcomes between the two treatment groups, with 81.3% (13 of 16) of vCPM patients and 37.5% (6 of 16) of hFDS patients (p = 0.0118) achieving complete wound closure by 8 weeks.

For larger wounds in the study (> 5 cm²), there were no significant differences in the proportion of patients who achieved complete wound closure, PAR at 28 and 56 days, or proportion of patients who achieved 50% or greater wound area reduction at day 28 between the two treatment arms. There were 15 patients in the vCPM treatment arm and 15 patients in the hFDS treatments arm used in these analyses.

Figure 2 presents a graphic summary of the wound closure endpoint at the end of treatment for vCPM and hFDS patients in the PP population. The graphic includes data for all wounds, wounds ≤ 5 cm², and wounds > 5 cm².

The mean per-patient product cost for wounds ≤ 5 cm² was calculated based on pricing for commercially available vCPM and hFDS product sizes. vCPM is available in multiple sizes to accommodate wounds as they decrease in size. hFDS is only available in one size. The mean calculated per-patient product cost for vCPM was $3,846 compared to $7,968 for hFDS (p < 0.0001).

Safety outcomes

The summary of AEs is presented in Table 5. Four AEs involved the study wound in the hFDS group, and only one AE involved the study wound in the vCPM group. All 5 of these AEs were wound-related infections. Six of seven serious adverse events (SAEs) in the hFDS group involved the index ulcer: five events of active osteomyelitis or cellulitis infection and one abscess. Four SAEs were reported in the vCPM treatment group. Only two of these involved the index ulcer: 1 osteomyelitis and 1 cellulitis event.

DISCUSSION

In this prospective, multicenter, single-blind study, we compared clinical outcomes and product cost between vCPM and hFDS for the treatment of chronic DFUs. The comparison of clinical outcomes between these two products is valuable, as they are both commonly used in the chronic wound care space and have been proven effective in prior clinical studies. hFDS is a bioengineered construct that consists of neonatal dermal fibroblasts cultured in vitro on a biodegradable, absorbable polyglactin mesh. As the fibroblasts proliferate onto the mesh, they secrete human dermal collagen, fibroactin, glycosaminoglycans, growth factors, and other proteins, embedding themselves in a self-produced dermal matrix. vCPM, a placental tissue allograft, contains a native amniotic 3-dimensional collagen rich matrix, endogenous growth factors, and viable cells including epithelial cells, fibroblasts, and mesenchymal stem cells. Overall, the compositions of hFDS and vCPM are very different; however, both skin substitutes are cryopreserved, contain viable cells, and are stored at −80°C. vCPM and hFDS have both demonstrated advantages over SOC alone for chronic DFUs in multicenter RCTs, which are the gold standard for evaluation of clinical efficacy. The hFDS multicenter RCT was conducted more than 10 years ago in 2003, with a reported closure rate of only 30% for hFDS. However, only a few advanced wound therapies were available at that time, making hFDS prevalent in DFU treatment protocols.
Since 2003, many more advanced skin substitutes have been developed, including placental products. The lack of comparative clinical trials makes treatment selection difficult for wound care providers. To date, only a few prospective studies comparing the efficacy of different skin substitutes have been conducted. In 2008, Landsman et al. conducted a study comparing the efficacy of Oasis® (Smith & Nephew, Plc, London, UK) and Dermagraft® in DFUs. In 2011, DiDomenico et al. compared wound closure outcomes of Apligraf® and TheraSkin®. Finally, in 2014, Zelen et al. compared clinical outcomes between Epi-Fix® (MiMedx Group, Marietta, GA), Apligraf®, and SOC. These studies, however, were all limited by relatively small sample sizes ranging from 26 patients to 60 patients in total. The present trial is the first report of comparative efficacy of vCPM versus hFDS for chronic DFU treatment.

Results of this study confirmed our hypothesis that vCPM is non-inferior to hFDS with eight weekly applications (Table 3). There were no significant differences in patient demographics between the two treatment groups (Table 1). However, a chance imbalance resulted in more plantar DFUs in the vCPM group than in the hFDS group (23 patients vs. 12 patients, \( p = 0.015 \)), and mean wound duration for vCPM patients was significantly higher compared to hFDS patients (199 days vs. 146 days, \( p = 0.022 \)).

Inherently, plantar wounds, specifically wounds on the heel, are harder to heal than nonplantar wounds, and wounds of longer duration have shown to be a strong negative predictor of wound healing. Despite these imbalances, the proportion of patients that achieved complete wound closure by 8 weeks trended higher in the vCPM treatment group compared to the hFDS treatment group (48.4% vs. 38.7%).

In 2013, Wilcox et al. analyzed a database containing approximately 59,000 DFUs and found that over 75% were \( \leq 5 \text{ cm}^2 \). As the majority of DFUs presented in real-world wound practice are within this size range, we evaluated clinical outcomes of wounds \( \leq 5 \text{ cm}^2 \). Interestingly, 5 cm² was also the median wound size in both treatment groups.

### Table 5. Summary of adverse events (ITT population)

| Adverse events (n) | vCPM | hFDS |
|--------------------|------|------|
| Index ulcer-related (n, %) | 1 (5.9%) | 4 (16.7%) |
| Serious adverse events (n) | 4 | 7 |
| Index ulcer-related (n, %) | 2 (50.0%) | 6 (85.7%) |
| Adverse events resulting in patient discontinuation from study (n, %) | 1 (2.6%) | 5 (13.5%) |

vCPM: viable cryopreserved placental membrane; hFDS: human fibroblast-derived dermal substitute; ITT: intent to treat; n: number; SOC: standard of care.
which is larger than in previously reported trials.\textsuperscript{13,16,22,33,34} Treatment with vCPM resulted in a higher proportion of patients achieving complete wound closure compared to hFDS patients (81.3\% vs. 37.5\%) which has not previously been reported. However, these findings are in line with previously published wound-closure rates for vCPM and hFDS which range from 62\% to 85.2\%, and 30\% to 50\%, respectively.\textsuperscript{13,18,22,33,34} Notably, mean wound sizes for these studies were smaller than 5 cm\(^2\).

For wounds >5 cm\(^2\), wound closure rates were higher in the hFDS group, however, there were no significant differences in the clinical outcomes between vCPM and hFDS treatment arms (\(p = 0.02148\)). Although normal physiological wound closure rates should remain the same for both vCPM and hFDS, it is reasonable to assume that vCPM should have a higher potential to normalize the chronic DFU microenvironment at a later stage of wound healing.

Using product pricing during the trial for wounds \(\leq 5\) cm\(^2\), preliminary findings for cost analysis showed that mean vCPM product cost would have been significantly lower than hFDS product cost ($3,846 vs. $7,968 per-patient, \(p < 0.0001\)) during the course of treatment if all 3 sizes (2 cm \(\times\) 3 cm, 1.5 cm \(\times\) 2 cm, and 16 mm) of vCPM had been utilized.

Given the rising incidence of diabetes in the U.S., therapies for DFUs should ideally be both efficacious and cost effective. In 2014, an economic analysis showed that Medicare and privately insured patients with DFUs consumed $11,710 and $16,883, respectively, in incremental annual health care costs compared to their matched controls without DFUs. This correlates to an annual payer burden of $9.1 billion to $13.2 billion.\textsuperscript{35} With such astronomical costs associated with DFUs in the U.S., advanced and effective wound-care therapies that result in lower per-patient costs and economic burdens are a top priority.

Complications significantly affect the cost of treatment, including wound infections, hospitalizations, and amputations. In 2016, Hicks et al. showed that 90\% of DFU hospital admissions were due to neuropathy and infection, and that hospital costs per DFU admission for patients with infection cost 38\% more than for other etiologies ($11,290 vs. $8,145).\textsuperscript{36}

In the present study, there were 10 AEs/SAEs related to infection of the index ulcer in the hFDS group and 3 in the vCPM group. Due to the small numbers of AEs in this trial, a formal cost analysis on AEs was not performed; however, data suggests that the added cost of treating these infectious AEs would significantly increase the overall per-patient cost, possibly more for patients in the hFDS treatment group who experienced more AEs.

The main limitations of this study include wound-closure assessment after 8 weekly applications, which is not common in clinical studies for DFUs, and the lack of a follow-up period after the treatment phase of the trial. In addition, the imbalance of the number of plantar wounds and chronicity of DFUs between the two groups could have negatively impacted clinical outcomes recorded for the vCPM treatment group. Though sample size was sufficient to meet the primary endpoint, it was not large enough to make definitive conclusions about analyses performed for wounds \(\leq 5\) cm\(^2\). These are interesting data findings and future studies will be needed to confirm preliminary results. Additionally, the single-blind design of the study, the lack of stratification by wound location and size for analyses, as well as the lack of specificity regarding wound location, are also recognized as limitations. As placental membrane products become more widespread in the wound care space, future comparative analyses using other placental products will provide valuable data for health care providers and payers.

In the current study, we demonstrated that vCPM is non-inferior to hFDS with regard to DFU closure when hFDS’s treatment regimen of 8 applications was used, but may have better outcomes for wounds \(\leq 5\) cm\(^2\). A cost analysis for these wounds demonstrated a lower cost per-patient for the vCPM product. Clinical and cost outcomes reported in this study may provide valuable guidance for wound care providers and payers.

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1. \(p\) values were determined from Wilcoxon rank-sum test.
2. \(cm\): centimeters; \(hFDS\): human fibroblast-derived dermal substitute; \(ITT\): intent to treat; \(n\): number; \(PP\): per protocol; \(vCPM\): viable cryopreserved placental membrane.
3. Difference = Difference in closure rates (vCPM group minus hFDS group).
4. The Confidence Interval (CI) for the difference is calculated using the Newcombe method. The sponsor will conclude non-inferiority if the lower limit of the 90\% CI (corresponding to a 95\% 1-sided lower bound) is greater than \(-15\%\).
5. CI: confidence interval; \(PP\): per protocol; \(hFDS\): human fibroblast-derived dermal substitute; \(n\): number; \(vCPM\): viable cryopreserved placental membrane.
6. \(p\) values for mean time to wound closure, mean percent area reduction at 8 weeks and mean number of applications were determined from a Wilcoxon rank-sum test.
7. \(p\) values are from a 2-sample t-test for continuous variables and chi-squared test or Fisher’s exact test for categorical variables. \(ABI\): ankle brachial index; \(BMI\): body mass index; \(DM\): diabetes mellitus; \(g/\text{dl}\): grams per deciliter; \(hFDS\): human fibroblast-derived dermal substitute; \(ITT\): intent to treat; \(n\): number; \(PP\): per protocol; \(vCPM\): viable cryopreserved placental membrane.
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