Use of a Dehydrated Amniotic Membrane Allograft in the Treatment of Lower Extremity Wounds: A Retrospective Cohort Study

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Abstract: Injuries to the skin are extensively costly to the health care system. When caused by metabolic and vascular compromise, these injuries are even more foreboding for patients. They can result in chronic inflammation, reduced mobility, and chronic pain. *Materials and Methods*. Twenty patients were selected from the author's patient population at the West Boca Center for Wound Healing for a retrospective cohort study. Patients underwent a run-in period of 2 weeks, where standard of care was used to clear the wound of bioburden. A dehydrated, human amniotic membrane (dHAM; WoundEx Membrane, Skye Biologics, Inc, El Segundo, CA) was applied at weeks 1 (2 weeks post run-in), 3, and 5, if necessary. Wound measurements and photographs were performed weekly. Data were collected through a standard form in each patient's medical record to improve reliability and reproducibility. The data extraction was performed by the author and to reduce bias. Reduction of bias was performed by selecting patients whose wounds already were established and in temporal sequence. *Results*. In this review of 20 patients treated with the dHAM, the author was able to effectively close all wounds in approximately 9.9 weeks (69.3 days). A linear relationship was discovered between wound size in cm^2 and days to closure. Diabetic foot ulcers closed on average in 11.8 weeks (82.6 days) and venous leg ulcers in 9.2 weeks (64.4 days). No adverse events were noted secondary to the dHAM application, which shows this is a safe and effective treatment option. As of the date of this publication, there is no recurrence of the ulcerations noted. *Conclusion*. The use of this particular dHAM allograft effectively closed diabetic foot ulcerations in 82.6 days and median wound closure in 69.3 days. This poses as an advantageous clinical benefit in the scope of treatment of lower extremity wounds.

Key words: amniotic allografts, wound regeneration, advanced wound care, diabetes, healing rate, remodeling

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Injuries to the skin are extensively costly to the health care system. When caused by metabolic and vascular compromise, these injuries are even more foreboding for patients and can result in chronic inflammation, reduced mobility, and chronic pain. Venous leg ulcers (VLUs) are the most common wound type in the United States and affect between

500 000 to 2 million people annually.[1] In the United Kingdom, for example, the median duration of VLUs is 9 months, and 20% of ulcers do not heal within 2 years.[2] The diagnosis of VLUs is clinically made based on anatomic location, morphology, and a series of characteristic skin changes. The diagnosis is confirmed by the appropriate laboratory studies, which may include functional assessment of the venous system. The gold standard for diagnosing venous disease is venography, which is performed infrequently because of expense, morbidity, and the availability of noninvasive tests.

Diabetic foot ulcers (DFUs) are less common, but more costly in the United States. An estimated 29.1 million people have diabetes mellitus,[3] and the estimated lifetime incidence of a DFU is 25%.[4] Diabetic foot ulcers continue to be a major cause of morbidity and immobility, as well as the leading cause of nontraumatic lower extremity amputation. The annual cost of DFUs in 2012 was $246 billion.[5] While $70 billion of this cost was associated with lost work production, the remaining $176 billion incurred as excess health care expenditures.[5] This population also has a 3-year cumulative mortality rate of 28%.[6]

Treatments designed for both wound types have focused on conservative modalities. Conservative treatment may include basic wound care guidelines (which can include surgical and nonsurgical debridement), collagens, foams, alginates, hydrogels, and hydrocolloids. Additional treatments of venous compression, diabetic foot offloading, and pressure-relief support surfaces may also be necessary. If conservative treatment fails, decisions regarding advanced tissue-based products for wounds may be required.

A novel amniotic allograft, derived from dehydrated human amniotic membrane (dHAM), has recently been made available for the treatment of chronic wounds. WoundEx Membrane (Skye Biologics, Inc, El Segundo, CA) is crafted with a proprietary and scientifically validated HydraTek Process, a technology that has been demonstrated to provide viable amniotic membrane allografts by retaining the majority of the natural collagens, growth factors, and bioactive molecules found in natural and unprocessed placental tissues.

Based on previous studies reporting the potential of amniotic membrane in wound healing[7-9] and its antimicrobial,[10,11] antifibrotic,[12] anti-inflammatory,[13] and natural immune-privileged[13,14] properties, the author assessed the effects of using this amniotic allograft in the treatment of the 20 lower extremity wounds.

Materials and Methods

Characteristics of amniotic membranes. The human placenta consists of a placental lobe and a placental sac. The placental sac is made up of 2 adjacent membranes, the amnion and chorion, which extend from the chorionic plate and the umbilical cord. These membranes contain the amniotic fluid and surround and protect the fetus during development. The amnion membrane consists of a layer of epithelial cells, a basement membrane, and an underlying avascular stromal layer containing mesenchymal cells and mesenchymal stem cells. Structurally, the stromal layer contains collagen types I, III, IV, V, and VI as well as laminins, proteoglycans, and fibronectin. The chorion membrane sits beneath the amnion and consists of a reticular layer, a basement membrane, and the underlying trophoblast. The reticular layer contains a similar set of structural proteins as found in the amniotic stromal layer, albeit with a different distribution, while the trophoblast is enriched for laminins and fibronectin.

The collagens and other fibrous protein components in the extracellular matrix (ECM) of the amniotic membranes provide a structural scaffold to support proliferation and regeneration. These tissues also contain growth factors, which modulate the immune response, control inflammation, inhibit matrix metalloproteinases production, support angiogenesis, promote ECM production and tissue proliferation, and assist in tissue remodeling.

The dHAM used is available in both an amnion membrane configuration (WX45) and a chorion-based membrane configuration (WX200). These membranes are processed by the technology and designed to resorb and incorporate faster, so their biologic components can work quicker.

The technology is designed to better preserve the tissue's natural biomechanical structure by scientifically controlling moisture levels versus traditional heat-baking or freeze-drying (lyophilization) systems; it also avoids the use of harsh chemical rinses or crosslinking agents. Further, while some amniotic membranes are marketed as "immune-privileged" based on the natural properties of the placentas, the technology allografts have been proven to be able to suppress an active immune response in vitro,[15] which is instrumental in modulating inflammation and potentially reducing the risk of rejection and graft failure.

History of clinical use. Historically, most clinical reports[16-20] on the use of placental tissues have discussed

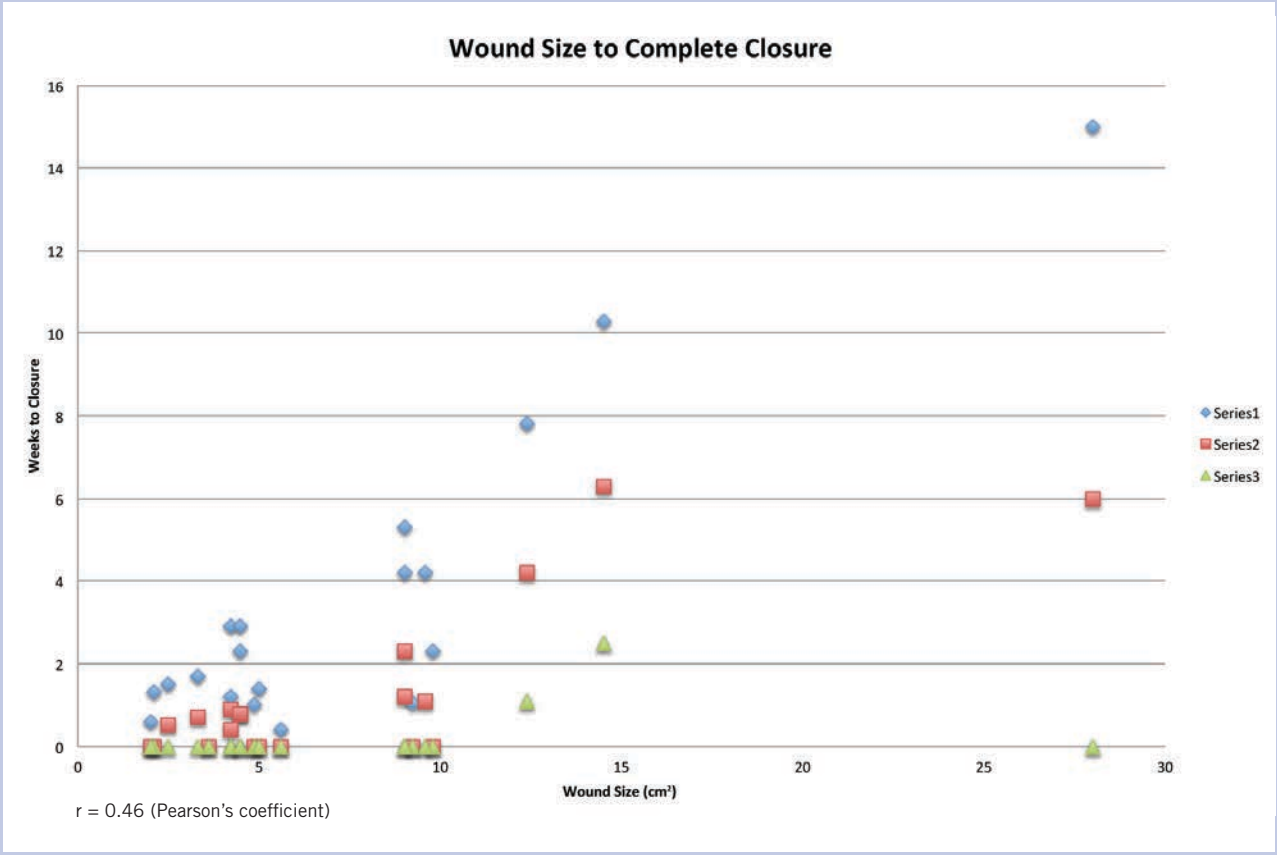

Figure. Weeks to closure correlated to wound size.

the use of amniotic membranes for an array of clinical applications since the 1920s.These uses include general surgery,[21,22] corneal surgery,[23-25] plastic surgery,[26] burn and wound care,[27-36] sports medicine,[34-37] foot and ankle procedures,[38,39] spine and dural repair,[40-46] nerve wrap or dural covering,[40,43,46] and tendon repair.[37,38]

Methods. The study was reviewed by Midlands Institutional Review Board (IRB) for approval, and they ruled this pilot retrospective study review of existing patient records was exempt from IRB review in accordance with 45CFR46.101B. The data collected were without identifiers or links to identifiers. In addition, the investigator conducted the records review, and as the patients' clinician, he had access to their records as part of routine clinical care. Twenty patients were selected for inclusion in a retrospective cohort study from the authors' patient population at a single clinical site. Male and female patients were randomly selected without bias to gender. Patients were identified as having a chronic ulceration involving the venous system of the lower extremities, a chronic DFU or ulcer of autoimmune origin nonvenous and nondiabetic. All patients were initially screened using the ankle-brachial index (ABI), medical history, and chronicity of wound of longer duration than 4 weeks. Inclusion criteria included VLUs and DFUs with a minimum of 4 weeks nonhealing, and other wound types not attributed to either venous leg or diabetic foot wounds. Exclusion criteria included acute infection, use of prior cellular-tissue products for wounds (CTPs), or ABI less than 0.6.

Patients underwent a run-in period of 2 weeks, where standard of care (SOC) was used to clear the wound of bioburden. The dHAM was applied at weeks 1 (2 weeks post run-in), 3, and 5, if necessary. Wound measurements and photographs of the wound were recorded weekly. Data were collected through a standard form in each patient's medical record to improve reliability and reproducibility. The data extraction was performed by the author and to reduce bias; reduction of bias was performed by selecting patients whose wounds already were established and in temporal sequence.

Results

Twenty patients met the inclusion criteria, and the age ranged from 55 to 100 years, with a median of 74.5 years. Of the 20 patients, 9 were male and 11 female. Body mass index (BMI) ranged from 24.85 to 41.19, with a median BMI of 28.65. Wound sizes ranged from 2.0 cm^2 to 14.5 cm^2, with a median of 7.45 cm^2. Wound types were as follows: VLU (n = 10), DFU (n = 8), and other autoimmune (n = 2). Of the 20 patients, 8 were diagnosed with peripheral arterial disease (40%), while in total, 14 patients (70%) had peripheral vascular disease. eTable 1 includes all demographic data.

eTable 2 demonstrates the healing rates of all patients in the study population. With all patients at 4 weeks, the average wound size reduced from 7.44 cm^2 to 3.43 cm^2, which is a 46% reduction in wound size. At 8 weeks, wound sizes reduced additionally on average to 1.27 cm^2, which reflects an overall wound reduction of 83%. By 12 weeks, average wound size reduced to 0.2 cm^2, resulting in an overall 98% complete healing rate. Of the 20 patients, 18 completely resolved by week 12, and the average healing rate for all wounds was 9.9 weeks. Average number of dHAM applications for all cases was 2.5, and the median number was 3. There were no adverse events related to the dHAM product, and no occurrences of major or minor amputations.

Figure demonstrates the plotted data set against size to weeks to closure. For the majority of sample data presented, a linear regression relationship with the dHAM use and direct time to heal is evident.

Statistical analysis. The relationships of wound size to week to closure were evaluated using Pearson's correlation coefficient and linear regression. Because the relationships were mostly linear, additional variables were calculated that divided each patient's time to heal and time to 50% closure of wound size. Independent *t* testing was performed and compared the time to heal and wound closure of size.

Discussion

It is widely accepted that healing chronic wounds requires the need for collagen ECM products for use in assisting the structural repair of these complicated wound types. The important components of these ECM products are the glycosaminoglycans, proteoglycans, fibronectins, and growth factors that promote granulation and epithelialization of dermal wounds.[7,8-10,27-36] The primary aim of this study was to evaluate the effectiveness of a dHAM matrix for the treatment of lower extremity wounds as well as to evaluate the safety of the tissue's use. The dHAM's effectiveness was determined by assessing and monitoring the number of days to wound closure and the number of tissues required to close the wound. Measuring any adverse events during the study assessed the safety of the graft.

In this retrospective cohort study of 20 patients, the author was able to effectively close all wounds in approximately 9.9 weeks (69.3 days). A linear relationship was discovered between wound size in cm^2 and days to closure. Diabetic foot ulcers closed on average in 11.8 weeks (82.6 days), and VLUs closed in 9.2 weeks (64.4 days). No adverse events were noted secondary to dHAM application, which shows this is a safe and effective treatment option. As of the date of this publication, there is no recurrence of the ulcerations noted.

Recent studies have advocated the use of other dehydrated acellular tissue products for wounds. Baldursson et al[47] published a retrospective trial of a fish collagen dermis in which 18 patients completed the trial study and showed only > 20% of surface area closure by week 5. Zelen et al[48] showed the ability of an open-structure human reticular acellular dermis matrix (HR-ADM) to facilitate wound closure in nonhealing DFUs versus DFUs treated with SOC. At the primary outcome time (6 weeks), 65% of the HR-ADM-treated DFUs healed (13/20) compared with 5% (1/20) of DFUs that received SOC alone. At 12 weeks, the proportions of DFUs healed were 80% and 20%, respectively.[48] The dHAM studied herein showed an 82.6-day closure rate for DFUs (100% closure at 12 weeks) in comparison data sets.

Limitations

This retrospective study has some disadvantages versus prospective studies. Among the disadvantages are that some key statistics cannot be measured, and significant biases may affect the selection of controls. In addition, major biases with retrospective cohort studies can impact the recall of former exposure to risk variables. Among the biases that can negatively impact the veracity of this type of study are selection bias and misclassification or information bias as a result of the retrospective aspect. With retrospective studies, the temporal relationship is frequently difficult to assess, particularly when discussing patients with DFUs versus patients with venous disease, or those with mixed disease. This is particularly problematic in this sample size, because it can be very difficult to make accurate comparisons between DFU and venous ulcer patient relationships if

records are recorded by another person outside of the sample selector.

Conclusion

This study found the use of a novel dHAM allograft appears to be safe and effective in the use of lower extremity wounds of venous, diabetic, and autoimmune origins. While this is a small subset study, the author acknowledges a larger cohort study is necessary to validate the findings seen in this trial. By week 12, 90% of these wounds closed, with a closure rate of 98%. More importantly, the closure rate of 46% at 4 weeks is demonstrative of accelerated wound healing in populations with severe comorbidities and obesity factors. The average wound healing time was 69.3 days. These findings support the use of this particular dHAM in the treatment of these patient populations.

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eTable 1. Patient demographics

Subject	Age	Gender	BMI	Location	Comorbidities
1	69	F	26.45	Foot	RA
2	100	M	27.89	Leg	PAD, PVD, VLU
3	78	F	26.63	Foot	RA
4	68	M	37.30	Foot	DM
5	74	M	25.84	Foot	DM
6	62	F	26.57	Leg	PVD, PAD, VLU
7	62	F	26.57	Leg	PVD, PAD, VLU
8	62	F	26.57	Leg	PVD, PAD, VLU
9	79	F	29.12	Leg	PVD, VLU
10	81	M	35.94	Foot	DM
11	90	F	26.66	Leg	PVD, VLU
12	82	M	26.32	Foot	OM,Trauma, DM
13	74	F	23.94	Leg	PVD,VLU
14	89	M	27.73	Leg	PVD, VLU
15	58	M	41.19	Foot	DFU
16	75	F	25.42	Leg	PVD,VLU
17	55	M	32.28	Foot	DFU
18	92	F	24.85	Leg/ankle	PVD, VLU, PAD
19	56	F	27.30	Leg	VLU, DM, PVD, Autoimmune
20	62	M	28.50	Foot	DFU, HIV

BMI: body mass index; F: female; M: male; RA: rheumatoid arthritis; PAD: peripheral artery disease; PVD: peripheral vascular disease; VLU: venus leg ulcer; DM: diabetes mellitus; OM: osteomyelitis; DFU: diabetic foot ulcer; HIV: human immunodeficiency virus

eTable 2. Healing rate data table (wound area in cm^2)						
Subject	**Size 0 wk**	**4 wk**	**8 wk**	**12 wk**	**Applications**	**Closure wk**
1	**2.1**	1.3	0	0	2	6
2	**4.88**	1	0	0	2	6
3	**2**	0.6	0	0	2	5
4	**28**	15	6	0	3	12
5	**4.2**	1.2	0.4	0	3	11
6	**5.6**	0.4	0	0	2	6
7	**9.24**	1.05	0	0	2	7
8	**9.8**	2.31	0	0	2	6
9	**9**	5.32	1.2	0	2	12
10	**3.6**	0	0	0	1	4
11	**3.3**	1.7	0.7	0	3	10
12	**14.5**	10.3	6.3	2.5	3	26
13	**9.6**	4.2	1.1	0	3	12
14	**4.5**	2.3	0.8	0	3	10
15	**2.5**	1.5	0.5	0	3	12
16	**12.4**	7.8	4.2	1.1	3	14
17	**4.2**	2.9	0.9	0	3	11
18	**4.5**	2.9	0.8	0	3	9
19	**5**	1.4	0	0	2	7
20	**9**	4.2	2.3	0	3	12