Use of a Dehydrated Amniotic Membrane Allograft on Lower Extremity Ulcers in Patients with Challenging Wounds: A Retrospective Case Series

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Abstract
Lower extremity ulcers in patients with diabetes mellitus may take a long time to heal despite the use of advanced topical therapies. A retrospective review of cases was conducted to assess the use of a dehydrated amniotic membrane allograft (DAMA) in a convenience sample of 9 wounds in 8 patients (5 men, 3 women, average age 62 years [range 31–81 years]) with diabetes mellitus and/or vascular disease. Wound data and patient characteristics were abstracted from medical records. Descriptive statistics were used to summarize the data. In 5 of 9 wounds, DAMA was applied after a failure to demonstrate a 50% reduction in area after 4 weeks of treatment with advanced wound care, offloading, and compression as indicated. In 4 wounds, DAMA was applied 2–4 weeks after presentation because of concerns about existing patient risk factors for nonhealing. Wounds were present for an average of 11 weeks (range 1–35 weeks) before application of DAMA. Mean baseline wound area and volume were 3.11 cm² (± 3.73) and 0.55 cm³ (± 0.58), respectively. All wounds healed in an average of 5.7 (± 2.9) weeks (range: 1–9 weeks) after a mean of 2.7 applications (± 1.7) (range 1–5 applications). No adverse events occurred. These observations suggest prospective, randomized, controlled clinical studies to compare the use of DAMA to other topical treatment modalities are warranted.

Keywords: case reports, wound healing, cell- and tissue-based therapy, foot ulcer, venous ulcer

Index: Ostomy Wound Management 2015;61(10):30–36

Potential Conflicts of Interest: Derma Sciences, Inc (Princeton, NJ) provided funding for editorial support services.
With respect to time to healing, a retrospective analysis of data from 2 randomized controlled trials (N = 120) noted a 50% reduction in wound area at 4 weeks as a critical cut-off point for evaluating DFU treatment success. Thus, with a primary goal of rapid wound closure to reduce the risk of complications and improve outcomes, based on the healing rates previously stated and further post-hoc and economic analyses of clinical trial data, the use of advanced wound care modalities such as CTPs is recommended if wound area is not reduced by 50% after 4 weeks of standard care with moist wound healing and offloading.1,11,12

Amniotic tissue has been used to treat a variety of wounds due to the many characteristics that make it suitable for use in tissue engineering. In vitro studies show amniotic tissue contains growth factors and biological macromolecules and possesses nonimmunogenic13 and anti-inflammatory14 properties. Pregnancy often is referred to as an immunological paradox based on the “immune privilege” of the placental organ due to the low risk of immune rejection.15 A review16 of the properties of amniotic membrane notes placental membrane cells do not express MHC Class II antigens, which are responsible for immunologic rejection of allografts in humans. In addition to the absence of MHC Class II molecules, recent in vitro scientific research16 suggests placental membranes secrete compounds that may actively mask the placenta and the fetus from immunological detection by the maternal cells. AMNIOEXCEL® (registered trademark of BioD, LLC made available by Derma Sciences Inc, Princeton, NJ) is a dehydrated amniotic membrane allograft (DAMA). DAMA is derived from the innermost layer of the placental sac that surrounds the fetus in the womb. DAMA is processed by BioD, LLC via a proprietary DryFlex® technique to make it easy to handle and conform to the wound bed immediately upon application without the need of prior hydration.17 In vivo and in vitro studies show DAMA supports wound healing.17-20 Its mechanism of action is based on the ability of amniotic membrane to provide a natural matrix for cellular attachment and assist in cell migration and proliferation.20 In vitro studies7,20,21 demonstrate reconstruction of the wound is mediated through regenerative cytokines, including epithelial growth factor, transforming growth factor-beta, fibroblast growth factor, and platelet-derived growth factors alpha and beta, which stimulate protein and collagen synthesis, collagenase activity, and chemotaxis of fibroblasts and smooth muscle cells. Because amniotic tissue contains growth factors and biological macromolecules in addition to its nonimmunogenic,13 anti-inflammatory,14 and antibacterial12 properties, it may play a role during the inflammatory, proliferation, and maturation phases of wound healing.21

The objective of this retrospective case series was to assess the clinical experience of using DAMA in a variety of wounds using a convenience sample of 9 challenging wounds in patients with comorbidities that impact healing (eg, diabetes mellitus, vascular disease) and/or have ceased to heal using other advanced wound care modalities.

Methods

Data from patients with lower extremity wounds treated between November 2013 and August 2014 at Wayne Memorial Wound Healing and Hyperbaric Center were collected retrospectively based on a convenience sample and as such followed no specific inclusion or exclusion criteria. All patients included in this series provided informed consent for the use of their information for educational and research purposes. IRB approval was obtained from the Wayne Memorial Hospital Institutional Review Board.

Standard care. The Center follows wound care guidelines/algorithms that are proprietary to Healologics Inc (Jacksonville, FL). The guidelines and algorithm are based on Sheehan et al’s13 findings from a prospective randomized controlled trial involving patients with DFUs (N = 203). The study found wounds that failed to demonstrate a 50% wound reduction after 4 weeks of treatment are unlikely to heal. Wound care is directed by a physician and follows the basic tenets of proper chronic wound care, including nutritional support, debridement, bioburden control, and the use of dressings that support moist wound healing. Specific situations will determine if multicomponent dressings are selected for the management of bioburden, infection, or pain in addition to the use of offloading and compression therapy as appropriate. In general, treatment choices were based on the wound and patient characteristics combined with the physician’s discretion.

Wounds referred to the center are usually challenging (eg, long duration, limited response to previous treatments, and patient comorbidities such as diabetes mellitus, vascular disease, atypical dermatologic conditions); thus, first-line treatment includes advanced dressings such as silver dressings, negative pressure wound therapy (NPWT), and protease-modulating dressings. For wounds that do not demonstrate a 50% reduction after 4 weeks of treatment, the next treatment option is CTPs.
Use of DAMA. DAMA was a newly available CTP at the Center. It was used in the first 5 patients whose wounds failed to demonstrate a 50% reduction in area after 4 weeks of treatment with advanced wound care, offloading, and other care. Following this initial experience with DAMA, it was applied 2–4 weeks after a wound presented in 4 additional patients whose risk factors (eg, the presence of comorbidities, vascular status, and overall health status) deemed quick wound closure a priority to assess where this new treatment option best fit in the treatment algorithm.

Procedure. Before DAMA application, the wound received sharp debridement to ensure it was free of necrotic tissue, detritus, and nonviable tissue and to assess for clinical signs and symptoms of infection (eg, heat, pain, redness and swelling, delayed healing, friable granulation tissue, and so on). 1

DAMA is stored at room temperature and is ready to use directly out of the package. The allograft membrane was carefully removed from the sterile package and trimmed to size, if necessary, to overlap the wound margins by approximately 1 mm. Once the membrane was placed in the wound, it self-adhered. A moistened cotton swab was used to remove any air bubbles. In the case of dry wounds, extra moisture (saline) was added to the wound bed by a single dose vial using a quantity sufficient to hydrate the entire graft (after the graph was secure) to help achieve optimal moisture balance. A nonadherent dressing was placed over the membrane (eg, a silicone contact layer) followed by a secondary dressing to absorb exudate (eg, foam dressing).

DAMA was applied every 2 weeks. One week following application, the wounds were assessed for signs of infection and the secondary dressings were changed. At each dressing change, wounds were measured with a ruler (length, width, and depth), photographed, and assessed per wound appearance (eg, percent of granulation tissue, visual inspection for

Table 1. Baseline patient and wound information

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Comorbidities</th>
<th>Wound duration (months)</th>
<th>Location</th>
<th>Start of dehydrated amniotic membrane allograft (DAMA)</th>
<th>Data available on past treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Area (cm²)</td>
<td>Volume (cm³)</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>60</td>
<td>Previous bilateral heal surgeries and post-operative infection, diabetes, ESRD, HTN</td>
<td>2</td>
<td>Calcaneous</td>
<td>11</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>32</td>
<td>Diabetes</td>
<td>1.5</td>
<td>Metatarsal fifth head</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>64</td>
<td>Diabetes, HTN</td>
<td>11</td>
<td>Malleolus</td>
<td>1.65</td>
<td>0.33</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>74</td>
<td>Sarcoidosis, PVD, phlebitis, DVT, left foot drop, psoriasis</td>
<td>4</td>
<td>Lower leg</td>
<td>1.57</td>
<td>0.16</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>47</td>
<td>Necrobiosis lipoidica, diabetes, venous stasis, HTN</td>
<td>3.5</td>
<td>Lower leg</td>
<td>1.73</td>
<td>0.52</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>78</td>
<td>Diabetes, CAD, cardiac surgeries (stents and pacemaker), HTN, dyslipidemia</td>
<td>4</td>
<td>Great toe</td>
<td>0.66</td>
<td>0.2</td>
</tr>
<tr>
<td>7A</td>
<td>Female</td>
<td>81</td>
<td>Kidney disease, HTN, dyslipidemia, GERD, CAD</td>
<td>2</td>
<td>Lower leg (Right distal anterior)</td>
<td>1.04</td>
<td>0.21</td>
</tr>
<tr>
<td>7B</td>
<td>Female</td>
<td>81</td>
<td>Kidney disease, HTN, dyslipidemia, GERD, CAD</td>
<td>2</td>
<td>Lower leg (Right proximal anterior)</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>60</td>
<td>Pulmonary embolism, Raynaud’s Syndrome, osteoarthritis</td>
<td>5.5</td>
<td>Abdomen (post-surgical)</td>
<td>8.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

CAD=coronary artery disease; DVT=deep vein thrombosis; ESRD=end-stage renal disease; GERD=gastroesophageal reflux disease; HTN=hypertension; PVD=peripheral vascular disease
Lower Extremity Ulcers Treated Using Amniotic Allograft

Signs of infection mentioned previously). All treatment plans were documented in the patient’s electronic medical file. Wound management included compression or offloading as needed based on best practice and physician discretion. All patient data were entered into i-heal™ Electronic Health Records Program 2.0 (Healogics Inc, Jacksonville, FL), which calculates area and volume.

Data Collection and Analysis. Demographics and patient and wound characteristics, including measurements, were manually abstracted from the patients’ medical records. Wound closure was defined as a wound that was reepithelialized, did not have any wound drainage, and did not require a dressing. Using Microsoft Excel, wound area and volume changes were calculated using descriptive statistics. Percent area reduction (PAR) and volume reduction at each time point were calculated using the difference from baseline divided by baseline measurement and multiplied by 100.

Results
Eight (8) patients (average age 62 years, range 31–81 years; 5 men; 3 women), with 9 wounds were included in the case series. The patients had a variety of comorbidities including diabetes mellitus, vascular disease, coronary artery disease, kidney disease, hypertension, and dyslipidemia. The patients’ wounds were present for an average of 11 weeks (range 1–35 weeks) before application of DAMA, and mean baseline wound area and volume were 3.11 cm² (± 3.73) and 0.55 cm³ (± 0.58), respectively (see Table 1).

Area and Volume Reduction. In all cases, wounds showed marked improvement, including an increase in the appearance of red, healthy granulation tissue and a decrease in wound size within 2 weeks of the first application of DAMA. By 4 weeks (1 or 2 applications), mean wound volume decreased from 0.48 cm³ (± 0.60) to 0.14 cm³ (± 0.23), an 85.6% (± 13.72%) volume reduction. Similarly, mean wound area decreased from 3.19 cm² (± 3.93) to 1.38 cm² (± 2.32) in 4 weeks, a 71.0% (± 6.29%) mean PAR (see Figure 1).

Time to Wound Closure. The wounds closed in an average of 5.7 (± 2.9) weeks (range: 1–9 weeks) after a mean of 2.7 applications (± 1.7) (range 1–5 applications) (see Table 2). All wounds were closed within 9 weeks.

Subset Analysis: Pretreatment Comparison. Wound measurement data were available for the weeks before DAMA application for 5 of the 9 wounds (cases 3, 4, 5, 6, and 8), allowing for comparison of the PAR achieved before and after the application of DAMA in this subset of patients. In these 5 wounds, the mean area reduced from to 5.06 cm² (± 5.45) to 2.88 cm² (± 3.21), representing a mean PAR of 43.1% (± 29.2) for the 6 weeks before DAMA application, compared to a mean decrease from 2.88 cm² (± 3.21) to 0.50 cm² (± 2.31) after initiating DAMA, representing an 82.6% (± 11.5%) PAR for the 6 weeks following DAMA application. The lack of closure before DAMA, despite the use of a variety of advanced wound care modalities including dressings, NPWT, and advanced skin and tissue substitutes, was noted. In many cases, patients had tried several advanced strategies with little or suboptimal response.

The following 2 cases are described in detail to provide representative examples of the cases included in this series.

Case Reports
Case 1 (Patient 1). In January 2014, 60-year-old Mr. B, (not his actual initial) who had a history of type 2 diabetes and end-stage renal disease, presented with infected bilateral heel wounds. They were surgically debrided and treated with local wound care and IV antibiotics. He was discharged from the hospital on February 27, 2014 with NPWT for postsurgical wound management and was to remain non-weight-bearing. On March 4, 2014, he presented to the wound care clinic for his first postsurgical assessment where 2 additional wounds similar to the original were

Table 2. Patient wound outcome information

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time to closure (weeks)</th>
<th>Number of dehydrated amniotic membrane allograft (DAMA) applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
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<td>3</td>
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<td>5</td>
</tr>
<tr>
<td>7A</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>7B</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

Mean Reduction in Wound Size (n=9)

Figure 1. Mean reduction in wound volume and area for all wounds between 2 and 6 weeks following first application of DAMA.
noted. His clinicians decided this was a good opportunity to investigate how DAMA compares to standard wound care (NPWT); the first DAMA was applied to one of the wounds (left heel) 1 week later. At that time, the wound measured 3.5 cm x 4 cm x 0.1 cm and was granular and clean (see Figure 2a). Within 2 weeks, 40% PAR was noted (see Figure 2b) and the wound continued to progress to closure at 8 weeks (see Figure 2c). By contrast, the wound treated with NPWT (right heel) with similar wound protocol as previously described decreased very slowly to its smallest size of 3.0 cm x 3.8 cm x 10.1 cm 3 weeks after initiating NPWT then increased again and plateaued at a size of 4 cm x 5.3 cm x 0.2 cm at weeks 16–20, at which point NPWT was stopped due to a lack of progress. Meanwhile, as of September 2014, the wound closed using DAMA remained closed. However, this comparison should be interpreted with caution because the wound treated with NPWT was larger at baseline (15.9 cm² versus 11.0 cm²).

Case 2 (Patient 3). Mr. D (not his actual initial), a 64-year-old patient with well-controlled diabetes and a history of chronic venous and arterial disease and recurrent ulcerations over the past 5 years, presented with a full-thickness venous leg ulcer on his right medial malleolus. The wound had developed 5 months prior and had been treated at another facility with topical antimicrobials and compression stockings. At presentation, the wound bed was covered with 100% slough and bioburden as determined by clinical and visual inspection and measured 3.8 cm x 1.5 cm x 0.2 cm. Following surgical debridement, the wound bed remained fibrotic, with slight periwound erythema and tenderness to palpation. Wound treatments included high-grade multilayer compression wraps and a bilayered bioengineered skin substitute, which was used 5 times over 14 weeks without a great deal of impact on wound healing. After a lack of marked progression following 2 months of advanced treatments, DAMA was applied at week 35 (see Figure 3a) and compression therapy was continued. DAMA was applied every 2 weeks with a total of 3 applications. At 4.5 weeks, marked improvement was noted with a decrease in wound size from 3.0 cm x 0.7 cm x 0.2 cm to 1.0 cm x 0.3 cm x 0.2 cm, which represented a 85.7% PAR (see Figure 3b). The wound closed after 7 weeks using DAMA (see Figure 3c).

Discussion
In all 9 wounds, despite slowed or absent improvement with regular wound care or use of multiple advanced modalities such as NPWT and other CTPs, the application of DAMA resulted in healing. In addition to the average 71.0% PAR noted at 4 weeks, all wounds achieved closure within 9 weeks, with an average time to closure of 5.7 weeks. This is particularly important in wounds in which healing has stalled or slowed or where risk factors, such as diabetes mellitus and vascular disease, make expedient closure a priority.

Data on the use of CTPs in difficult-to-heal wounds demonstrate similar or decreased rates of healing. Notably, in a large randomized controlled trial, DAMA was shown to have a statistically significant effect on reducing the time to healing when compared to standard care. The results of this study support the use of DAMA in the treatment of chronic wounds, particularly in cases where traditional treatments have failed.
prospective trial\textsuperscript{24} of 208 patients with DFUs, 56\% of wounds were healed at 12 weeks. This is similar to the rates seen in other randomized controlled trials\textsuperscript{7,22-23} of DFUs with healing rates of 30\% to 56\% at 12 weeks based on almost 300 wounds total. In addition, randomized controlled trials involving more than 300 patients with venous leg ulcers reported healing rates of 47\% to 63\% at 6 months with CTPs.\textsuperscript{25,26}

Two recent case studies\textsuperscript{18,27} describing CTPs derived from placental tissue used in venous and DFUs in a total of 7 patients demonstrate a decrease in wound size following 1 to 3 applications, similar to the overall time to heal and number of applications observed in the current cases. Furthermore, a prospective, randomized, comparative parallel group study\textsuperscript{28} of a dehydrated amniotic membrane in the management of DFUs of at least 4 weeks’ duration (N = 25) found wound areas were reduced by a mean of 97.1\% in the treated group (applied every 2 weeks) compared to 32\% in the group receiving standard care of moist wound therapy with silver dressing (changed daily or as needed) after 4 weeks and by 98.4\% versus 1.8\% (ie, wounds increased in size), respectively, at 6 weeks. A retrospective analysis\textsuperscript{29} of a different dehydrated amniotic membrane in the treatment of various chronic wounds found after 12 weeks of care, 76.1\% of wounds were closed (67.6\% of venous leg ulcers and 85.2\% of DFUs). A recent retrospective review\textsuperscript{19} of medical records compared the effectiveness of bioengineered living cellular construct and a dehydrated amniotic/chorionic membrane in 218 patients and found the bioengineered construct reduced median time to closure (13.3 weeks versus 26 weeks) and a significantly higher proportion of wounds healed at both 12 weeks (48\% versus 28\%) and 24 weeks (72\% versus 47\%) (P = 0.01), suggesting CTPs vary in their efficacy.

Lastly, the authors’ clinical experience with DAMA found it easy to use and to require minimal maintenance; wounds were checked every week. DAMA was kept in place for 2 weeks and only secondary dressings were changed more frequently as needed. No adverse events were observed. Overall, the use of DAMA provided a convenience benefit for both the Wound Care Center and the patients.

**Limitations**

The results in this case series cannot be extrapolated to other patient populations. Controlled clinical studies are needed to compare the effect of DAMA to other topical treatment modalities in the care of chronic wounds.

**Figure 3.** a) Case 2: Time of first dehydrated amniotic membrane allograft (DAMA) application (March 27, 2014); b): 2 weeks later (April 10, 2014): second DAMA application; c) 7 weeks of DAMA. Wound closed (reepithelialized without drainage or dressing requirements).
Conclusion
In these clinical cases, following the application of DAMA, all wounds proceeded to heal within 9 weeks. All wounds were considered difficult-to-heal, in part due to underlying venous and arterial disease and/or poorly controlled diabetes mellitus, and had previously failed to close following the use of multiple advanced wound care strategies. These observations suggest prospective, randomized, controlled clinical studies to compare the use of DAMA to other topical treatment modalities are warranted.

Acknowledgments
The authors thank Derma Sciences, Inc (Princeton, NJ) for funding editorial support services.

References