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Case Report

Application of Dehydrated Amniotic Membrane Allografts in Advanced Diabetic Foot Ulceration: Case Report and Review of Literature

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Abstract: Management of diabetic foot ulcers (DFUs) presents challenges to even the most experienced wound care providers. Because of the chronic, non-healing nature of many DFUs, advances in the treatment and care of this disease process are particularly relevant. This case study aims to report the efficacy of the application of dehydrated amniotic membrane allograft (DAMA) to a diabetic foot ulcer. The patient in this study is a 44-year-old male who presented with an aggressive infection on his right foot, which resulted in an open wound of 18 months duration. This patient received weekly applications of dual-layer DAMA over seven weeks. Upon examination at the initial application, the wound was classified as a Wagner grade 3 with necrosis of the underlying muscle. Upon inspection at the final visit, the wound was closed entirely. The results that were shown include improvements in the size, depth, edges, necrotic tissue amount, and epithelization of the wound. This case study demonstrates that the ap-plication of DAMA has the potential to augment the body's natural DFU healing response; however, future nonrandomized and randomized controlled trials are needed to establish its efficacy further.

Keywords: Diabetic Foot Ulcer; Amniotic Membrane Allograft

1. Introduction

Diabetic foot ulcers are foot lesions that may affect the foot's skin, soft tissue, and bones. These lesions are caused by multifactorial etiologies as part of the microvascular complications of diabetes. As a result, an aggravated infection can occur in diabetic patients. Despite treatments, many DFUs remain active and unsuppressed. With approximately 37.3 million diabetic adults in the United States, physicians are faced with an epidemic of their own [1]. Given the projected increase of 1.4 million new diagnoses of diabetes mellitus each year, advancing knowledge and care for the disease and its related conditions is especially relevant [2].

In addition, many DFUs have a high recurrence rate, with 65% of the ulcers returning within five years [3]. The inability to heal DFUs presents a severe danger to patients as anywhere from 5-24% of untreated DFUs can lead to limb amputation within 6-18 months [4]. These infections can lead to long-term impairment and possible lower-limb amputation without timely and correct management.

With a median healing time of 12 weeks, complications of DFUs often result in amputation. The Centers for Disease Control Prevention (CDC) reported that in 2018 alone, 154,000 diabetic patients were discharged from hospitals with a recent lower extremity amputation [5]. If the diabetic-related wounds were addressed promptly and efficiently, these amputations might have been preventable. The path to reducing limb amputation, in addition to the healthcare costs associated with amputation, must originate from early and aggressive treatment of diabetic foot ulcerations. Since diabetic wounds heal slowly by nature, any process that promotes a quicker healing time ultimately improves a

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patient's long-term quality of life. Despite typical treatment in a multi-disciplinary wound clinic, many patients with diabetic foot ulcers suffer from further wound complications or the severity of invasive amputations. The current standard of care for DFUs is debridement, followed by a moist dressing covering, wound off-loading, and a vascular assessment [6]. Understanding that necrotic tissue complicates and prolongs the healing process, debridement of necrotic tissue is imperative to the initial treatment of DFUs. While these interventions are currently classified as the standard of care, one must consider the limitations of each of these regimens. The increased healing time and augmented risk of amputation associated with the standard of care only solidifies the need for new DFU treatment alternatives. Regenerative medicine is such an alternative.

The use of placental tissues in regenerative medicine has increased exponentially since the first use of amnion as a skin graft in 1910 [7]. Human amniotic membrane is a collagen-based extracellular matrix derived from the maternal placenta and is composed of two layers, the chorion and the amnion. The amnion, the innermost placental layer, protects against fluid loss and mechanical injury to the developing embryo. Consequently, the use of DAMA has excellent potential to decrease both the healing time and complications associated with treating diabetic wounds.

Despite the prevalence of diabetes-related ulcerations within the diabetic community and the severity of consequences they cause, DFUs and their management and treatment are a lesser researched topic than other disease processes. Further education and research on improving the treatment for DFUs would indefinitely benefit the diabetic community.

2. Case Presentation Section

2.1 Dehydrated Human Amniotic Membrane Allografts

Following the standards established by the U.S. Food and Drug Administration (FDA) and the American Association of Tissue Banks (AATB), human amnions were obtained from consenting C-section donors. An independent certified laboratory tested all the donations for infectious disease in accordance with Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 CFR part 493, and FDA regulations. In ad-dition, each birth mother was tested for Hepatitis B Core Antibody (HBcAb), Hepatitis B Surface Antigen (HbsAg), Hepatitis C Antibody (HCV), Human Immunodeficiency Virus Antibody (HIV1/HIV-2 Plus O), Human T-Lymphotropic Virus Antibody (HLTV-I/11), Syphilis (RPR), HIV1/HCV/HBV, NAT, and West Nile Virus (WNV). Each test was performed with an FDA-Approved testing kit (see Appendix A). All test results were negative or non-reactive. The donated amnion was aseptically processed and did not involve the addition of cells, tissues, or articles other than the exceptions outlined in 21 CFR Part 1271.10(a)(3). The amnion was placed on a sterile drying tray and desiccated in a high nitrogen concentration drying chamber in a temperature-controlled area (10C-25C), with the air compressor set to 60-145 PSI. The patches are desiccated until the relative humidity reading is steady for one hour. The sterility of the final processed product is tested at an independent laboratory, Eurofins VRL Laboratories. In addition, the desiccated allografts are sterilized via electron beam irradiation before distribution.

2.2 Patient History

A 44-year-old male presents with a medical history of end-stage renal disease and Type 2 diabetes mellitus. The patient developed an aggressive infection of the right foot and underwent a trans-metatarsal amputation in 2019. The patient reported the occurrence of a large blister on the stump of his foot after the surgery. Due to the lack of health insurance, his wound remained open for 18 months. The patient reported numbness, tingling, and shooting sensations in the right foot. Severe pain was also noted when walking and removing shoe gear. The wound remained open despite the previous

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standard of care attempts, including using an off-loading pad in the shoe, dry sterile dressings, and sharp wound debridement with calcium alginate and betadine.

The patient received the first DAMA patch on January 25th, 2022. Upon initial examination, the DFU measurement was 5.5cm x 4.5 cm x 0.3cm and classified as Wagner grade 3 with necrosis of the underlying muscle. Over seven weeks, the patient received weekly applications of dual-layer human amniotic membrane allografts. The wound was noted as completely closed at the patient's final appointment. The duration of the wound closure was 49 days from the initial allograft application. No adverse events or severe adverse effects from the amniotic membrane allograft application were reported.

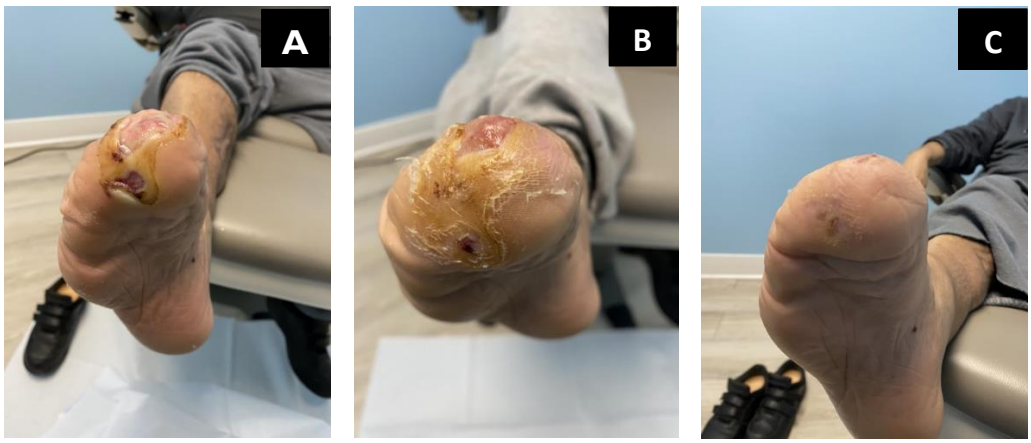

Figure 1. Progression of the Diabetic Foot Ulcer throughout Amniotic Membrane Allograft Applications. (A) Date of Exam: 02/01/2022; DFU measurements: 5.3cm x 4.3cm x 0.1cm. (B) Date of Exam: 02/22/2022; DFU measurements: 4.2cm x 3.5cm x 0.1cm. (C) Date of Exam: 03/15/2022; DFU measurements: DFU is now closed.

Date of Examination	DFU measurements
01/25/2022	5.5cm x 4.5cm x 0.3cm
02/01/2022	5.3cm x 4.3cm x 0.1cm
02/08/2022	5.1cm x 4.4cm x 0.1cm
02/15/2022	5.0cm x 3.9cm x 0.1cm
02/22/2022	4.2cm x 3.5cm x 0.1cm
03/01/2022	3.1cm x 1.8cm x 0.1cm
03/08/2022	0.9cm x 0.8cm x superficial
03/15/2022	0.0cm x 0.0cm x superficial

Table 1. Measurements of the DFU at each time point.

3. Discussion

As observed in the present case study, treating chronic DFUs with dehydrated amniotic membrane allografts is an effective modality to afford a barrier layer over wounds and augment the body's natural healing process. This is evident through the continual decrease in size over the seven amniotic membrane allograft applications. Each allograft was applied based on medical necessity. Weekly allograft applications were terminated upon wound closure, as evident in Figure 1. The healing process of the DFU can be observed qualitatively in Figure 1 and quantitatively in Table 1.

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The use of human amniotic membrane allografts as an effective method in DFU treatment is again supported by a 2016 randomized controlled trial (RCT) comparing the treatment standard of care to the utilization of dual-layer amniotic membrane allografts [8]. In this RCT, allograft reapplication occurred as often as once per week based upon the physician's judgment, similar to our single patient report. This RCT resulted in 45.5% of patients who received DFU standard of care (adhesive dressing and compression) and the amniotic membrane allograft achieved full closure of the DFU. In contrast, 0% of the patients who received only the standard of care procedure had complete wound closure. This RCT, along with our single patient report, provides a foundation for future research in treating DFUs with DAMA.

While amniotic membrane allografts have proven to augment the body's ability to regenerate the structural tissue defects associated with DFUs, they are also comparable in cost to the standard of care, which averages about $17,245 [9]. Not only is the standard of care for DFUs less effective, but it is also high in cost and typically relies on inpatient expenditures. Medicare recognizes the medical necessity of amniotic membrane allografts in the treatment of both DFUs and venous stasis ulcers. Consequently, many patients can rely on Medicare to assist with the costs associated with DFU treatment. This presents the opportunity for human amniotic membrane allografts to be utilized in rural and underserved communities where DFU treatment is typically delayed due to high costs and lack of supplies. This could exponentially decrease the risk of amputations in diabetic patients in these rural and underserved communities. In the future, we plan to conduct a prospective study examining the efficacy of our products in underserved communities.

4. Results

Individual elements of the wound were examined during all seven allograft applications. These elements included measurement of the wound size, depth, edges, necrotic tissue amount, and epithelization. All five elements decreased from the initial allograft application to the final wound closure (Figure 2 and Figure 3). Upon initial examination in January, the wound measured 5.5 cm x 4.5 cm x 0.3 cm (Table 1), with full-thickness skin loss involving damage or necrosis of subcutaneous tissue. The edges of the wound were distinct and attached with a clearly visible outline. Necrotic tissue covered less than 25% of the wound bed. At the final examination in March, the wound was noted as being superficial, measuring 0.0 cm x 0.0 cm x 0.0 cm (Table 1), with intact skin and non-blanchable erythema. No edges or necrotic tissue were present. While improvements were seen in all five categories, perhaps the most notable of these elements was epithelialization. The DFU was classified as a 5 upon initial examination in January, correlating to less than 25% of the wound being covered. Over the 49 days, epithelization of the wound increased from less than 25% to 100% wound coverage by the final patient visit (Table 2). This large-scale epithelization of the DFU provides the protective barrier essential to eliminating pathogens during the healing process. The patient experienced complete wound epithelization within seven weeks of weekly allograft applications. This is compared to the 18 months before application when the patient did not have any epithelization advancements. Evidently, the application of dehydrated amniotic membrane allografts was essential in augmenting the healing process of the DFU.

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	Application #1	Application #2	Application #3	Application #4	Application #5	Application #6	Application #7	Final Visit
Size	3	3	3	2	2	2	1	1
Depth	3	3	3	3	3	3	3	1
Edges	2	2	2	2	2	2	2	1
Necrotic Tissue Amount	2	2	1	1	1	1	1	1
Epithelization	5	5	5	4	4	4	4	1

Table 2. Progression of Size, Depth, Edges, Necrotic Tissue Amount, and Epithelization in DFUs over 49 days.

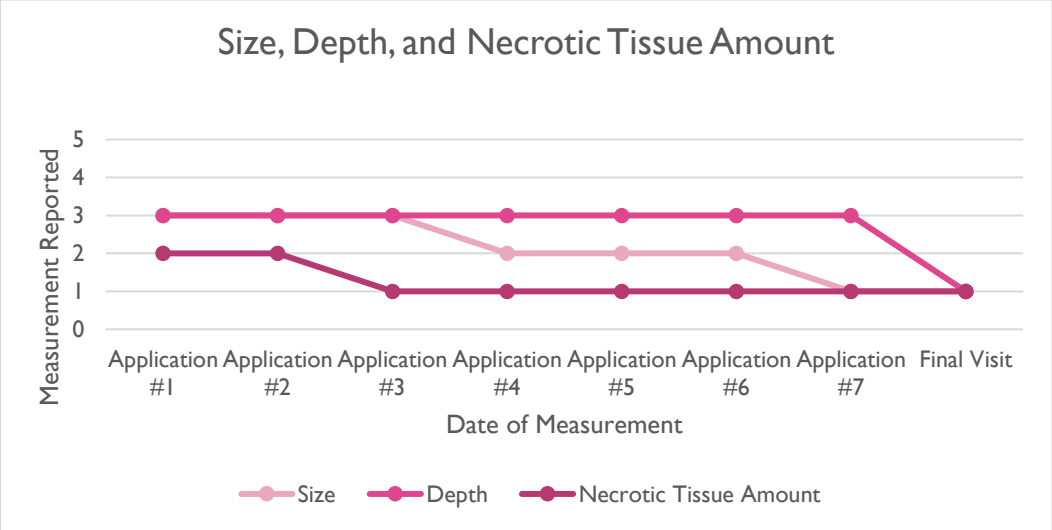

Figure 2. Progression of Size, Depth, and Necrotic Tissue Amount in DFU over 49 days. Reference Appendix B for the key correlated to each value.

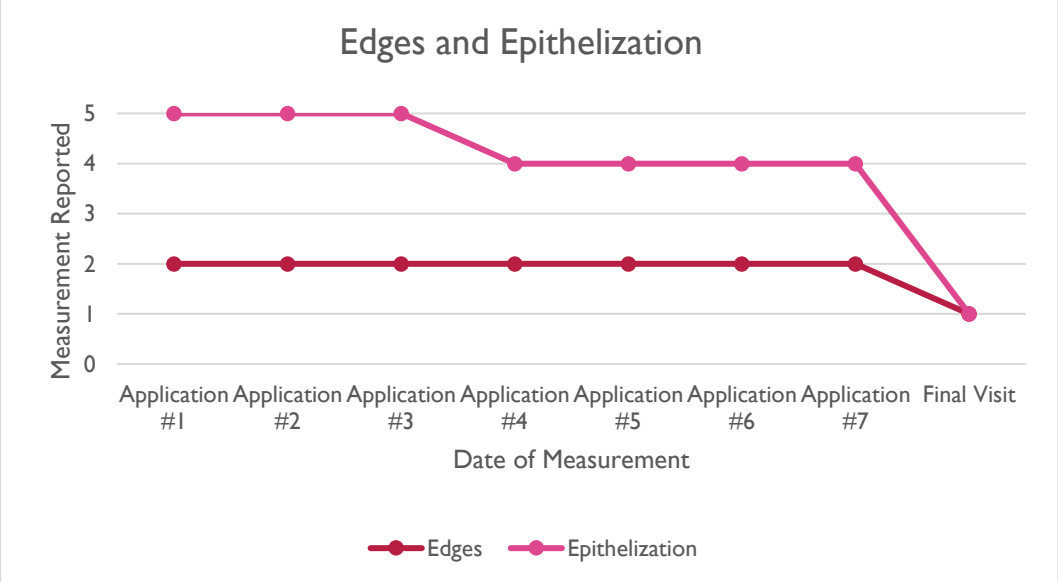

Figure 3. Progression of Edges and Epithelization in DFU over 49 days. Reference Appendix B for the key correlated to each value.

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Our results are in accordance with other published studies demonstrating the potential of DAMA for treating DFU. Over the course of seven applications, our patient's wound decreased drastically in each category of measurement. In comparison, a study displayed that the use of DAMA worked to help the body promote tissue reconstruction, which resulted in wound closure. The duration of each participant's wound before joining the study spanned 24-84 months. Of the 14 individuals in the study who had an amniotic patch placed on their wound, all 14 patients experienced wound closure within 14-60 days [7].

Additionally, a retrospective case series examined the effects of DAMA on DFUs of eight patients. Each patient experienced wound closure in a mean time of 9.2 weeks after the initial DAMA application. Of the study group, four out of eight patients received three applications, and each encountered wound closure in a mean time of 8.3 weeks [10].

Furthermore, in 2019, Joseph Caporusso demonstrated that the use of DAMA leads to successful wound closure in patients with diabetic foot ulcers greater than one year in duration, with the previous failure of prior conservative treatment [11]. The utilization of an amniotic membrane allograft in chronic DFUs resulted in the complete closure of the wound for patients with a median healing time of five weeks [11].

Each of these studies shows that DAMA has undeniable benefits relating to wound closure. The results gathered from each outside study are in accordance with the results analyzed in this case report. However, the patient in our single-patient study experienced a quicker wound closure than several participants in the previously mentioned studies.

5. Conclusions

The utilization of amniotic membrane allografts in treating chronic DFUs produces favorable results and is consistent with current medical literature. In the presented case study, the use of amniotic membrane allografts augmented the DFU healing process. The patient failed the standard of care methods for 18 months and achieved full closure within seven weeks of allograft application. Future applications for early, preventative use of amniotic membrane allografts in addition to the current standard of care for DFUs present a novel opportunity to reduce long-term morbidity and amputation risk in diabetic patients.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data can be found in Appendix A and Appendix B.

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Conflicts of Interest: All authors are associated with Regenative Labs. Regenative Labs was involved in the design of the study, data analysis, and writing. An independent physician performed treatment and data collection at Keir Foot and Ankle Clinic. Regenative Labs influenced the decision to publish.

Appendix A

Test Kits

1. HBcAb: Catalog number: 06P06, Abbott Laboratories, Abbott Park, IL, USA
2. HbsAg: Catalog number: 06P02, Abbott Laboratories, Abbott Park, IL, USA
3. HCV: Catalog number: 06P04, Abbott Laboratories, Abbott Park, IL, USA
4. HIV1, HIV2, plus O: Catalog number: 06P01, Abbott Laboratories, Abbott Park, IL, USA
5. HTLV-I/II: Catalog number: 06P07, Abbott Laboratories, Abbott Park, IL, USA
6. RPR: Catalog number: 900025, Arlington Scientific, Springville, UT, USA

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7. HIV1, HCV, HBV, NAT: Catalog number: 303330, 303331, 303719, 303334, 303344
8. WNV: Catalog number: 07001061190, Roche Diagnostics, Indianapolis, IN, USA

Appendix B

Wound Size

1. Length x width less than 4 square cm cm2
2. Length x width between 4 and 16 square cm
3. Length x width between 16.1 and 36 square cm
4. Length x width between 36.1 and 80 square cm
5. Length x width greater than 80 square cm

Depth

1. Non-blanchable erythema on intact skin.
2. Partial-thickness skin loss involving epidermis and/or dermis
3. Full-thickness skin loss involving damage or necrosis of subcutaneous tissue; may extend down to but not through underlying fascia; and/or mixed partial and full-thickness and/or tissue layers obscured by granulation tissue.
4. Obscured by necrosis
5. Full-thickness skin loss with extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures.

Edges

1. Instinct, diffuse, none clearly visible
2. Distinct, outline clearly visible, attached, even with wound base
3. Well-defined, not attached to wound base
4. Well-defined, not attached to wound base, rolled under, thickened
5. Well-defined, fibrotic, scarred, or hyperkeratotic

Necrotic Tissue Amount

1. None visible
2. < 25% of wound bed covered
3. 25% to 50% of wound covered
4. >50% and <75% of wound covered
5. 75% to 100% of wound covered

Epithelialization

1. 100% wound covered, surface intact
2. 75% to < 100% wound covered &/or epithelial tissue extends >0.5cm into wound bed
3. 50% to <75% wound covered &/or epithelial tissue extends to < 0.5cm into wound bed
4. 25% to < 50% wound covered
5. < 25% wound covered.

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