A Summary of the Current High-Quality Evidence Examining the Clinical Impact of Human Amnion/Chorion Skin Substitutes on Diabetic Foot Ulcers

Outline

Purpose of this document

In the wake of the evidence reviewed in the most recently rescinded local coverage determination titled, “Skin Substitute Grafts/Cellular and/or Tissue-Based Products for the Treatment of Diabetic Foot Ulcers and Venous Leg Ulcers,” Legacy Medical Consultants sought to independently conduct a systematic review of high-quality evidence (e.g., randomized controlled trials, systematic reviews, meta-analyses) looking at the clinical utility of human placenta-derived, amnion- and/or chorion-based skin substitute grafts for the management of lower extremity diabetic ulcers. After reviewing the studies described in the rescinded LCD, it was noted that several high-quality manuscripts were not included in the analysis, and we therefore performed a systematic review of all the English-language randomized trials from January 1, 2013 through March 15, 2023.

Executive summary

There are 11 randomized controlled trials looking at the efficacy of human placenta-derived, amnion- and/or chorion-based skin substitute grafts for the clinical management of lower extremity diabetic ulcers compared to a standard of care and 1 randomized controlled trial looking at the relative clinical efficacy of either weekly or biweekly application. Additionally, there are 4 systematic reviews and meta-analyses that have independently analyzed the impact of this class of skin substitute products on several outcomes of interest including proportion of wounds achieving full closure

defined as 100% re-epithelialization of the skin and time to wound closure. Based on the published results from the studies, 10 of 11 randomized controlled trials demonstrated significant improvement of this primary outcome (with the 1 trial not demonstrating statistically significant efficacy due to being underpowered; Game et al. 2021) as well as 4/4 systematic reviews and meta-analyses showed superiority of human placenta-derived, amnion- and/or chorion-based skin substitute grafts across several outcomes including proportion of wounds achieving full closure, time to complete healing, and cost-effectiveness compared to standard of care.

Specifically, according to one systematic review and meta-analysis by Mohammed et al. (2022) that compared dehydrated human amnion and chorion allograft (DHACA) to standard of care (SOC), which was defined as the quartet of pressure relief/offloading, debridement, infection management, and revascularization when indicated, the authors examined the pooled effect estimate from 11 randomized controlled trials. The analysis showed that DHACA was superior to SOC with regards to complete wound healing at both 6 weeks (RR = 3.78; 95% CI: [2.51, 5.70]; $p<0.00001$) and at 12 weeks (RR = 2.00; 95% CI: [1.67, 2.39], $p<0.00001$) after intervention. Further, the authors found that the meta-analysis favored the DHACA treatment with regards to the average time to heal in the 12^{th} week (mean difference = -12.07, 95%CI: [-19.23, -4.91], p=0.001) as well as wound size reduction (mean difference = 1.18, 95%CI: [-0,10, 2.26], p=0.03). This systematic review and meta-analysis demonstrated that using DHACA with SOC is safer and more effective than using SOC alone for patients with diabetic foot ulcers.

When examining the patient populations within each of these trials, the average ages for each study ranged from 55.1 years old to 63.3 years old with the standard deviations for each study ranging from 7 years to 18.3 years. Of all patients enrolled, approximately 70% of patients were male and 30% of patients were female, which is slightly higher than but rather consistent with the literature showing diabetic foot ulcers occur at a rate 1.5 times higher in men than women (Zhang et al. 2017, Rossboth et al. 2020, Lin et al. 2020). This demographic breakdown shows that many of these patients are either currently eligible for Medicare (age $\geq$65) or will be eligible within a few years.

The authors of Mohammed et al. 2022 assessed the risk of bias using the tools described in the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0. The six domains described in the handbook include random sequence generation, allocation sequence concealment, blinding of study participants and personnel, blinding of outcome assessors, incomplete outcome data, selective outcomes reporting, and other potential sources of bias. The authors flagged that most studies had low risk in the domains of random sequence generation, incomplete outcome data, selective reporting, and other bias; high risk in the domain of blinding of participants and personnel; and

unclear (or mixed) risk in the domain of allocation concealment and blinding of outcome assessment (see Figure 2 of Mohammed et al. 2022).

Although the lack of blinding can introduce bias into the experimental design of these randomized controlled trials, blinding can be difficult in studies looking at wound care treatments as they often involve physical products such as bandages, dressings, or topical agents that are visually distinctive, and these products might be unique in appearance, smell, or mode of application making it difficult to mask their identity from both the patients and the caregivers. Further, when traditionally implementing blinding for self- or provider-administered medications, creating a placebo or sham products that mimics the appearance and feel of the actual product may be more feasible, but this can be logistically complex and costly to produce a placebo skin substitute graft that looks and feels like a real one.

Citations

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Background on current scope of the problem

Lower extremity diabetic ulcers (LEDUs) are a common complication affecting millions of people in the United States. The annual incidence of active foot ulcers in patients with diabetes is approximately 6.0% among Medicare beneficiaries and 5.0% among the veteran population (Margolis et al., 2011; Boyko et al., 2006). Although the proportion of patients with diabetes and a history of foot ulcers is much higher than those being actively treated, there is an estimated 1.0-3.5 million people with a history of foot ulcers in the United States alone (International Diabetes Federation, 2015; History of foot ulcer among persons with diabetes – US, 2003). Overall, the lifetime incidence of foot ulcers occurring in a patient with diabetes is estimated to be 15-34% (Armstrong et al., 2017). Roughly half of all ulcers get infected, and about 20% of these infections necessitate hospital admission; additionally, 15% to 20% of serious infections result in amputation of a lower limb (Petersen et al., 2022, Skrepnek et al., 2015, Senneville et al., 2023). About 20% of people with a diabetic foot ulcer will undergo a lower extremity amputation, either minor (e.g., part of the foot) or major (e.g., above foot) (McDermott et al., 2023). In total, over 150,000 non-traumatic lower extremity amputations are performed every year in people with diabetes in the U.S. (CDC 2022). Individuals with a diabetic foot ulcer face a 30% chance of mortality within five years, and this rate increases to over 70% for those who undergo an amputation above the foot (Armstrong et al., 2020).

The current standard of care of lower extremity diabetic ulcers is two-fold: treatment of active ulcers using the current standard of care as well as implementing interventions to reduce the risk of foot ulcer recurrence. Current standard of care for wound management includes: debridement to eliminate nonviable tissue, foreign materials and bacterial components; off-loading to reduce weight bearing on the ulcer of interest with the use of either total contact casting or a knee-high walker; wound dressings to promote a moist environment conducive to tissue growth and epithelial migration without causing excessive maceration; early infection (e.g., cellulitis, osteomyelitis) control via the use of antibiotics as indicated by the managing provider to reduce the risk of hospitalization and amputation; revascularization aimed at restoring pulsatile arterial flow to the foot in patients with severe peripheral artery disease; and potentially the use of adjunctive therapies such as hyperbaric oxygen therapy and negative pressure wound therapy (Armstrong et al., 2023, Kruse et al., 2006).

A deeper dive into lower extremity diabetic wound treatment highlights the three major management categories: debridement of nonviable tissue, offloading pressure, and controlling infection (Kruse et al., 2006). **Debridement** refers to the removal of necrotic and other non-viable tissue with the goal of decreasing infection risk and reducing wound-perpetuating pressure. After debridement, irrigation of the wound and

application of dressings allows for the wound to begin the healing process in a favorable environment. Second, **offloading pressure** from the foot and lower extremity, in the form of total contact casts for example, helps to facilitate healing by reducing the number of recurring insults to the tissue. Lastly, **infection prevention and management** are crucial to prevent life- and limb-threatening episodes. Proper antibiotic stewardship (or escalation if severe enough) can help prevent the development of antibiotic resistance. In addition to these three pillars, other considerations include environmental moisture control, optimizing nutritional status, addressing social determinants of health, and surgical revascularization when indicated. Factors associated with poor wound healing response include end-stage organ disease (CHF, PAD, ESRD requiring RRT) and dependence on assistance ambulatory devices (Prompers et al., 2008). These principles are echoed by numerous US domestic and international professional societies including the International Working Group for the Diabetic Foot (IWGDF), Infectious Disease Society of America (IDSA), American Orthopaedic Foot and Ankle Society (AOFAS), American Diabetes Association (ADA), the American Association of Clinical Endocrinologists (AACE), Society for Vascular Surgery (SVS), American Podiatric Medical Association (APMA), and Society for Vascular Medicine (SVM).

Diabetes care, and more specifically LEDUs, generates a significant economic burden on the healthcare system. According to a 2017 study, total estimated cost of diagnosed diabetes in 2017 was $327 billion, with $237 billion in direct medical costs and $90 in indirect opportunity costs (Yang et al, 2018). Diving deeper, the high incidence and prevalence of LEDUs cannot be understated. Although well-established, the current standard of care, as outlined above, still leads to significant economic consequences with spending for various treatments of diabetic wounds varying drastically. Specifically for non-healing diabetic wounds, costs ranged from $6.2-18.7 billion without considering treatment for additional infections (Nussbaum et al, 2018). One study examined the cost effectiveness of hypothetical nerve decompression (ND) implementation to minimize neuropathic diabetic foot ulcer (nDFU) recurrence as compared to the current $6.17 billion annual nDFU expense and found that widespread adoption of ND after nDFU healing could reduce annual DFU occurrences by at least 21% in the third year and 24% by year 5, representing calculated cost savings of $1.30 billion (year 3) to $1.48 billion (year 5) (Rankin et al., 2015).

Intervening early on diabetic ulcers can result in avoidance of significant clinical burden. For example, one study that compared paired Medicare patients to paired commercially insured patients over a 12-month follow-up period showed that patients with diabetic foot ulcer had more days hospitalized (+138.2% Medicare, +173.5% private), days requiring home health care (+85.4% Medicare, +230.0% private), ED visits (+40.6% Medicare, +109.0% private), and outpatient office visits (+35.1%

Medicare, +42.5% private) than their insurance-matched controls (Rice et al, 2014). Additionally, the early clinical intervention would likely result in significant cost reductions for caring for these patients. For example, one study found that in comparison to diabetic patients without foot ulcers, the cost of care for patients with a foot ulcer is 5.4 times higher in the year after the first ulcer episode and 2.8 times higher in the second year. Additionally, this study found that the costs for the treatment of the highest-grade ulcers are 8 times higher than for treating low-grade ulcers (Driver et al, 2008).

The advent of advanced treatment (AT) which is comprised of cellular and acellular skin substitutes, derived either from animal tissue or human placental tissue sources, offers a new avenue of escalation treatment for chronic LEDUs unresponsive to the standard of care. These products are regulated under 510(k) and PMA processes from the FDA. The field of advanced treatment includes a wide range of products differentiated by a complex nomenclature.

In their systematic review and meta-analysis, Santema et al. (2016) described the range of skin substitutes products consisting of “bioengineered or artificial skin, autografts (taken from the patient), allografts (taken from another person) or xenografts (taken from animals).” Offering additional detail for these groups, Jones et al. (2013) describe in a systematic review and meta-analysis looking at skin grafting for venous ulcers that these products can be differentiated further to include:

1. **Autografts:** From the patient during a minor surgical procedure (pinch grafts, split-thickness mesh grafts, full-thickness skin grafts) or after growing the patients' cells (including keratinocytes from hair follicles) to form a thin film in the laboratory (cultured keratinocyte autograft, or a cultured epidermal autograft)
2. **Allografts (allogeneic):** From other human sources (cultured keratinocytes, cultured epidermal fibroblasts), grown in the laboratory and kept ready for use when necessary.
3. **Xenografts:** Typically from pigs, as their skin has a similar structure to human skin.
4. **Artificial skin (tissue-engineered skin, bioengineered skin or human skin equivalents):** Products typically feature a matrix into which cells important for skin repair are embedded and can be single, bi-layer, or tri-layer products.

One recent nationally representative, propensity score matched retrospective study of Medicare beneficiaries receiving care for LEDUs examined the impact of AT compared to no AT (NAT; no advanced treatment, e.g., standard of care) on several clinical outcomes. This study showed that patients with diabetes who were treated with AT for a LEDU were noted to have experienced fewer minor amputations and a 50% reduction in

major amputations compared with those treated with NAT (AT: n=490 (3.9%), NAT: n=551 (4.3%), p=0.0367 and AT: n=197 (1.6%), NAT: n=402 (3.2%), p<0.0001, respectively). They also experienced significantly fewer readmissions (AT: n=508 (4.0%), NAT: n=805 (6.4%), p<0.0001) and ED visits (AT: n=2322 (18.3%), NAT: n=2932 (23.1%), p<0.0001) compared with those treated with NAT (Armstrong et al., 2021). Further, in a second propensity score matched group, AT patients that followed full parameters for use (FPFU) demonstrated significant reductions in minor and major amputations by >50% with AT when FPFU compared with NAT (AT: n=22 (1.9%), NAT: n=47 (4.2%), p=0.0040 and AT: n<11 (<1%), NAT: n=30 (2.7%), p=0.0008, respectively). Using AT FPFU was associated with significantly reduced hospital readmissions (AT: n=27 (2.4%), NAT: n=73 (6.5%), p<0.0001) and ED visits compared with NAT (AT: n=161 (14.2%), NAT: n=237 (21.0%), p=0.0004). Major amputations were similar between AT FPFU and AT not FPFU (AT FPFU: n<11 (<1%), AT not FPFU: n=18 (1.6%), p=0.1006), while minor amputations were reduced with AT FPFU (AT FPFU: n=22 (1.9%), AT not FPFU: n=51 (4.5%), p=0.0020) (Armstrong et al., 2021). This type of benefit in rates of amputation, ED visits, and hospital readmissions would likely have significant and beneficial economic impact. For example, in 2010, the total estimated cost of both providing and caring for a major amputation even after 1 year was nearly $105,000 (Rothenberg et al., 2020). Additionally, one study estimated that 49-85% of diabetic foot ulcer-related amputations could have been prevented (Lavery et al., 2006). With how incrementally impactful AT has been demonstrated to be, we argue that treating LEDUs with AT offers one of the highest value solutions on the market.

In this systematic review specifically, we will be analyzing the landscape of evidence including randomized controlled trials, systematic reviews, and meta-analyses looking at the role of human placenta-derived, amnion- and/or chorion-based skin substitute grafts for the clinical management of lower extremity diabetic ulcers.

Citations

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How this analysis was conducted

This evidence review outlined below analyzes human placenta-derived, amnion- and/or chorion-based skin substitute grafts for the clinical management of lower extremity diabetic ulcers in the Medicare population. The evidence focuses on whether these products demonstrate improvement in health outcomes as defined by quality of life and patient functioning.

A literature search of MEDLINE/PubMed was conducted using the following key words: Non-healing; wound; chronic; diabetic foot; foot ulcer; guidelines; wound healing; skin substitutes; human amnion; human chorion; placenta; placental-derived; skin substitute; randomized trial; standard of care; skin grafts; wound dressing; human derived products; FDA regulations. An additional source for references include the LCD titled, “Skin Substitute Grafts/Cellular and/or Tissue-Based Products for the Treatment of Diabetic Foot Ulcers and Venous Leg Ulcers” (L35041). The literature search was filtered to locate English-language only, full-text articles specific to diabetic foot ulcers within the last 10 years (2013-2024) filtered to find randomized clinical trials, systematic reviews, and meta-analyses.

Framework for analysis

- **Importance**: The abstract should begin with a sentence or 2 explaining the clinical (or other) importance of the study question.
- **Objective**: State the precise objective or study question addressed in the report (e.g., "To determine whether..."). If more than 1 objective is addressed, the main objective should be indicated, and only key secondary objectives stated. If an *a priori* hypothesis was tested, it should be stated.
- **Design**: Describe the basic design of the study and include the specific study type (e.g., randomized clinical trial, cohort, cross-sectional, case-control, case series, survey, meta-analysis, bibliometric analysis). State the years of the study and the duration of follow-up. For older studies (e.g., those completed >3 years ago), add the date of the analysis being reported. If applicable, include the name of the study (e.g., the Framingham Heart Study). As relevant, indicate whether observers were blinded to patient groupings, particularly for subjective measurements.
- **Setting**: Describe the study setting to assist readers to determine the applicability of the report to other circumstances, for example, multicenter, population-based, primary care or referral center(s), etc.
- **Inclusion Criteria**: Describe which members of the target population are eligible to be in the study
- **Exclusion Criteria**: Describe which members of the target population are not eligible to be in the study

- **Participants**: State the clinical disorders, important eligibility criteria, and key sociodemographic features of patients (or other study participants). The numbers of eligible participants and how they were selected should be provided, including the number approached but who refused or were excluded. For selection procedures, these terms should be used, if appropriate: random sample (where random refers to a formal, randomized selection in which all eligible individuals have a fixed and usually equal chance of selection); population-based sample; referred sample; consecutive sample; volunteer sample; convenience sample. If matching is used for comparison groups, characteristics that are matched should be specified. In follow-up studies, the proportion of participants who completed the study must be indicated.
- **Intervention(s) (for clinical trials)**: The essential features of any interventions, or exposures, should be described, including their method and duration. The intervention, or exposure, should be named by its most common clinical name, and nonproprietary drug names should be used. In each trial, standard of care (SOC) was typically defined by the individual trial protocol. In general, standard of care consisted of a combination of wound debridement of non-viable tissue, use of non-adherent wet-to-dry wound dressings for infection control through barrier protection, off-loading of ulcers, and the occasional use of compression stockings.
- **Main Outcome(s) and Measure(s)**: Indicate the primary study outcome measurement(s) as planned before data collection began. If the manuscript does not report the main planned outcomes of a study, this fact should be stated and the reason indicated. State clearly if the hypothesis being tested was formulated during or after data collection. Explain outcomes or measurements unfamiliar to a general medical readership.
- **Results**: Summary demographic information (e.g., characteristics such as sex and age) and the number of study participants should be reported in the first sentence of the Results paragraph. The main outcomes of the study should be reported and quantified, including final included/analyzed sample. When possible, present numerical results (e.g., absolute numbers and/or rates) with appropriate indicators of uncertainty, such as confidence intervals. Use means and standard deviations (SDs) for normally distributed data and medians and ranges or interquartile ranges (IQRs) for data that are not normally distributed. Avoid solely reporting the results of statistical hypothesis testing, such as P values, which fail to convey important quantitative information. For most studies, P values should follow the reporting of comparisons of absolute numbers or rates and measures of uncertainty (e.g., 0.8%, 95% CI −0.2% to 1.8%; P =.13). P values should never be presented alone without the data that are being

compared. Measures of relative risk also may be reported (e.g., relative risk, hazard ratios) and should include confidence intervals. Studies of screening and diagnostic tests should report sensitivity, specificity, and likelihood ratio. If predictive value or accuracy is reported, prevalence or pretest likelihood should be given as well. All randomized clinical trials should include the results of intention-to-treat analysis as well. In intervention studies, the number of patients withdrawn because of adverse effects should be given. Approaches such as number needed to treat to achieve a unit of benefit may be included when appropriate. All surveys should include response/participation rates.

- **Adverse Events**: In a similar framework to results above, adverse events and severe adverse events as defined by the study under review are broken out and reported separately to analyze safety.
- **Conclusions and Relevance**: Provide only conclusions of the study that are directly supported by the results. Give equal emphasis to positive and negative findings of equal scientific merit. Also, provide a statement of relevance indicating implications for clinical practice or health policy, avoiding speculation and overgeneralization. The relevance statement may also indicate whether additional study is required before the information should be used in clinical settings.
- **Limitations**: Limitations of study as expressed by the authors of the study.
- **Trial Registration**: For clinical trials only (not non-trial observational studies), the name of the trial registry, registration number, and URL of the registry must be included. IRB information is included in this section as well (IRB exemption, institution granting IRB approval, and IRB protocol number, if applicable).
- **Funding Source**: Identification of the funding source if this information was provided by the manuscript.

List of AMA citations for reviewed literature (January 1, 2013 - March 15, 2023)

Randomized controlled trials

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Systematic reviews and meta-analyses

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4. Paggiaro AO, Menezes AG, Ferrassi AD, De Carvalho VF, Gemperli R. Biological effects of amniotic membrane on diabetic foot wounds: a systematic review [published correction appears in J Wound Care. 2020 May 2;29(5):306]. J Wound Care. 2018;27(Sup2):S19-S25.

Summary of Evidence

Randomized controlled trials

1. Zelen CM, Serena TE, Denoziere G, Fetterolf DE. A prospective randomised comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. Int Wound J. 2013;10(5):502-507.
    a. **Importance:** This study addresses the significant challenge of healing diabetic foot ulcers, which are a common complication in diabetic patients and can lead to severe morbidity and economic burden.
    b. **Objective:** To compare the healing characteristics of diabetic foot ulcers treated with dehydrated human amniotic membrane allografts (EpiFix) versus standard care.
    c. **Design:** This was an IRB-approved, prospective, stratified, randomized, parallel group, non-blinded, single-center clinical trial, comparing two treatment groups for diabetic foot ulcers over a duration of 6 weeks.
    d. **Setting:** The study was conducted at a single center specializing in diabetic foot care in Southwest Virginia.
    e. **Inclusion Criteria:** Patients with a diabetic foot ulcer of at least 4-week duration, without infection and having adequate arterial perfusion, were included. Study inclusion criteria included the following characteristics: age 18 or older; able and willing to provide consent and agree to comply with study procedures and follow-up evaluations; ulcer size >1 and <25 cm^2; ulcer duration of ≥4 weeks; no clinical signs of infection; serum Cr <3.0 mg/dl; HbA1c < 12%; adequate circulation to the affected extremity as demonstrated by dorsum transcutaneous oxygen test (TcPO2) ≥30 mmHg, ankle-brachial index (ABI) between 0.7 and 1.2 or triphasic or biphasic Doppler arterial waveforms at the ankle of affected leg.
    f. **Exclusion Criteria:** Exclusions included participation in another clinical trial; Charcot foot; index ulcer probing to bone; currently receiving radiation or chemotherapy; known or suspected malignancy of current ulcer; diagnosis of autoimmune connective tissue disease; received a biomedical or topical growth factor for their wound within the previous 30 days; pregnant or breast feeding; taking medications considered to be immune system modulators and allergy to gentamicin or streptomycin.
    g. **Participants:** 25 subjects with type 1 or type 2 diabetes presenting for care of a diabetic foot ulcer on any part of the foot, aged 18 or older.
    h. **Intervention(s)/Exposure(s):** Treatment involved either standard care alone (n=12) or standard care plus the application of EpiFix (n=13), a dehydrated human amniotic membrane allograft. Standard of care was defined as wound debridement, appropriate moist wound therapy adhering to standardized guidelines with the use of Silvasorb gel and Aquacel AG at discretion of clinician and compression dressings.
    i. **Main Outcome(s) and Measure(s):** Primary outcomes included reduction of wound size and the proportion of ulcers completely healed after 4 and 6 weeks. A final evaluation of study outcomes occurred at 12 weeks for those patients with continued enrollment.
    j. **Results:** The EpiFix group showed significantly greater wound size reduction and higher rates of complete healing at both 4 and 6 weeks compared to the standard care group. At 4 weeks, the average ulcer surface area reduction was 32.0% ± 47.3% for the 12 subjects of the SOC group and 97.1% ± 7.0% for the 13 subjects of the EpiFix group ($p<0.001$). At 6 weeks, the average ulcer surface area reduction was −1.8% ± 70.3% for the 12 subjects of

the SOC group and 98.4% ± 5.8% for the 13 subjects of the EpiFix group ($p<0.001$). At 4 weeks, none of the subjects from the SOC group (0%) was healed, whereas 10 of the 13 subjects in the EpiFix group (77%) had wounds that had completely epithelialized ($p<0.001$). At 6 weeks, 1 of the 12 subjects from the SOC group (8%) was healed and 12 of the 13 subjects in the EpiFix group (92%) were healed ($p<0.001$).

k. **Adverse Events:** Two patients in the SOC group developed cellulitis of the effected extremity, which was treated with sharp debridement and antibiotics. One SOC patient had a gastrointestinal bleed and one experienced acute pyelonephritis. The one patient in the EpiFix group experienced pneumonia, respiratory distress and acute renal failure during the study period, although this was not believed to be related to the use of the amniotic membrane allograft.

l. **Conclusions and Relevance:** The use of EpiFix in addition to standard care significantly improves healing rates of diabetic foot ulcers compared to standard care alone, indicating its efficacy and potential benefits in clinical practice.

m. **Limitations:** The study's limitations include its small sample size and the lack of comparison with other advanced wound care products. Further research is recommended to confirm these findings and explore the effectiveness of EpiFix in other patient populations and conditions.

n. **Trial Registration:** The study was registered at ClinicalTrials.gov with the number NCT01552499. Western IRB provided IRB approval.

o. **Funding Source**: No funding information was provided in the study.

2. Zelen CM, Serena TE, Snyder RJ. A prospective, randomised comparative study of weekly versus biweekly application of dehydrated human amnion/chorion membrane allograft in the management of diabetic foot ulcers. Int Wound J. 2014;11(2):122-128. doi:10.1111/iwj.12242

a. **Importance**: Diabetic ulcers present a significant challenge in medical care, often leading to severe morbidity and economic burden. The study addresses the critical need for effective treatments to accelerate healing and reduce long-term complications.

b. **Objective**: The study aimed to determine if weekly applications of dHACM allografts are more effective than biweekly applications in reducing the time to heal diabetic foot ulcers.

c. **Design**: This was a prospective, randomized, comparative, non-blinded, single-center clinical trial conducted from September 2012 to October 2013. The primary outcome measured was the mean time to healing, with observers not blinded to patient groupings.

d. **Setting**: The trial was conducted in a single center in Southwest Virginia, which may influence the applicability of results to other settings due to regional differences in patient populations and healthcare practices.

e. **Inclusion Criteria**: Inclusion criteria included patients aged 18 or older, type 1 or type 2 diabetes, able and willing to provide consent and agree to comply with study procedures and follow-up evaluations, ulcers of size ≥1 and <25 cm^2, ulcer duration of ≥4 weeks unresponsive to standard care, no clinical signs of infection, serum Cr <3.0 mg/dL, HgA1c <12%, and adequate circulation to the affected extremity as demonstrated by dorsum transcutaneous oxygen test (TcPO2) ≥ 30 mmHg, or ABI between 0.7 and 1.2, or triphasic or biphasic doppler arterial waveforms at the ankle of the affected leg.

f. **Exclusion Criteria**: Exclusion criteria included current participation in another clinical trial, Charcot foot, index ulcer probing to bone, current receipt of radiation or chemotherapy, known or suspected malignancy of current ulcer, diagnosis of autoimmune connective tissue

disease, receipt of a biomedical or topical growth factor for their wound within previous 30 days, pregnant or breast feeding, taking medications considered to be immune system modulators, and allergy or known sensitivity to Gentamicin or Streptomycin.

g. **Participants:** The study involved patients with diabetic foot ulcers, controlling for a number of specific sociodemographic features and clinical disorders, leading to 40 participants who were enrolled and randomized to weekly or biweekly treatment groups.
h. **Intervention(s)/Exposure(s)**: The intervention consisted of the application of dHACM allograft either weekly (n=20) or biweekly (n=20), in addition to standard wound care protocols, including offloading and use of a non-adherent, moist dressing with compressive wrapping.
i. **Main Outcome(s) and Measure(s)**: The primary outcome was the mean time to complete healing of the ulcers defined as complete re-epithelialization of the wound without drainage or need for dressing. Secondary outcomes examined were percentage of diabetic ulcers completely healed by 4, 6 and 12 weeks in each group and number of dHACM allografts used.
j. **Results**: Although overall healing rates were similar between the groups, time to healing was shorter for those receiving weekly application of dHACM versus biweekly application (2.4 ± 1·8 weeks versus 4.1 ± 2·9 weeks, respectively), p=0.039. Illustrating the effect of weekly application, no differences were observed in median wound size between the groups at week 0 (first dHACM application; p= 0.303) and at week 1 follow-up (p=0.128), but wound size was significantly smaller by week 2 (p=0.0007) for those patients receiving a second dHACM application at week 1. While a similar number of grafts were used on each healed wound (biweekly group = 2.4 ± 1·5 versus 2.3 ± 1·8 – weekly group, p=0.841), those wounds receiving weekly dHACM healed 41.5% faster than those treated with dHACM biweekly. Rates of healing are compared by week. A significantly greater number of patients receiving weekly dHACM were healed at weeks 2, 3 and 4 compared with those receiving biweekly dHACM application.
k. **Adverse Events**: During the study period, four patients in the biweekly group and two patients in the weekly group experienced a total of eight adverse events and three hospitalizations, although none of these were attributed to the dHACM allograft. Wound related events included three reports of blisters from the offloading boot and one wound infection which was treated with antibiotics and sharp debridement during hospitalization. One patient experienced two hospitalizations: one for urinary tract infection and one related to Anasarca. One other urinary tract infection was reported. One patient developed anemia and septicemia. During hospitalization, all study procedures and evaluations continued to be conducted by the primary study investigator (CMZ). No patients were withdrawn from the study because of adverse events.
l. **Conclusions and Relevance**: Weekly application of dHACM has demonstrated superior clinical effectiveness when compared with biweekly application in the treatment of chronic neurotropic ulcers of the lower extremity in diabetic patients. These results support those of earlier studies with overall healing rates of over 92%. The low volume of wastage and ease of use is further evidence towards cost effectiveness. Therefore, dHACM is a desirable treatment option from both a clinical and economic perspective that should be considered by clinicians that treat diabetic foot ulcers.

m. **Limitations**: The study's small sample size and the lack of a standard care comparison group are notable limitations. Further, multicentre trials are suggested to confirm these results and assess the treatment's effectiveness across diverse settings.
n. **Trial Registration**: The trial was registered with ClinicalTrials.gov (NCT01657474). The study was reviewed and approved by Western IRB.
o. **Funding Source**: The document does not specify the funding sources.

3. Lavery LA, Fulmer J, Shebetka KA, et al. The efficacy and safety of Grafix(®) for the treatment of chronic diabetic foot ulcers: results of a multi-centre, controlled, randomised, blinded, clinical trial. Int Wound J. 2014;11(5):554-560. doi:10.1111/iwj.12329
    a. **Importance:** This study examines the efficacy and safety of Grafix®, a cryopreserved placental membrane, in treating chronic DFUs.
    b. **Objective:** The primary objective was to evaluate the proportion of patients achieving complete wound closure by 12 weeks using Grafix® compared to standard wound care. Secondary objectives included assessing the time to wound closure, adverse events, and wound closure in the crossover phase.
    c. **Design:** This was a multi-center, controlled, randomized, blinded clinical trial that enrolled patients from May 2012 through April 2013. The study's sample size was based on assumed closure rates in the control arm (30%) and the Grafix group (50%), with a 30% dropout rate.
    d. **Setting:** The study was conducted across various medical research and clinical centers, enhancing the generalizability of the findings to a broader patient population with DFUs.
    e. **Inclusion Criteria:** Inclusion criteria consisted of confirmed type I or type II diabetes, patients age between 18 and 80 years, index wound present between 4 and 52 weeks, wound located below the malleoli on plantar or dorsal surface of the foot and ulcer between 1 and 15cm^2.
    f. **Exclusion Criteria:** Main exclusion criteria included hemoglobin A1c >12%, evidence of active infection including osteomyelitis or cellulitis, inadequate circulation to the affected foot defined by an ankle brachial index <0.70 or >1.30, or toe brachial index ≤0.50 or Doppler study with inadequate arterial pulsation, exposed muscle, tendon, bone or joint capsule and reduction of wound area by ≥30% during the screening period.
    g. **Participants:** 139 patients were evaluated during screening, with 97 patients randomized: 50 received Grafix, and 47 received standard wound therapy. Baseline characteristics were similar across both groups.
    h. **Intervention(s)/Exposure(s):** Patients were treated with either Grafix® (a human viable wound matrix made of cryopreserved placental membrane) or standard wound care. Standard wound care consisted of surgical debridement, mechanical off-loading, and use of non-adherent dressings.
    i. **Main Outcome(s) and Measure(s):** The primary endpoint was complete wound closure defined as 100% re-epithelialization with no wound drainage, as determined by the site investigator and confirmed by a central wound core laboratory. The secondary endpoints included the time to initial wound closure among patients who received Grafix compared to those who received control as measured by Kaplan-Meier analysis, proportion of patients who achieved 50% or greater reduction in wound size by 28 days, and the number of applications needed for closure and wound recurrence after initial wound healing.
    j. **Results:** The proportion of patients who achieved complete wound closure was significantly higher in the Grafix group compared to the standard wound care group (62% vs. 21%, p=0.0001). The median time to healing was shorter in the Grafix group compared to the

standard wound care group (42 days vs. 69.5 days, p=0.019). Additionally, fewer Grafix patients experienced adverse events compared with control patients (44% vs. 66%, p=0.031). Wound-related infections were lower in the Grafix group (18% vs. 36.2%, p=0.044). However, among the study participants that had healed, there was no significant difference in the percentage of ulcers that remained closed.

k. **Adverse Events**: Fewer Grafix patients experienced at least one adverse events compared with control patients (44.0% versus 66.0%, p=0.031). Among the patients randomized to Grafix, there were significantly fewer patients with wound-related infections (Grafix, 9 of 50, 18.0%, versus control, 17 of 47, 36.2%, p=0.044) and fewer hospitalizations related to infections in the Grafix group than control (6% versus 15%, p=0.15).
l. **Conclusions and Relevance:** In this multicenter, randomized clinical trial, the use of Grafix showed a significantly higher wound healing rate, faster healing and fewer wound-related infections among the patients who received cryopreserved placental membrane compared to those who received standard wound care.
m. **Limitations:** In this well-controlled study, the primary limitation includes the open-label nature and potential for bias introduced by the industry funding.
n. **Trial Registration**: There is no associated NCT reported with this manuscript.
o. **Funding**: This study was funded by Osiris Therapeutics, Inc. All the authors received research funding from Osiris Therapeutics, Inc. for their participation in the study.

4. Zelen CM, Gould L, Serena TE, Carter MJ, Keller J, Li WW. A prospective, randomised, controlled, multi-centre comparative effectiveness study of healing using dehydrated human amnion/chorion membrane allograft, bioengineered skin substitute or standard of care for treatment of chronic lower extremity diabetic ulcers. Int Wound J. 2015;12(6):724-732.
    a. **Importance**: Diabetic ulcers pose significant health and economic burdens, often leading to severe complications such as amputation. Rapid and complete healing is the primary treatment goal to mitigate these risks.
    b. **Objective**: To compare the healing effectiveness of weekly applications of Apligraf (bioengineered skin substitute), EpiFix (dehydrated human amnion/chorion membrane allograft), or standard wound care (collagen-alginate dressing) for chronic lower extremity diabetic ulcers over 4 and 6 weeks.
    c. **Design**: A prospective, randomized, controlled, parallel group, multicenter clinical trial conducted at three sites, focusing on the percent change in complete wound healing after 4 and 6 weeks of treatment.
    d. **Setting**: The study was conducted at three outpatient wound care centers in Virginia, USA, under the direction of a principal investigator with all patients signing an IRB-approved consent form.
    e. **Inclusion Criteria**: Inclusion criteria included patients aged 18 or older, type 1 or type 2 diabetes, able and willing to provide consent and agree to comply with study procedures and follow-up evaluations, ulcers of size ≥1 and <25 cm^2, ulcer duration of ≥4 weeks unresponsive to standard care, no clinical signs of infection, serum Cr <3.0 mg/dL, HgA1c <12%, and adequate circulation to the affected extremity as demonstrated by dorsum transcutaneous oxygen test (TcPO2) ≥ 30 mmHg, or ABI between 0.7 and 1.2, or triphasic or biphasic doppler arterial waveforms at the ankle of the affected leg.

f. **Exclusion Criteria**: Exclusion criteria included current participation in another clinical trial, index wound duration >52 weeks without intermittent healing, index ulcer probing to tendon, muscle, capsule, or bone, current receipt of radiation or chemotherapy, known or suspected malignancy of current ulcer, diagnosis of autoimmune connective tissue disease, use of biomedical/topical growth factor within previous 30 days, pregnant or breast feeding, taking medications considered to be immune system modulators, allergy or known sensitivity to Gentamicin, Streptomycin, bovine collagen or components of linear polysaccharide shipping medium, wounds improving more than 20% over the 2-week run-in period of the trial using standard of care dressing and Camboot offloading, patient taking Cox-2 inhibitors, and planned use of Dakin's solution, mafenide acetate, scarlet red dressing, tincoban, zinc sulphate, povidone-iodine solution, polymyxin/ nystatin or chlorhexidine during the trial.
g. **Participants**: 65 subjects entered the 2-week run-in period, with 60 being randomized (20 per group) after exclusion based on predefined criteria.
h. **Intervention(s)**: Weekly applications of either Apligraf (n=20), EpiFix (n=20), or standard wound care with collagen-alginate dressing (n=20).
i. **Main Outcome(s) and Measure(s)**: Primary outcome was the percent change in complete wound healing after 4 and 6 weeks. Secondary outcomes included percent change in wound area per week, velocity of wound closure, and cost of product used.
j. **Results**: Complete healing occurred by week 4 in 35.0% (7/20) of patients receiving Apligraf, 85% (17/20) of patients receiving EpiFix, and 30% (6/20) of patients receiving standard care. Lower extremity wounds treated with EpiFix had significantly higher rates of complete healing within 4 weeks compared to wounds treated with Apligraf (Hochberg-adjusted p=0.001) or standard care (Hochberg-adjusted p=0.001). After 6 weeks of treatment initiation, patients treated with EpiFix continued to have the highest rates of complete healing at 95% (19/20) versus 45·0% (9/20) for patients receiving Apligraf, and 35% (7/20) for patients receiving standard care (Hochberg-adjusted p=0.0006 and p=0.0001, respectively). For time to heaoing, a Kaplan-Meier analysis was performed to compare the performance of the three groups. The Log-Rank test of equality of the healing function over study groups produced a chi-square test statistic of 36.766 ($p<0.0001$). When adjusting for multiple comparisons of each treatment to the other using the Hochberg method, the comparison of EpiFix to Apligraf and standard care for healing rate was significantly in favor of EpiFix ($p\leq0.0001$). Based on the Kaplan–Meier analysis for those patients that healed completely, the estimated median healing time was 49 days (95%CI: 28–63 days) for the group treated with Apligraf, 13 days for those receiving EpiFix (95% CI: 7 – 21 days) and 49 days for patients receiving standard care (95%CI: 28 – 70 days). The mean number of grafts used per patient was lower in the EpiFix group (2.5) compared to the Apligraf group (6), resulting in a lower cost of treatment.
k. **Adverse Events**: Five adverse events were documented. One patient in the EpiFix group developed cellulitis and infection on the affected foot. Two patients in the Apligraf group were hospitalized for reasons unrelated to the study wound, one with a urinary tract infection and the other with wound infection on the non-study foot, resulting in a trans-metatarsal amputation. Both patients remained in the study. Two patients in the standard care group had adverse events. One developed cellulitis on the left ankle unrelated to the study wound, and was treated with antibiotics as an outpatient. Another patient in the standard care group was hospitalized for treatment of infection in the study wound and was treated with sharp debridement and IV antibiotics.

l. **Conclusions and Relevance**: The study demonstrates the clinical and cost-effectiveness superiority of EpiFix over Apligraf and standard care for the treatment of diabetic lower extremity ulcers, advocating its use for rapid and complete healing.
m. **Limitations**: Limitations include the inability to blind clinical researchers, the pilot nature, and small sample size which may limit the ability to draw definitive conclusions.
n. **Trial Registration**: The study was registered with ClinicalTrials.gov (NCT01921491) and approved by the Western IRB.
o. **Funding Source**: The study was sponsored and funded by MiMedx Group, Inc.

5. Zelen CM, Serena TE, Gould L, et al. Treatment of chronic diabetic lower extremity ulcers with advanced therapies: a prospective, randomised, controlled, multi-centre comparative study examining clinical efficacy and cost. Int Wound J. 2016;13(2):272-282.
    a. **Importance**: The study evaluates the clinical efficacy and cost-effectiveness of advanced therapies in treating chronic diabetic lower extremity ulcers, a major complication of diabetes that can lead to severe health consequences.
    b. **Objective**: The primary objectives were to compare the time-to-heal, rates of complete healing, and costs of advanced wound therapies between bioengineered skin substitutes (BSS) and dehydrated human amnion/chorion membrane (dHACM) allografts.
    c. **Design**: A prospective, randomized, controlled, parallel-group, multicenter comparative effectiveness trial. The study included 100 patients and was designed to provide a strong statistical examination of clinical factors affecting wound healing.
    d. **Setting**: The trial was conducted at four wound care centers with three in Virginia and one in Oklahoma.
    e. **Inclusion Criteria**: Inclusion criteria consisted of the following: age 18 or older, type I or type II diabetes, ulcer size ≥1 cm2 and <25 cm2, ulcer duration of ≥4 weeks and is unresponsive to standard wound care, no clinical signs of infection, serum Cr <3·0 mg/dl, HgA1c <12%, adequate circulation to the affected extremity (defined as dorsum transcutaneous oxygen test (TcPO2) ≥ 30 mmHg, ABI between 0.7 and 1.2, or triphasic or biphasic doppler arterial waveforms at the ankle of affected leg), and able and willing to provide consent and agrees to comply with study procedures and follow-up evaluations.
    f. **Exclusion Criteria**: Exclusion criteria consisted of the following: current participation in another clinical trial, index wound duration of >52 weeks without intermittent healing, index ulcer probing to tendon, muscle, capsule or bone, currently receiving radiation or chemotherapy, known or suspected malignancy of current ulcer, diagnosis of autoimmune connective tissue disease, use of biomedical/topical growth factor within previous 30 days, pregnant or breast feeding, patient taking medications considered to be immune system modulators, allergy or known sensitivity to Gentamicin, Streptomycin, bovine collagen or components of linear polysaccharide shipping medium, wounds improving >20% over the 2-week run-in period of the trial using standard of care dressing and Camboot offloading, patient taking Cox-2 inhibitors, or planned use of Dakin's solution, Mafenide Acetate, Scarlet Red Dressing, Tincoban, Zinc Sulfate, Povidone-iodine solution, Mafenide Acetate, Polymyxin/Nystatin or Chlorhexidine during trial.
    g. **Participants**: In this study, after exclusions, 100 patients were included for analysis. These patients had been randomized 1:1:1 to receive either BSS (n=33), dHACM (n=32), or standard wound care (n=35).

h. **Intervention(s)/Exposure(s)**: Treatment with either BSS, dHACM, or standard of care. Standard of care was defined as daily dressing changes performed by patients with collagen-alginate dressings and gauze and wound offloading with a cast walker.
i. **Main Outcome(s) and Measure(s)**: The primary objective of this comparative effectiveness study was to compare healing characteristics between groups treated weekly with BSS or dHACM and SWC during the 12-week study period. The secondary objectives of this study were to compare the direct costs of these advanced wound therapies and to examine clinical factors associated with more rapid healing at 12 weeks. An intent-to-treat analysis was used, which included all patients as originally allocated after randomization and those who received at least one treatment.
j. **Results**: Within the 12-week study period, complete healing occurred in 73% of subjects treated with BSS (24/33), 97% of subjects treated with dHACM (31/32) and 51% of subjects receiving SWC alone (18/35) (adjusted p=0.00019). Additionally, mean time-to-heal within 12 weeks were 47.9 days (95%CI: 38.2–57.7) for the BSS group, 23.6 days (95%CI: 17.0–30.2) for the dHACM group and 57.4 days (95%CI: 48.2–66.6) for the SWC group (adjusted $p=3.2*10^{-7}$). Multivariate analysis demonstrated that subjects treated with dHACM had a significantly higher probability of healing their wounds (HR: 5.66; adjusted $p=1.3*10^{-7}$) compared to SWC alone, whereas subjects treated with BSS did not differ significantly compared to SWC alone. Using the same model in which the reference for group was dHACM instead of SWC, the HR for BSS was significantly reduced (HR: 0.30; unadjusted $p=5.8*10^{-5}$), indicating superiority of dHACM. A Kaplan-Meier plot of time-to-heal within 12 weeks by the study group, after adjusting for significant covariates, demonstrated a superior wound-healing trajectory for dHACM compared to BSS or SWC. Fewer grafts were required to achieve complete closure in the dHACM group, resulting in an average reduction in the cost of graft material compared with the BSS group.
k. **Adverse Events:** Ten adverse events were reported during the study. Seven of the reported adverse events were wound/foot infections with two of these resulting in hospitalization of the subject. Two of these infections resulted in the withdrawal of the subject from the study. One of the adverse events was a urinary tract infection (UTI) that necessitated admission of the subject to the hospital for treatment. Two of the remaining adverse events were injuries: one was sustained in a car accident that resulted in hospitalization of the subject and one was local trauma of the study foot with bruising. Four of these adverse events were considered serious because they resulted in hospitalization of the subject. Two subjects were hospitalized for wound infections (both were in the SWC treatment group) with one subject diagnosed with osteomyelitis, requiring withdrawal from the study. As mentioned above, the remaining two subjects were hospitalized for a UTI (BSS group) and a car accident (dHACM group). None of the reported adverse events were considered product related.
l. **Conclusions and Relevance**: The study concludes that dHACM is superior to BSS in both clinical and cost-effectiveness for the treatment of chronic lower extremity ulcers in diabetic patients. These findings support the use of dHACM as a beneficial treatment option for chronic diabetic ulcers.
m. **Limitations**: Limitations of the study include short follow-up time of only 1 week after complete healing, lack of data examining wound recidivism, cost data obtained from a CMS reimbursement schedule do not reflect the actual cost of material across all clinical settings, and lack of ancillary cost examination related to differences in product handling, storage and application procedures, which may have further impacted costs.

n. **Trial Registration**: Written consent was obtained from all subjects prior to any study-related procedures. The trial was pre-registered in ClinicalTrials.gov (NCT01921491), conducted in compliance with applicable regulatory requirements, in accordance with the provisions of the Declaration of Helsinki and in adherence to Good Clinical Practice (GCP).
o. **Funding Source**: No information was reported about funding, but the products included are EpiFix from Mimedx and Apligraf from Organogenesis.

6. Snyder RJ, Shimozaki K, Tallis A, et al. A Prospective, Randomized, Multicenter, Controlled Evaluation of the Use of Dehydrated Amniotic Membrane Allograft Compared to Standard of Care for the Closure of Chronic Diabetic Foot Ulcers. Wounds. 2016;28(3):70-77.
    a. **Importance:** This study investigates the effectiveness of dehydrated amniotic membrane allograft (DAMA) in treating chronic diabetic foot ulcers, aiming to reduce the risk of amputation and improve healing outcomes.
    b. **Objective**: The study was designed to describe the natural history (efficacy, safety, and impact on wound closure rates) of DAMA+SOC compared to SOC alone in subjects with chronic grade 1 or 2 DFUs.
    c. **Design**: Prospective, randomized, multicenter, open-label, parallel group trial conducted over 6 weeks, assessing the wound closure rates of DAMA plus standard care against standard care alone.
    d. **Setting**: Conducted across eight clinical sites in the United States, focusing on outpatient wound care.
    e. **Inclusion Criteria**: Inclusion criteria included be an ambulatory person of at least 18 years of age at the time of informed consent, have type 1 or type 2 diabetes mellitus, have an HbA1c of < 12%, have at least 1 wound that: a) is Wagner grade 1 or superficial 2 (ie, without bone, tendon, or joint exposure), b) has a duration of at least 1 month, c) has no clinical signs of infection or osteomyelitis, d) is between 1 cm2 and 25 cm2 in area, e) closed < 30% in area during the screening period, and f) is located on the foot, distal to malleolus, have adequate circulation to the affected extremity as demonstrated by an ABI between 0.7 and 1.2, or triphasic or biphasic Doppler arterial waveform at the ankle of the affected leg, or dorsum transcutaneous oxygen test ≥ 30 mmHg, have a serum creatinine of < 3.0 mg/dL or CrCl > 30 mL/min, and have the ability and willingness to understand and comply with study procedures and give written, informed consent prior to enrollment in the study or initiation of study procedures.
    f. **Exclusion Criteria**: Exclusion criteria consisted of the following: have participated in another clinical trial within 30 days prior to consent, have an active Charcot deformity of the study foot (e.g., foot is erythematous, warm, edematous, and is actively remodeling), are receiving radiation or chemotherapy of any kind, have a known or suspected malignancy of a current ulcer, are pregnant or breast feeding, have an active malignant disease, are receiving hemodialysis or peritoneal dialysis, have sickle cell anemia or Raynaud's syndrome, have a diagnosis of autoimmune connective tissue disease, have received a biologic agent, growth factor, xenograft, or skin equivalent to the ulcer 30 days prior to consent, have exposed bone, tendon, or joint capsule in the study ulcer, or are taking medications considered to be immune system modulators.

g. **Participants**: 29 subjects with chronic diabetic foot ulcers were stratified by ulcer size (1-10 cm2 and 10-25 cm2) randomized into two groups: DAMA plus standard care (n=15) and standard care alone (n=14).
h. **Intervention(s)/Exposure(s)**: The intervention group received DAMA with SOC, while the control group received SOC alone. SOC consisted of debridement of necrotic/nonviable tissue and hemostasis, moist wound dressings, offloading where appropriate with a DH Walker boot, and infection surveillance and management.
i. **Main Outcome(s) and Measure(s)**: The primary endpoint of the study was the proportion of subjects with complete wound closure prior to or on week 6 after initiation of treatment, which was defined as 100% complete skin re-epithelialization without drainage or dressing requirements.
j. **Results**: In the ITT population, 33% of the DAMA+SOC group achieved complete wound closure by week 6 compared to 0% in the standard care group (p=0.017). Further, subjects in the DAMA+SOC achieved wound closure more rapidly than did those allocated to SOC alone ($P < 0.0001$) based upon the Kaplan-Meier analysis. The subjects in the DAMA group had an average of 4.3 ± 1.7 applications of DAMA, with one piece applied weekly.
k. **Adverse Events**: No treatment-related adverse events were reported. The incidence of adverse events did not differ significantly between the groups, indicating the safety of DAMA in this patient population. Six subjects in the SOC alone cohort and 4 subjects in the DAMA+SOC cohort (ITT population) experienced treatment-emergent adverse events. In the DAMA+SOC cohort, these events included wound infection, localized infection, osteomyelitis, prolonged bleeding, cellulitis, and atrial flutter. Treatment-emergent adverse events observed in the SOC-alone cohort included tendon injury, skin ulcer, diabetic foot infection, cellulitis, and deep vein thrombosis. The incidence of adverse events was not different between the groups and, given the nature of the underlying diabetic disease and associated comorbidities, these events were not unexpected.
l. **Conclusions and Relevance**: DAMA plus standard care significantly improved the closure rates of chronic diabetic foot ulcers compared to standard care alone, suggesting DAMA as a safe and effective treatment option.
m. **Limitations**: The study was limited by its small sample size and open-label design, which may affect the generalizability and interpretation of the results.
n. **Trial Registration**: ClinicalTrials.gov Identifier was NCT02209051. The IRB protocol was approved by the following IRBs: Western IRB, Beth Israel Deaconess-Plymouth IRB, Duke University Health System IRB, and Wayne Memorial Hospital IRB.
o. **Funding Source**: The study was wholly supported by Derma Sciences, Princeton, NJ.

7. DiDomenico LA, Orgill DP, Galiano RD, et al. Aseptically processed placental membrane improves healing of diabetic foot ulcerations: Prospective randomized clinical trial. Plast Reconstr Surg Glob Open. 2016;4:e1095.
    a. **Importance**: The study investigates an advanced treatment for diabetic foot ulcers, focusing on the efficacy and cost-effectiveness of aseptically processed placental membrane, a significant concern due to the complications associated with diabetic foot ulcers.
    b. **Objective**: To evaluate the effectiveness of aseptically processed dehydrated human amnion and chorion allograft (dHACA) in comparison to standard of care (SOC) for healing nonhealing diabetic foot ulcers (DFUs).
    c. **Design**: A prospective, randomized clinical trial with patients treated for 12 weeks, assessing wound closure rates of DFUs with dHACA plus SOC versus SOC alone.

d. **Setting**: The study was performed at five outpatient wound care centers in the United States.
e. **Inclusion Criteria**: Inclusion criteria consisted for the following: male or female age 18 or older, type 1 or type 2 diabetes mellitus (ADA diagnostic criteria), signed informed consent, patient's wound diabetic in origin and larger than 1 cm2, wound present for a minimum of 4 week duration, with documented failure of prior treatment to heal the wound, wound has no signs of infection, wound present anatomically on the foot as defined by beginning below the malleoli of the ankle, additional wounds may be present but not within 3 cm of the study wound, serum creatinine <3.0 mg/dL, HbA1c <12% at randomization, patient has adequate circulation to the affected extremity, as demonstrated by 1 of the following within the past 60 days: Dorsum transcutaneous oxygen test >30 mm Hg; ABI with results of >0.7 and <1.2; or Doppler arterial waveforms, which are triphasic or biphasic at the ankle of affected leg, patient is of legal consenting age, and patient is willing to provide informed consent and is willing to participate in all procedures and follow-up evaluations necessary to complete the study.
f. **Exclusion Criteria**: Exclusion criteria consisted of the following: wound probing to bone (UT grade IIIA–D), index wound >25 cm2, HbA1c >12% within previous 90 days, serum Cr >3.0 mg/dL, patients with a known history of poor compliance with medical treatments, patients previously randomized into this study, or presently participating in another clinical trial, patients currently receiving radiation therapy or chemotherapy, patients with known or suspected local skin malignancy to the index wound, patients with uncontrolled autoimmune connective tissues diseases, non-revascularizable surgical sites, active infection at index wound site, any pathology that would limit the blood supply and compromise healing, patients who have received a biomedical or topical growth factor for their wound within the previous 30 days, patients who are pregnant or breast feeding, patients who are taking medications that are considered immune system modulators that could affect graft incorporation, patients taking a Cox-2 inhibitor, and patients with wounds healing >20% during the screening period.
g. **Participants**: Forty patients with DFUs were randomized into two groups: dHACA plus SOC (n=20) or SOC alone (n=20).
h. **Intervention(s)/Exposure(s)**: Weekly treatment with dHACA (AmnioBand) plus SOC versus SOC alone for up to 12 weeks.
i. **Main Outcome(s) and Measure(s)**: The primary endpoint was the proportion of wounds healed at 6 weeks. Secondary endpoints included proportion of wounds healed at 12 weeks, time to heal within 6 and 12 weeks, number of graft applications, graft wastage, and cost of treatment per healed wound.
j. **Results**: At 6 weeks, 70% of dHACA-treated DFUs healed compared with 15% treated with SOC alone (p=0.001). The odds ratio for healing in dHACA + SOC–treated patients compared with SOC patients was 17 (95% confidence interval [CI], 3.1–93) (p=0.001). At 12 weeks, 85% of the DFUs in the dHACA + SOC group had healed compared with 25% in the SOC group (not statistically tested). At 6 weeks, mean time to heal for the dHACA–SOC group was 30 days (95% CI, 24–35) compared with 40 days (95% CI, 37–43) for the SOC group (P = 0.00073). At 12 weeks, mean time to heal between the groups had widened considerably: dHACA + SOC, 36 days (95% CI, 27–46); SOC, 70 days (95% CI, 59–81; P = 0.00073. At 6 weeks, PAR for the SOC group had reached 48% ± 65%, whereas the value for the dHACA + SOC group was 87% ± 30%. The corresponding figures at 12 weeks were little changed: 41 ± 72 and 87 ± 31, respectively. By 12 weeks, 85% of dHACA group DFUs healed versus 25% in the SOC group. The dHACA group showed a mean time to heal of 36

days at 12 weeks. At 6 weeks, the mean number of grafts used per wound for the dHACA + SOC group was 3.1 (±1.7). The mean cost of product to heal DFUs was $1091 (±$619; n = 14). At 12 weeks, the mean number of grafts used per healed wound for the dHACA + SOC group was 3.8 (±2.2). The mean cost of product to heal was $1400 (±$1100; n = 17).

k. **Adverse Events**: Four adverse events occurred: 1 in the dHACA + SOC group (5%) and 3 in the SOC group (15%). All adverse events that progressed into SAEs involved localized pedal infections initially treated with antibiotics. There were a total of 2 SAEs, 1 in the dHACA + SOC group and 1 in the SOC, both involving foot infections that progressed to os- teomyelitis; both were treated with OR debridement and IV antibiotics. No adverse events were found to be graft related.
l. **Conclusions and Relevance**: Aseptically processed dHACA significantly improves the healing of DFUs over SOC alone, with minimal adverse events and reasonable cost.
m. **Limitations**: Small sample size, lack of blinding (patient and investigator), and lack of a soft-tissue matrices comparator could bias results. The study suggests further research to confirm findings in a larger, more diverse population.
n. **Trial Registration**: ClinicalTrials.gov Identifier was NCT02399826. IRB approval was granted by Western IRB (protocol number, 20150073).
o. **Funding Source**: The study was funded by the Musculoskeletal Transplant Foundation.

8. DiDomenico LA, Orgill DP, Galiano RD, et al. Use of an aseptically processed, dehydrated human amnion and chorion membrane improves likelihood and rate of healing in chronic diabetic foot ulcers: A prospective randomised multi-centre clinical trial in 80 patients. Int Wound J. 2018;15:950-957.
    a. **Importance**: The study focuses on the potential of aseptically processed, dehydrated human amnion and chorion membrane (dHACA) to enhance healing in chronic diabetic foot ulcers, a condition posing significant risk and healthcare burden.
    b. **Objective**: To assess the efficacy of dHACA, in addition to standard care, on the healing rates of non-healing diabetic foot ulcers compared to standard care alone.
    c. **Design**: A parallel, 2-group, prospective, randomized, controlled, multicenter trial in which eligible patients with 1 or more neuropathic DFUs were randomized 1:1 to receive either dHACA with SOC or SOC alone over 12 weeks.
    d. **Setting**: Conducted in five outpatient wound care centers across the United States.
    e. **Inclusion Criteria**: Inclusion criteria consisted for the following: male or female age 18 or older, type 1 or type 2 diabetes mellitus (ADA diagnostic criteria), signed informed consent, patient's wound diabetic in origin and larger than 1 cm2, wound present for a minimum of 4 week duration, with documented failure of prior treatment to heal the wound, wound has no signs of infection, wound present anatomically on the foot as defined by beginning below the malleoli of the ankle, additional wounds may be present but not within 3 cm of the study wound, serum creatinine <3.0 mg/dL, HbA1c <12% at randomization, patient has adequate circulation to the affected extremity, as demonstrated by 1 of the following within the past 60 days: Dorsum transcutaneous oxygen test >30 mm Hg; ABI with results of >0.7 and <1.2; or Doppler arterial waveforms, which are triphasic or biphasic at the ankle of affected leg, patient is of legal consenting age, and patient is willing to provide informed consent and is willing to participate in all procedures and follow-up evaluations necessary to complete the study.

f. **Exclusion Criteria**: Exclusion criteria consisted of the following: wound probing to bone (UT grade IIIA–D), index wound >25 cm2, HbA1c >12% within previous 90 days, serum Cr >3.0 mg/dL, patients with a known history of poor compliance with medical treatments, patients previously randomized into this study, or presently participating in another clinical trial, patients currently receiving radiation therapy or chemotherapy, patients with known or suspected local skin malignancy to the index wound, patients with uncontrolled autoimmune connective tissues diseases, non-revascularizable surgical sites, active infection at index wound site, any pathology that would limit the blood supply and compromise healing, patients who have received a biomedical or topical growth factor for their wound within the previous 30 days, patients who are pregnant or breast feeding, patients who are taking medications that are considered immune system modulators that could affect graft incorporation, patients taking a Cox-2 inhibitor, and patients with wounds healing >20% during the screening period.
g. **Participants**: Eighty patients with diabetic foot ulcers, randomized into two groups.
h. **Intervention(s)/Exposure(s)**: Application of dHACA (AmnioBand) plus standard care (n = 40) versus standard care alone (n = 40). Standard of care was defined as surgical debridement to remove all necrotic tissue, screening for infection and probing of the wound for bone, receipt of collagen alginate primary dressing, and off-loading using a removable diabetic offloading cam-walker.
i. **Main Outcome(s) and Measure(s)**: The primary endpoint was the percentage of wounds healed at 6 weeks between the two treatment groups. Secondary endpoints included healing at 12 weeks, time to heal within 6 and 12 weeks, number of graft applications, graft wastage, and cost to closure for healed wounds.
j. **Results**: At 6 weeks, with regard to the primary endpoint of complete wound healing, 68% (27/40) of the dHACA plus SOC-treated DFUs had healed compared with 8 of 40 (20%) with SOC alone ($p=1.9*10-5$). At 12 weeks, 85% of the DFUs in the dHACA plus SOC group had healed (34/40) compared with 33% (13/40) in the SOC group ($p=6.0*10-6$). At 6 weeks, mean time-to-heal for the dHACA plus SOC group was 29.2 days (95% CI: 25.1-33.4) compared with 39.5 days (95% CI: 37.4-41.5) for the SOC group ($p=1.6*10-5$). At 12 weeks, the difference in mean time to heal between the groups had lengthened: dHACA plus SOC: 37.0 days (95% CI: 29.5-44.4); SOC: 67.3 days (95% CI: 59.0 -79.6; $p=6.0*10-6$). The median time to heal within 12 weeks for the dHACA plus SOC cohort was 35.0 days (95% CI: 26.5-43.5). The hazard ratio (HR) for dHACA plus SOC treatment was 4.25 (95%CI: 0.44-0.79; $p=2.5*10-5$) compared with SOC as treatment after controlling for initial wound area. At 12 weeks, mean PAR for the SOC group was 45.6 (SD: 74.2) compared with 90.3 (SD: 13.65) for the dHACA plus SOC group ($p=6.0*10-6$). Mean cost of the tissue to heal a DFU was $1771. The dHACA plus SOC group used a mean of 3.7 (SD: 1.97) grafts per wound at 6 weeks, and by 12 weeks, the mean number of grafts used per healed wound for the dHACA plus SOC group was 4.0 (SD: 2.56).
k. **Adverse Events**: Eleven AEs occurred, with 3 in the dHACA plus SOC group (8%) and 8 in the SOC group (20%). All AEs involved localized pedal infections initially treated with antibiotics. There were a total of 4 serious adverse events (SAEs), with 1 in the dHACA plus SOC group (3%) and 3 in the SOC (8%). The SAEs involving foot infections all required hospitalization, and the majority progressed to osteomyelitis and were treated with IV antibiotics and OR debridement as necessary. There were no adverse events found to be graft related.

l. **Conclusions and Relevance**: dHACA significantly enhances the healing rates of chronic diabetic foot ulcers compared to standard care alone.
m. **Limitations**: Included the study's open-label design and the withdrawal of patients not showing sufficient improvement at 6 weeks rather than continuing through 12 weeks of treatment. Future studies looking at an additional comparator arm of an advanced skin substitute may provide greater evidence about the relative merit of dHACA.
n. **Trial Registration**: ClinicalTrials.gov Identifier NCT02399826. Approved by the Western Institutional Review Board on January 29, 2015, with the protocol number 20150073.
o. **Funding Source**: Funded by the Musculoskeletal Transplant Foundation, Grant/Award Number: 002.

9. Serena TE, Yaakov R, Moore S, et al. A randomized controlled clinical trial of a hypothermically stored amniotic membrane for use in diabetic foot ulcers. J Comp Eff Res. 2020;9(1):23-34.
    a. **Importance**: The study explores the effectiveness of hypothermically stored amniotic membrane (HSAM) in treating diabetic foot ulcers (DFUs), a condition that significantly impacts patient quality of life and healthcare costs.
    b. **Objective**: The purpose of this randomized controlled trial (RCT) was to investigate the comparative effectiveness of HSAM and SOC versus SOC alone in the treatment of DFUs over a 16 week study period (12-week treatment phase and a 4 week follow-up phase).
    c. **Design**: A multicenter, prospective, randomized controlled trial assessing clinical outcomes of DFUs treated with HSAM plus SOC against SOC alone.
    d. **Setting**: Conducted across 14 clinical centers.
    e. **Inclusion Criteria**: Inclusion criteria included the following: subjects with type I or II diabetes, DFU of greater than or equal to 4-weeks duration at presentation that was unresponsive to SOC, DFU located below the medial aspect of the malleolus extending at least through the epidermis into dermis, subcutaneous tissue, muscle, or tendon but not into bone, HbA1c <12%, ulcer between 1 and 25 cm2, minimum age of 18 years, adequate lower extremity perfusion as evidenced by transcutaneous oxygen measurement (TCOM) or a skin perfusion pressure (SPP) measurement of ≥30 mmHg, or an ankle-brachial index (ABI) between 0.7 and ≤1.3 or toe-brachial index (TBI) of ≥0.6 within 3 months of the first Screening Visit, no evidence of unresolved gross soft-tissue infection or osteomyelitis as ruled out by x-rays, CAT scan or MRI, no evidence of underlying comorbid conditions that would adversely affect wound closure (cancer, Raynaud's Syndrome, severe venous insufficiency or uncorrected arterial insufficiency), and abstaining from concomitant medications that would compromise wound closure (cytotoxic drugs or chemotherapeutics).
    f. **Exclusion Criteria**: Exclusion criteria consisted of the following: evidence of skin cancer within or adjacent to the ulcer site, symptoms of osteomyelitis, ulcers of the calcaneus, renal impairment (creatinine >2.5 mg/dl), hepatic impairment (≥2 x ULN), hematologic disorders, cellulitis, ulcers with sinus tracts, active deep vein thrombosis, uncontrolled diabetes and severely immune compromised subjects.
    g. **Participants**: 76 subjects with DFUs were randomized into two groups: HSAM plus SOC (n=38) and SOC alone (n=38).
    h. **Intervention(s)/Exposure(s)**: Application of HSAM in conjunction with SOC versus SOC alone. SOC was defined as debridement, infection elimination, use of dressings and offloading by total contact casting. Study SOC followed guideline treatment that addressed

debridement/devitalized tissue, infection or inflammation, moisture balance and wound edge preparation/wound depth (the DIME paradigm).

i. **Main Outcome(s) and Measure(s)**: Primary endpoints were the frequency of and time to wound closure by 16 weeks (12 weeks in the treatment phase of the study, 4 weeks in the follow-up phase). Secondary outcomes included reductions in ulcer area and depth.

j. **Results**: The primary unadjusted end point of wound closure for HSAM-treated ulcers was significantly greater than SOC by 12 weeks (55 vs 29%; p = 0.02) and 16 weeks (58 vs 29%; p = 0.01) respectively. The K–M median time to wound closure for HSAM-treated ulcers was 11 weeks. For SOC-treated ulcers, the K–M median time to wound closure was not attained by 16 weeks (i.e., 50% of patients in the SOC group failed to demonstrate wound closure by the end of study, 16 weeks). A forward selection Cox proportional hazards regression model that adjusted for treatment, age, sex, BMI, area, depth, volume and age of the ulcer at baseline, was performed to compute the hazard ratio (HR) or probability of attaining wound closure over the entire course of the study (study day 0 through to study week 16). The Cox regression hazard ratio (HR = 1.75 [1.16–2.70]) showed a 75% greater weekly probability of wound closure in favor of the HSAM group. Cox adjusted survival data for wound closure showed that HSAM was superior to SOC at 4 weeks (11% vs 3%), 8 weeks (36% vs 23%), 12 weeks (60% vs 38%) and 16 weeks (63% vs 38%); p = 0.04. DFUs treated in the study showed an incidence of achieving greater than a 60% reduction in baseline area (82 vs 58%; p = 0.02) and depth (65 vs 39%; p = 0.04) for the HSAM-treated and the SOC-treated wounds respectively. In addition, the incidence of ulcers demonstrating greater than a 75% reduction in baseline volume was greater in the HSAM-treated subjects compared with the SOC-treated subjects (81 vs 58%; p = 0.06; Figure 2). Group comparisons showed that the HSAM-treated group demonstrated greater mean percent decreases at the end of study from baseline in ulcer area (78 vs 55%), depth (59 vs 39%) and volume (74 vs 67%) compared with the SOC group.

k. **Adverse Events**: Not specifically mentioned, indicating the need to review the full article for details on safety evaluations.

l. **Conclusions and Relevance**: HSAM treatment significantly enhances the healing process of DFUs compared to SOC, offering a promising treatment option.

m. **Limitations**: Limitations include the lack of blinding in using a skin substitute compared with standard bandages as the primary wound contact material. The open-label design and the need for further studies to confirm these findings in broader populations and settings can be improved.

n. **Trial Registration**: Western IRB provided IRB approval for this study.

o. **Funding Source**: The study was funded by Organogenesis Inc., with disclosures of consultancy roles for some authors.

10. Tettelbach W, Cazzell S, Reyzelman AM, et al. A confirmatory study on the efficacy of dehydrated human amnion/chorion membrane dHACM allograft in the management of diabetic foot ulcers: A prospective, multicentre, randomised, controlled study of 110 patients from 14 wound clinics. Int Wound J. 2019;16:19-29.

a. **Importance:** Diabetic foot ulcers represent a significant healthcare challenge, leading to hospitalization, amputation, and substantial healthcare costs. Advanced wound care therapies, including dehydrated human amnion/chorion membrane (dHACM), have been explored to enhance healing rates and outcomes.

b. **Objective:** The purpose of the study was to confirm the efficacy of dHACM in treating chronic lower extremity ulcers in persons with diabetes, comparing its effectiveness to standard care in a more diverse and comprehensive patient population across multiple clinical settings.
c. **Design:** This was a prospective, randomized, controlled, multicenter clinical trial conducted across 14 wound care centers in the United States, focusing on patients with lower extremity ulcers of at least 4 weeks duration.
d. **Setting:** Study subjects were enrolled at 14 study sites in the United States. Study sites were located in California, Virginia, Ohio, Texas, Massachusetts, Oregon, and Alabama, and both hospital-based and private clinic settings in urban and rural areas were represented.
e. **Inclusion Criteria:** Inclusion criteria consisted of adults aged 18 or older, type 1 or type 2 diabetes, able and willing to provide consent and agrees to comply with study procedures and follow-up evaluations, ulcers sized $\geq$1 cm^2 and $\leq$25 cm^2, ulcer duration of $\geq$4 weeks unresponsive to standard wound care, no clinical signs of infection, serum Cr <3.0 mg/dL, HgA1c <12%, and adequate circulation to the affected extremity as demonstrated by dorsum transcutaneous oxygen test (TcPO2) ≥ 30 mm Hg, ABI between 0.7 and 1.2, or triphasic or biphasic Doppler arterial waveforms at the ankle of affected leg.
f. **Exclusion Criteria:** Exclusion criteria encompassed current participation in another clinical trial, index wound duration of >52 weeks without intermittent healing, index ulcer probing to tendon, muscle, capsule, or bone, currently receiving radiation or chemotherapy, known or suspected malignancy of the current ulcer, diagnosis of autoimmune connective tissue disease, use of biomedical/topical growth factor within previous 30 days, pregnant or breast feeding, taking medications considered to be immune system modulators, allergy or known sensitivity to gentamicin or streptomycin, wounds improving greater than 25% over the 2-week run-in period of the trial using standard of care dressing and Camboot offloading, patient taking COX-2 inhibitors, and planned use of Dakin's solution, Mafenide acetate, scarlet red dressing, Tincoban, zinc sulfate, povidone-iodine solution, Mafenide acetate, Polymyxin/nystatin, or chlorhexidine during trial.
g. **Participants:** The study involved 110 patients with type 1 or type 2 diabetes presenting with lower extremity ulcers below the ankle, meeting the specific inclusion and exclusion criteria. They were randomized 1:1 to the dHACM group (n=54) and non-dHACM group (n=56).
h. **Intervention(s):** Patients were randomized to receive weekly applications of dHACM in addition to standard wound care and offloading, or standard care with alginate wound dressings and appropriate offloading, for 12 weeks. Standard of care consisted of moist dressings, debridement, offloading of weight-bearing ulcers, infection prevention, and patient education on proper foot care.
i. **Main Outcome(s) and Measure(s):** The primary outcome was the percentage of ulcers completely healed within 12 weeks. Secondary outcomes included healing rates at different intervals, time to heal, and ulcer recurrence at 16 weeks.
j. **Results:** The primary study outcome was percentage of study ulcers completely healed in 12 weeks, with both ITT and per-protocol participants receiving weekly dHACM significantly more likely to completely heal than those not receiving dHACM (ITT—70% versus 50%, P = 0.0338, per-protocol—81% versus 55%, P = 0.0093). A Kaplan–Meier analysis was performed to compare the time-to-healing performance with/without dHACM, showing a significantly improved time to healing with the use of allograft (log-rank p=0.0187). Cox regression analysis showed that dHACM-treated subjects were more than twice as likely to heal completely within 12 weeks than no-dHACM subjects (HR: 2.15, 95% confidence interval 1.30–3.57, P = 0.003). At the final follow up at 16 weeks, 95% of dHACM-healed ulcers and

86% of healed ulcers in the no-dHACM group remained closed. The median number of grafts applied per healed wound was 5 (range 1–12).

k. **Adverse Events:** The study recorded 230 adverse events. The most common adverse event was development of an additional ulcer (n = 34). A total of 112 adverse events occurred in subjects receiving dHACM and 118 in subjects not receiving dHACM. Fifty-three adverse events (23%) were ulcer-related. In the dHACM-treated subjects, 30 adverse events were ulcer-related. In subjects not receiving dHACM, 23 events were ulcer-related. Of the 53 ulcer-related events, 30 were infectious events. There were 11 target ulcer infections (6 dHACM-treated and 5 no-dHACM), 15 cases of cellulitis (7 dHACM-treated and 8 no-dHACM), and 4 cases of osteomyelitis (3 dHACM- treated and 1 no-dHACM). There were three events classified as possibly being product related. These included one case of wound maceration and two positive wound cultures (1 Providencia Stuartii, 1 Pseudomonas Aeruginosa).

l. **Conclusions and Relevance:** The study confirms that dHACM is an effective treatment for diabetic foot ulcers, offering superior healing rates compared to standard care alone, across a diverse patient population and clinical settings.

m. **Limitations:** Limitations included potential biases due to the open-label design, variations in debridement quality, and inability to monitor offloading compliance strictly.

n. **Trial Registration:** The study was registered at ClinicalTrials.gov with the identifier NCT01693133 and approved by Chesapeake IRB or site's local IRB.

o. **Funding Source:** The study was sponsored by MiMedx Group, Inc.

11. Thompson P, Hanson DS, Langemo D, Anderson J. Comparing Human Amniotic Allograft and Standard Wound Care When Using Total Contact Casting in the Treatment of Patients with Diabetic Foot Ulcers. Adv Skin Wound Care. 2019;32(6):272-277.

a. **Importance:** The study investigates the efficacy of combining human amniotic allograft (HAA) with total contact casting (TCC) versus TCC with standard wound care in treating diabetic foot ulcers, highlighting the potential for improved healing strategies in this high-risk population.

b. **Objective:** The purpose of this study was to compare the healing effectiveness and outcomes between diabetic foot ulcers treated with TCC and HAA versus TCC with standard wound care.

c. **Design:** This is a prospective, randomized study conducted at a Midwestern wound care clinic, involving adult outpatients with diabetic foot ulcers, assessing mean ulcer surface area, time to closure, recurrence rates, satisfaction with TCC, infection rates, and hemoglobin A1c levels.

d. **Setting:** The study was carried out in a university-affiliated rural Midwestern wound care clinic.

e. **Inclusion Criteria:** Inclusion criteria consisted of adults 18 years or older with a diagnosis of type 1 or 2 diabetes and a diabetic foot ulcer on the plantar surface larger than 0.5 cm who had not shown a 50% reduction in wound area after 4 weeks of standard treatment. Other inclusion criteria consisted of random blood glucose of 450 mg/dL or less, a hemoglobin A1c (HbA1c) of 15% or lower drawn within the 4 weeks prior to study enrollment, and evidence of adequate per- fusion to the subject’s foot as established with an ankle-brachial index of between 0.7 and 1.2. Participants were excluded if they had gangrene on any area of the

affected foot or evidence of infection not currently being treated as determined by their primary care provider.

f. **Exclusion Criteria:** Participants were excluded if they had gangrene on any area of the affected foot or evidence of infection not currently being treated as determined by their primary care provider.
g. **Participants:** After screening 270 patients for inclusion, 13 patients were randomized into two intervention groups.
h. **Intervention(s)/Exposure(s):** Group A received TCC and HAA (n=7); Group B received TCC and standard wound care (n=6). Standard wound care typically consists of wound assessment, surgical debridement, cleansing, topical treatment, and a protective dressing.
i. **Main Outcome(s) and Measure(s):** The study measured difference in time to closure, HbA1c, diabetic foot ulcer closure, recurrence of diabetic foot ulcers, wound drainage, and participant satisfaction.
j. **Results:** The majority of participants experienced wound closure during the course of the study (92.3%). Two participants did not achieve closure, both of whom had Charcot foot. Group A, which had a higher mean hemoglobin A1c at study outset (9.63% vs. 8.47%), experienced a longer mean time to closure (29.50 days) compared with group B (26.20 days). The 90-day recurrence rates were different for the two groups, with only one recurrence for group A (14.29%) but five recurring ulcers in group B (83.33%). Wound drainage was monitored at every visit. The majority of participants in group B had no drainage (83.3%) or a small amount (16.7%) compared with group A participants, where 42.9% had moderate drainage, 28.6% had a small amount, and 28.6% had none.
k. **Adverse Events:** Specific details on adverse events were not provided in the summary, indicating the need to review the full article for comprehensive safety evaluations.
l. **Conclusions and Relevance:** While not statistically significant due to sample size, there was a trend suggesting the effectiveness of HAA with TCC in treating diabetic foot ulcers, meriting further investigation.
m. **Limitations:** Small sample size, in addition to homogeneity of the sample, limits the generalizability of these findings. The sample size was limited because of strict inclusion criteria, but also because of delayed referrals from primary care providers; by the time of referral, some patients were already experiencing complications such as infection and osteomyelitis, which in turn excluded them from the study. Additionally, this study did not examine statistical significance to see if these differences between groups were meaningful.
n. **Trial Registration:** This study sought IRB approval through its associated university and medical center review boards.
o. **Funding Source:** The study was supported in part by Derma Sciences Inc., which provided allografts for all subjects in the study.

12. Game F, Gray K, Chokkalingam K, et al. The effectiveness of a new dried human amnion derived membrane in addition to standard care in treating diabetic foot ulcers: A patient and assessor blind, randomised controlled pilot study. Int Wound J. 2021;18:692-700.
    a. **Importance:** Diabetic foot ulcers represent a significant source of disability, distress, and healthcare cost, often resulting in delayed healing and amputations. While standard treatments include antimicrobial treatment, vascular intervention, offloading, and regular debridement, many ulcers remain unhealed, highlighting the need for innovative treatment options.

b. **Objective:** The aim of this pilot study was therefore to investigate whether the biweekly addition of this dHAM product to standard care versus standard care alone led to a greater increase in the proport
c. ion of participants achieving healing of their index diabetic foot ulcer within 12 weeks,
d. **Design:** The study was designed as a multicenter, prospective, patient and observer blind, randomized controlled pilot trial. Participants were randomized to receive either standard care plus dHAM or standard care alone. Healing within 12 weeks was the primary outcome, with percentage wound area reduction as a secondary outcome.
e. **Setting:** Participants were recruited from two specialist diabetic foot clinics in the United Kingdom.
f. **Inclusion Criteria:** Patients were eligible for inclusion only if ALL of the following criteria applied: patients with diabetes (according to WHO criteria) aged 18 years or over, at least one full thickness ulcer below the malleolus of either foot, present for 4 weeks or more, at least one palpable pulse on the foot of the index limb or an ABPI >0.9, minimum ulcer diameter of 5 mm, maximum ulcer diameter of 20 mm, and able to attend clinic on a fortnightly basis.
g. **Exclusion Criteria:** The participant was not eligible to enter the trial if ANY of the following applied: eGFR <20 mL/min, HbA1c >108 mmol/mol, planned revascularization during the course of the study or within the 4 weeks preceding the start of the study, an ulcer of etiology other than diabetes, depth of ulcer to bone, suspected or confirmed osteomyelitis, severe infection of the index ulcer, active Charcot of the foot of the index ulcer, the need for negative pressure wound therapy, on treatment with systemic steroids at a dose > equivalent of 5 mg prednisolone for more than 5 days and/or systemic cytotoxic agents, unwilling or unable to attend all trial visits, unwilling or unable to give written informed consent, lacks the mental capacity to give consent, any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the trial, may influence the result of the trial or the participant's ability to participate in the trial, and participants who have participated in another research trial involving a wound healing product within the past 12 weeks.
h. **Participants:** Thirty-one individuals were randomized 1:1 to receive either dHAM (n=15) or usual care (n=16), with a mean age of 59.8 years, 81% male, and 87% having type 2 diabetes.
i. **Intervention(s) or Exposure(s):** The intervention group received the application of dHAM directly to the index ulcer bi-weekly following any necessary sharp debridement, in addition to standard care.
j. **Main Outcome(s) and Measure(s):** The primary outcome was the proportion of ulcers healed within 12 weeks. Secondary ulcer-related outcomes included time to healing in those that healed within the 12 weeks active intervention period, the number (%) of ulcers healed within 6 weeks, the percentage change in ulcer area from baseline assessed from digital images of acetate tracings using Image J, the incidence of secondary infection of the index ulcer, and pain in the area of the ulcer assessed by a 100 mm Visual analogue scale (VAS). Secondary patient-related outcomes also included adverse and serious adverse events, the incidence of major (above ankle) and minor (below ankle) amputation.
k. **Results:** Within 12 weeks, there was no statistically significant difference between the 4 (27%) index ulcers in the dHAM group that had healed and the 1 (6.3%) index ulcers in the standard care group (p=0.172). The percentage of wound area reduction was significantly higher in the dHAM group (p=0.0014). There was no significant difference in index wound

healed within 12 weeks, time to healing of those that healed, index wound healed within 6 weeks, number of SAEs, and number of AEs. The study's sample size was determined based on being sufficient for pilot studies, and statistical methods were applied to compare healing rates and wound area reduction between groups.

l. **Adverse Events:** No significant difference in adverse events (AEs), including serious adverse events (SAEs), was observed between the two groups, indicating a comparable safety profile between the dHAM treatment and standard care.

m. **Conclusions and Relevance:** The pilot trial results suggest that the addition of dHAM to standard care is a safe and promising treatment for diabetic foot ulcers, showing an increase in healing rates and wound area reduction. These findings are encouraging for the design of a future, statistically powered, definitive trial.

n. **Limitations:** The main study limitations include the inability to blind clinical researchers and the use of a placebo for the amniotic membrane, although patient blinding was successfully achieved. The pilot nature and small sample size limit the ability to draw definitive conclusions.

o. **Trial Registration:** The study sponsor was University Hospitals of Derby and Burton NHS Foundation Trust. The study was approved by the National Health Research Authority and East Midlands-Derby Research Ethics Committee; IRAS Project ID: 235573, and was registered with Clinicaltrials.gov under NCT03483467.

p. **Funding Source:** The study received funding from NuVision Biotherapies Ltd, Medilink East Midlands Ltd, and the East Midlands Academic Health Science Network. The funding was provided through an unrestricted grant, part of which was funded by the European Regional Development Fund (ERDF). The funders had no role in the study's design, execution, or decision to publish the results

Systematic reviews and meta-analyses

1. Mohammed YA, Farouk HK, Gbreel MI, et al. Human amniotic membrane products for patients with diabetic foot ulcers. do they help? a systematic review and meta-analysis. J Foot Ankle Res. 2022;15(1):71. Published 2022 Sep 14.

    a. **Objective**: Many studies have shown that DHACA as a treatment for diabetic foot ulcers is more effective than standard wound care alone. For further evaluation of the efficacy and time-sensitivity of DHACAs in patients suffering from DFU, the authors performed a systematic review and meta-analysis to compare dehydrated human amnion and chorion allograft (DHACA) plus the standard of wound care (SOC) with the SOC alone.

    b. **Materials and Methods**: The authors performed a systematic review and meta-analysis on the use of dehydrated human amnion/chorion membrane for the treatment of DFU using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines in reporting their study. The authors used four different databases for the literature search (PubMed, Scopus, Cochrane, and Web of Science) to search for the following keywords: Diabetic foot ulcer, human amnion membrane, amniotic allograft, Grafix, AmnioBand, EpiFix. English-written human-based randomized clinical trials (RCTs) were included in the study. Diabetic patients with foot ulcers were the target population. The intervention was human amnion, chorion, placental membrane, or any brand using them like Grafix, GrafixPL PRIME, AmnioBand, Stravix, biological dressing, bio implant dressing, or EpiFix. The comparator was any effective measurement like SOC. The authors excluded conference abstracts, books, single-armed clinical trials, animal studies, and studies on non-diabetic patients. The risk of bias was assessed according to the Cochrane risk of bias tool described in the Cochrane

Handbook for Systematic Reviews of Interventions 5.1.0 and reported this bias as low risk, high risk, or unclear risk of bias. The primary outcomes were the percentage of complete wound healing by the 6th and 12th week and the mean time to heal within the 1st, 6th, and 12th weeks. The secondary outcomes included the Kaplan–Meier plot of time to heal within the 1st, 6th, and 12th week, and wound size reduction.

c. **Results**:
    i. **Studies Included:** Of 2,477 records identified through database searching, 43 full-text articles were assessed for eligibility after removal of duplicates and title and abstract screening. Subsequently, 32 full-text articles were excluded due to non-randomized trials, ulcers were not related to diabetes, texts not available, trials were actively recruiting, control groups were not SOC, and outcomes were not of interest. Thus, 11 studies in total were included for qualitative and quantitative analysis.
    ii. **Complete Wound Healing**: The pooled results of the included studies showed a significant difference between DHACA plus SOC and the SOC alone, favoring the experimental group after the 6th and 12th weeks of follow-up (RR = 3.78; 95%CI: [2.51, 5.70], P < 0.00001) and (RR = 2.00; 95% CI: [1.67, 2.39], P < 0.00001) respectively. The pooled studies were homogenous in the 6th week while heterogenous in the 12th week (I2 = 0%, P = 0.61) and (I2 = 43%, P = 0.01), respectively.
    iii. **Adverse Events**: The analysis showed a significant difference between DHACA with SOC group and the SOC group favoring the experimental group (RR = 0.82, 95% CI: [0.70, 0.96], P = 0.01). The pooled studies' results were homogeneous (I2 = 29%, P = 0.19).
    iv. **Wound Size Reduction**: The pooled analysis of wound size reduction significantly favored DHACA with SOC over the SOC alone (MD = 1.18; 95% CI: [0.10, 2.26], P = 0.03). The pooled studies were heterogeneous, and the heterogeneity could not be resolved (I2 = 79%, P = 0.003).
    v. **Time to Heal**: The analysis favored the DHACA group over the control group after the 1st week of follow-up (RR = 5.74; 95%CI: [2.04, 16.18], P = 0.0009) as well as after the 6th and 12th weeks (RR = 3.00; 95%CI: [2.26, 3.98], P = 0.00001), (RR = 1.82; 95%CI: [1.46, 2.27], P = 0.00001) respectively. The results were significant in the three durations of follow-up with no inter-heterogeneity among the studies in the 1st, 6th, and 12th weeks (I2 = 0%, P = 0.98), (I2 = 5%, P = 0.39), and (I2 = 17%, P = 0.31) respectively.
    vi. **Kaplan-Meier Plot of Time to Heal**: The pooled effect estimate of the included studies showed no significant difference between the two groups in the 4th week (MD = -3.42; 95%CI: [-8.82, 1.97], P = 0.21), and the 6th week (MD = -2.92; 95% CI: [-6.10, 0.26], P = 0.07). On the other hand, the analysis favored the experimental group in the 12th week of follow-up (MD = -12.07; 95% CI: [-19.23, -4.91], P = 0.001). The results of pooled studies were heterogenous in the analyses of the 4th, 6th, and 12th weeks (I2 = 92%, P < 0.00001), (I2 = 66%, P = 0.01), and (I2 = 71%, P = 0.004) respectively.
d. **Conclusions**: DHACA with SOC has better efficacy than SOC alone in enhancing wound healing, reducing the mean time to wound healing, and diminishing the risk of adverse events.

2. Haugh AM, Witt JG, Hauch A, et al. Amnion Membrane in Diabetic Foot Wounds: A Meta-analysis. Plast Reconstr Surg Glob Open. 2017;5(4):e1302. Published 2017 Apr 25.
    a. **Objective:** The goal of this review was to describe commercially available products compared with standard wound care in randomized controlled trials and synthesize the results of those studies with a meta-analysis. The authors present a meta-analysis of 5 prospective trials that compared amniotic products with standard of care (SOC) in patients with nonhealing DFUs and conducted a cost analysis for their use.
    b. **Materials and Methods**: This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Randomized controlled trials comparing amniotic tissue products with SOC for use in nonhealing DFUs published in peer-reviewed English language journals were included in the review. Electronic searches were performed using PubMed, Cochrane Central Register of Controlled Trials, and the Cochrane Database of Systematic Reviews. The following keywords were used: “dehydrated amnion/chorion membrane,” “amniotic membrane diabetic foot ulcers,” “Grafix,” “Epifix,” and “amniotic membrane wound healing.” The outcome measure was the proportion of patients with healed ulcers after a set time period. All papers included in this analysis defined healing as complete re-epithelization of the wound. Papers included in this meta-analysis had varied endpoints at which they assessed whether wounds had healed completely. The trial endpoint for selected studies was either 6 weeks or 12 weeks. Publication bias was not assessed due to the small number of trials in our meta-analysis. The Cochrane meta-analysis guidelines suggest the use of Egger’s test for publication bias for analyses with more than 10 studies. A meta-analysis of these 5 randomized controlled trials comparing amniotic tissue products to SOC was performed using a random effects model.
    c. **Results**:
        i. **Studies Included**: A search of 3 databases identified 596 potentially relevant articles. Application of selection criteria led to the selection of 5 randomized controlled trials. The 5 randomized controlled trials represented 311 patients. Of these patients, 52 were treated using bioengineered skin substitutes and were excluded, so data from 259 patients treated with either amniotic tissue products or SOC were included in this review.
        ii. **Heterogeneity**: Chi-squared analysis of our 5 studies demonstrated an I2 of 50.5%, indicating moderate heterogeneity between the studies (heterogeneity chi-squared = 8.08 (df = 4, P = 0.089).
        iii. **Wound Healing**: The pooled RR of healing, which was defined as the proportion of patients with complete wound re-epithelization, with amniotic products compared with control was 2.7496 (2.05725–3.66524). A test of RR = 1 demonstrated a P value of <0.001, indicating that the increased RR of healing seen with amniotic products is statistically significant.
        iv. **Cost Analysis**: One study found that patients required an average of 2.5 applications of Epifix to achieve complete healing. The cost of these products has been estimated to be between $500 and $1000, therefore estimating the average cost per patient to be between $1250 and $2500. In their cost analysis, the authors of this study found that the average cost per healed DFU in the amniotic membrane group was $1517. In comparison, the average 1 year per patient medical cost of DFUs is estimated at $28,000 in the literature. This increased financial burden arises from a mixture of

increased emergency room visits, hospital admissions, home healthcare, and outpatient physician and advanced care visits. Patients who fail to heal with traditional SOC are at risk for chronic ulcers or limb loss, with the average cost of major amputations approaching $19,000 per patient per admission.

d. **Conclusion**: The authors of this review conclude that the use of amniotic membrane to improve wound healing with potentially significant cost savings is warranted. Amniotic membrane and amnion itself are valuable products that should be revisited for their regenerative and antibacterial properties, not only for DFUs, but for other high-risk surgical procedures including cardiac surgery, abdominal surgery, and implant-based procedures.

3. Laurent I, Astère M, Wang KR, Cheng QF, Li QF. Efficacy and Time Sensitivity of Amniotic Membrane treatment in Patients with Diabetic Foot Ulcers: A Systematic Review and Meta-analysis. Diabetes Ther. 2017;8(5):967-979.
    a. **Objective**: The objective of this study was to assess the efficacy and time sensitivity of human amnion/chorion membrane treatment in patients with chronic DFUs compared to standard of care (HACM + SOC vs. SOC alone).
    b. **Materials and Methods**: The Cochrane Library, PubMed, Embase and Web of Science databases were systematically searched to identify relevant articles up to 10 April 2017 using the search terms “diabetic foot ulcers” OR “diabetic foot” AND “amniotic membrane” OR “amnion” OR “bioimplant dressing” OR “Grafix” OR “EpiFix” AND “standard therapy “or “standard of care.” The eligibility criteria for an included study were: 1) prospective randomized control trials (RCTs); 2) studies comparing human amniotic membrane + standard therapy versus standard therapy in patients with DFUs; 3) studies in which an assessment of wound healing rates was conducted at least within a period of no less than 4 weeks and no more than 12 weeks; 4) studies published in English; 5) studies with complete outcome. Studies were excluded when they did not fulfill these inclusion criteria. Thus, all randomized controlled trials (RCTs) comparing human amnion/chorion membrane + standard therapy and standard therapy alone in patients with DFUs were included in the analysis. Eligible studies were reviewed, and data extracted into standard form. The Cochrane Collaboration's tool for assessing the risk of bias was used. Data were analyzed using a random effect model.
    c. **Results**:
        i. **Studies Identified**: The initial search of the four databases identified 352 published studies. Of these, 305 studies were deemed not relevant using the title and abstract. Of these remaining 47 studies, seven RCTS were ultimately included in the meta-analysis due to concerns about overlapping papers.
        ii. **Incomplete Healing**: There were far fewer unhealed wounds in patients receiving amniotic membrane + SOC treatment than in those receiving SOC alone. The overall effect in the group assessed at 4 weeks was $Z = 4.14$ ($P < 0.0001$; OR 0.05; 95% CI 0.01–0.21). The overall effect in the group assessed at 6 weeks was $Z = 4.28$ ($P < 0.0001$; OR 0.07; 95% CI 0.02–0.23). The overall effect in the group assessed at 12 weeks was $Z = 4.96$ ($P < 0.00001$; OR 0.10; 95% CI 0.04–0.24. The heterogeneity in each subgroup was not statistically significant. In the subgroup assessed at 4 weeks, $I 2 = 0\%$ ($P = 0.32$); in the subgroup assessed at 6 weeks, $I 2 = 47\%$ ($P = 0.11$); in the subgroup assessed at 12 weeks, $I 2 = 24\%$ ($P = 0.27$). In the test for subgroup differences, the heterogeneity was also not statistically significant. For the subgroup differences, $I 2 = 0\%$ ($P = 0.76$).

d. **Conclusion**: The results showed that patients receiving amniotic membrane with standard therapy had far fewer incomplete healing wounds than those receiving standard of care alone. Assessment of the wound healing state at 4 and 6 weeks revealed that the wound healing state was almost the same, but there was a net difference of wound healing state at 12 weeks. Human amnion/chorion membrane + standard of care treatment heals DFUs significantly faster than standard of care alone. When using the amnion in patients with DFUs, the optimal times to assess progress in wound healing should be 4 and 12 weeks.

4. Paggiaro AO, Menezes AG, Ferrassi AD, De Carvalho VF, Gemperli R. Biological effects of amniotic membrane on diabetic foot wounds: a systematic review [published correction appears in J Wound Care. 2020 May 2;29(5):306]. J Wound Care. 2018;27(Sup2):S19-S25.
    a. **Objective**: The amniotic membrane has biological properties that are beneficial to the wound healing process of diabetic foot ulcers (DFU). Our aim is to analyse the scientific evidence found in literature on the use of the amniotic membrane to stimulate DFU healing.
    b. **Materials and Methods**: A systematic review of amniotic membrane's influence was undertaken following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist for control. Using the search terms ‘placenta’ ‘diabetic foot’ ‘amnion’ and biological dressing’ and assessing the outcomes ‘wound healing’ and ‘wound healing time’, in DFU, the systematic review and meta-analysis was performed. The authors used the following inclusion criteria for studies: randomized controlled trial studies (RCT) that used amniotic membrane dressings in the treatment of DFUs and evaluated the wound healing outcome; articles published in full on national and international journals; indexed on the databases Lilacs, BVS, and PubMed published in Portuguese and English, between 2007 and 2017. The choice to search from 2007 onwards was to include as many articles as possible. The exclusion criteria were: articles, editorials and letters published in the form of a summary, unpublished studies, case reports, case series, transversal studies, observational and experimental (on animals). Following the inclusion and exclusion criteria, randomized controlled trials (RCT) were identified, and the risk of bias was analyzed according to the Cochrane risk of bias tool. The authors conducted a meta-analysis of two outcomes to evaluate the level of evidence: 1) wound healing proportion (number of patients whose wounds healed, or not, in the control and experimental groups using relative risk), and 2) difference on the average wound healing time (difference in average estimates of the control and experimental group solely based on the cases which healed considering the time, in days, for the outcome to happen).
    c. **Results**:
        i. **Included Studies**: After the search, 122 studies were identified with only 71 remaining after removal of duplicates. Of the 71 remaining, 64 studies did not meet inclusion criteria and one additional study was excluded because it used the same data from a more recent study leading to 6 studies finally included in the meta-analysis, consisting of 331 patients
        ii. **Wound Healing**: The experimental group shows a risk relative of wound healing around 2.32 [95% confidence interval (95% CI):1.78–3.02] times higher than the conventional dressings group.
        iii. **Average Wound Healing Time**: The meta-analysis showed that the average healing time for the experimental group was 32.4 days less than the conventional dressings group [CI: -41.05 to -23.71].

d. **Conclusion**: There is statistical evidence to support the effectiveness of amniotic membrane in comparison with other conventional dressings. In addition, there is a clear tendency for the use of amniotic membrane treatment to result in a larger number of DFUs healing at a quicker rate.

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