**Study Protocol**

**Date: 9-22-2016**

**Study Title: A Prospective Randomized Comparative Parallel Study of Amniotic Membrane in the Management of Venous Leg Ulcerations**

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**Background:** Venous leg ulcerations (VLUs) caused by chronic venous insufficiency (CVI) often results in chronic, non-healing wounds which impart significant socio-economic burden within the United States (U.S.) and around the world. The standard of care (SOC) for VLUs include debridement to clean the wound bed of devitalized tissue, application of passive dressing to protect the wound bed and maintain a moist environment. Also of significant importance is the use of compression therapy (including compression stockings and compression pumps) to minimize leg edema and encourage venous return to reduce venous hypertension. Despite these efforts wound healing rates are slow and often do not meet the gold standard of a 50% reduction in wound size by 4 weeks of SOC therapy. Often this lack of progress or stalled wounds increase the possibility of secondary infections, surgical intervention or hospitalization. Human amniotic membrane allografts have a history of success in use as specialized wound therapy for diabetic ulcers, venous leg ulcerations, burns, and post-surgical wounds. It has been demonstrated that the amnion used in wound care provides human extracellular matrix components, essential growth factors and which play an essential role in wound healing.

Venous ulcers have devastating consequences on the economy and healthcare system. VLUs account for 70-90% of all major lower extremity chronic wounds, affecting up to 1% of the American population and is especially prevalent in the elderly and the veteran population.[1,2] VLUs commonly require months to heal, cause poorer quality of life as compared to age-matched controls,[3] and frequently recur resulting in a loss of 2 million working days and direct healthcare costs exceeding $3 billion annually in the U.S.[4-6] VLUs that do not heal within 6 months have only a 22% chance of healing.[7] Furthermore, healing time has been demonstrated to be an independent risk factor for ulcer recurrence.[8]

VLUs are the result of several factors to include malfunctioning of venous valves and the sequela of venous hypertension. While the exact precipitating event(s) resulting in the formation of VLUs is unclear; it is accepted that the basic pathophysiology is the dysfunction of the leaflet-like one-way valves in the venous return system in the lower legs. As a result, back flow or reflux is common and ultimately causes venous hypertension. The veins dilate and under

pressure causes fluid, blood components, serum proteins to become trapped within the extravascular space resulting in reduced oxygen delivered to the local extracellular matrix.[9] Furthermore, it is believed that white blood cells infiltrate the area and can become trapped and activated leading again to a pro-inflammatory environment with further occlusion of the micro-circulation in the affected area again leading to local tissue ischemia.[10] Studies also indicate that keratinocytes fail to migrate from wound edges in this environment and thusly complete wound healing is often delayed or absent.[11]

Commonly utilized wound dressings do not actively promote accelerated wound healing. The current SOC treatment to conservatively address VLUs include the use of compression stocking, compression multi-layer wraps, compression pumps in conjunction with wound dressings and elevation of the legs whenever practical.[4] Commercially available conventional (wet-to-dry gauze) and more recent specialized wound dressings (hydrogel, foam dressings, hydrocolloid dressings) typically manage the wound bed in reference to protection and moisture balance (moist environment). Meta-analysis of studies evaluating the use of wound dressing with compression garments did not show any effect on ulcer healing rates, thus indicating the need for a wound dressing that biologically augments the wound environment to promote accelerated healing. In as much, biologic adjuncts to compression therapy including skin grafting and granulocyte-macrophage colony stimulating factors have shown to improve healing outcomes in patients with VLUs.[12, 13]

Amniotic membrane is a unique material, and its composition contains collagen types IV, V, and VII. Amniotic membrane is a structural extracellular matrix (ECM), which also contains specialized proteins, fibronectins, laminins, proteoglycans, and glycosaminoglycan. In addition, amniotic membranes delivers well-known essential healing growth factors like epidermal growth factor (EGF), transforming growth factor beta (TGF-β), fibroblast growth factors (FGFs) and platelet-derived growth factors (PDGFs) to the wound surface. In their natural state, these growth factors increase cell signaling and promote epithelialization of the wound bed. The amniotic membrane (AM) surround the developing fetus and is commonly discarded as medical waste following the birth of full-term babies. Historically, AM allografts have found clinical utility over the last century in humans for treating ocular wounds, skin ulcerations, burns, and a variety of other wounds.[14-18] The AM has been shown to possess anti-bacterial, anti-inflammatory, anti-adhesive, and immunomodulatory properties which make it an ideal candidate for use in wound care for the treatment of VLUs.[19-25] Preliminary investigations include multiple case studies (including patients with VLUs) utilizing dehydrated AM allografts. Several of these studies included patients that were refractory to other modalities of wound therapy.[26-31] Unpublished pre-clinical characterization of AmnioExCel™ indicate the presence of growth factors involved in tissue healing, anti-inflammatory protein molecules, protease inhibitors and the ability to promote cellular migration.

**Purpose:** The purpose of this study is to evaluate and compare healing characteristics and efficacy (wound size reduction and rate of complete healing) following the application of a

dehydrated human amnion membrane (dHAM - AmnioExCel$^{TM}$) as a biologic adjunct to the SOC versus SOC only (an absorptive alginate primary dressing in conjunction with a class II multi-layer compression wrap - Coban$^{TM}$ 2), in patients with VLUs.

**Hypothesis:** It is hypothesized that the application dHAM in conjunction with SOC will result in improved VLU healing as compared to SOC treatment alone.

**Objective:** The primary objective of the study will be to compare healing of wounds treated with AmnioExCel$^{TM}$ in conjunction with SOC and SOC alone. Primary outcome measures will include determining the average percent reduction in wound area (computed with respect to wound area at time "0") and the average proportion of wounds completely healed at 4, 8 and 12 weeks for each study group. Patient pain scores will be evaluated as a secondary outcome measure.

**Study Design:** Single center, prospective, non-blinded, randomized controlled, parallel study.

**Participant Population:** Male or female participants that have chronic VLUs.

**Participant enrollment:** 20 per study group (40 patients total)

**Study Duration:** It is anticipated that the study duration (including continuous enrollment, patient screening and follow-up and data analysis) will span a period of approximately 1 year.

**Inclusion Criteria:**

1. Male or female, 18 years or older
2. At least one VLU with a total surface area between 2 $cm^2$ and 20 $cm^2$
3. VLU present for at least 1 month
4. Presence of a VLU extending through the full thickness of the skin but not down to muscle, tendons, or bones
5. Ulcer has a clean, granulating base with minimal adherent slough
6. VLU has been treated with compression therapy for at least 14 days
7. The study VLU has < 35% area reduction with SOC treatment for the duration ≥ 4 weeks screening period
8. At least one of the following within the last 6 months:
    a. An Ankle-Brachial Index (ABI) of > 0.75
    b. Dorsalis Pedis (DP) systolic pressure ≥ 80 mm Hg for diabetic patients or ≥ 60 mm Hg for non-diabetic patients on study limb
    c. Posterior Tibial (PT) systolic pressure ≥ 80 mm Hg for diabetic patients or ≥ 60 mm Hg for non-diabetic patients on study limb
    d. Great toe systolic pressure ≥ 40 mm Hg
9. Willingness to comply with the protocol, attend all follow-up visits, complete all protocol related assessments and provide informed consent

**Exclusion Criteria:**

1. Presence of an active infection of the skin on the target limb such as cellulitis requiring antibiotics
2. Ulcer caused by a medical condition other than venous insufficiency
3. Ulcer suspicious for cancer
4. Known history of AIDS or HIV
5. Previously treated with tissue engineered materials (e.g., Apligraft, Dermagraft or EpiFix) or other scaffold materials (e.g., Oasis or Puraply)
6. Receipt of a biologic agent, growth factor or skin substitute within the prior 30 days
7. Known sensitivity to ethanol.
8. Uncontrolled diabetes mellitus with a HgBA1c of > 10% within the past 3 months
9. Rheumatoid arthritis
10. Significant lower extremity arterial occlusive disease that would preclude the use of compression therapy
11. NYHA Class III and IV congestive heart failure (CHF)
12. Ulcers on the dorsum of the foot or with more than 50% of the ulcer below the malleolus
13. Currently receiving radiation therapy or chemotherapy
14. Receiving immune modulators
15. Currently pregnant or trying to get pregnant
16. Breast feeding
17. Not willing to provide written informed consent or remain in compliance with the study protocol requirements

**Study Procedures and Methods:**

**Recruitment:** Patients with VLUs will be identified in the Vascular Health Alliance Wound Healing Center by the PI or Co-Investigators. Participation in this study is voluntary and no incentives, monetary or otherwise, will be offered for patient participation.

**Randomization:** Randomization will occur after a 4-week screening period has elapsed. Participants will be randomly assigned via the *RANDBETWEEN* function in Microsoft Excel. This function returns random integers between a defined range of numbers. For the study, the number range will be defined as being between 1 and 2 where a randomly assigned value of 1 will result in the placement of a patient into the standard of care group and an assignment of 2 will result in the placement of a patient into the AmnioExCel™ treatment group. To ensure an equal patient enrollment in each group, after a maximum of 20 patients are assigned into one treatment group, the remainder of the patients will be placed into the unfilled study group.

- Treatment cohort 1 will receive the standard of care (SOC) treatment alone for the enrolled VLU
- Treatment cohort 2 will receive SOC treatment with the study product (AmnioExCel™). If any participant randomized to this group has multiple ulcers only one ulcer will be treated with AmnioExCel™. The remainder will receive the SOC treatment only.

**Study Procedures:** All participants will receive SOC wound treatment during a 4 week screening period. Post randomization all participants will be seen by the study physician at least every 14 days for up to 12 weeks in order to have wounds evaluated and possible cleaning debridement performed. All participants will have the wounds cleaned with wound cleanser and dressings changed every week by the nursing staff at the Wound Healing Center. Representative images of the ulcerations will be captured via digital image capture during each weekly visit. Study related visits will occur every week for up to 12 weeks. The study team will take every step to ensure that no elements of the digital images can identify the participants. Standard dressing techniques will be consistently used for the duration of the study across all participants. AmnioExCel™ will be reapplied at weekly intervals in treatment cohort 2 if the study VLU has not completely healed (complete healing: indicated via 100% re-epithelialization without drainage) at those time-points. Percent reduction in wound area compared to day 0 (baseline) values and proportion of wounds completely healed will be determined from wound images captured at each weekly visit. Pain assessments will also be administered at each weekly visit using a Wong-Baker Faces Pain Scale throughout the study duration. Any evidence of infection or cellulitis will be closely monitored. End of study for each participant will occur at week 12.

**Schedule of Events:**

| | Screening | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 | Visit 7 | Visit 8 | Visit 9 | Visit 10 | Visit 11 | Visit 12 |
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| **Study Activity** | 4 wks | Randomization | 2 wks | 3 wks | 4 wks | 5 wks | 6 wks | 7 wks | 8 wks | 9 wks | 10 wks | 11 wks | 12 wks |
| **Informed Consent** | X | | | | | | | | | | | | |
| **SOC Therapy** | X | | | | | | | | | | | | |
| **Inclusion/Exclusion Criteria** | X | X | | | | | | | | | | | |
| **Medical History** | | X | | | | | | | | | | | |
| **Medications** | | X | X | X | X | X | X | X | X | X | X | X | X |
| **Concomitant Therapy** | | X | X | X | X | X | X | X | X | X | X | X | X |
| **Physical Examination** | | X | X | | X | | X | | X | | X | | X |

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| **Dressing Changes** | | X | X | X | X | X | X | X | X | X | X | X | X |
| **Ulcer Measurement** | | X | X | X | X | X | X | X | X | X | X | X | X |
| **AmnioExCel™ (if randomized to this cohort)** | | X | X | X | X | X | X | X | X | X | X | X | X |
| **Wound Pain Questionnaire** | | X | X | X | X | X | X | X | X | X | X | X | X |
| **Quality of Life** | | X | X | X | X | X | X | X | X | X | X | X | X |

**Study Product Description:**

AmnioExCel™ will be provided by BioD, LLC for use in this study. AmnioExCel™ is a Human Cellular and Tissue based Product (HCT/P) as defined by US FDA Title 21 of the Code of Federal Regulations, Part 1271. It is intended for use as a wound covering and is intended for homologous use at the direction of a physician. The material is a dehydrated human amnion allograft that is aseptically processed and terminally sterilized in compliance with current Good Tissue Practices (cGTP) from amniotic membranes obtained from donated human placenta upon determination of donor eligibility/suitability according to established methods. AmnioExCel™ is sterilely packaged for single patient, one time use only and should be stored in its original packaging at room temperature until ready for use. Once opened, AmnioExCel™ must be used immediately or discarded.

**Adverse Events:** Defined as any unfavorable or any unintended sign, symptom or disease that is reported by the patient to have occurred, or a worsening of a preexisting condition. Adverse events will be recorded and detailed. Reactions or outcomes that potentially involve the use of AmnioExCel™ will be reported to BioDlogics, LLC Customer Service Department.

**Potential Risks:** Although donor tissue is evaluated and processed following strict FDA guidelines, the donor screening process methods are limited and may not detect all diseases. As with any allograft, complications at the graft site may occur post operatively that are not readily apparent. These include, but are not limited to:

- Transmission of communicable diseases, include those of unknown etiology
- Transmission of infectious agents such as viruses, bacteria and fungi
- Immune rejection of, or allergic reaction to, implanted HCT/P.

**Potential Benefits:** It is not possible to know if there will be any potential benefit beyond what is expected for treatment utilizing the standard of care; however it is possible that wound healing may be improved or accelerated.

**Participant withdrawal:** Participants may refuse to participate or choose to withdrawal from the study at any time without fear of being penalized or losing benefits. Participants may be withdrawn from the study at any time if it is in the study Physician's judgment that withdrawal is in the participant's best interest, if the participant's medical condition changes or if the participant no longer follows the study instructions.

**Informed Consent:** The formal consent of a participant, using the GHS IRB approved informed consent form template, will be obtained by the study Physician before the participant undergoes any study procedures. The consent form will be signed and personally dated by the participant, a witness and the person who conducted the informed consent discussion. The original signed informed consent form will be retained in the participant's study records. A photocopy of the informed consent form will be scanned into the electronic system at the Vascular Health Alliance office. A photocopy of the signed informed consent form will be given to the participant for their records at the time of consent.

**Safety and Reporting:** Adverse Events, Serious Adverse Events and Unanticipated Adverse Device Effects will be reported to the GHS IRB according to OHRP policies 18.0 "Reporting of Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events" and 40.0 Appendices "Reportable Event Reporting Requirements - Appendix A."

**Protocol Deviations:** Protocol Deviations will be reported to the GHS IRB according to OHRP policy 20.0 "Non-compliance with Regulations, IRB Policies, Procedures or Decisions."

**Privacy and Confidentiality:** All data for this study will be kept confidential.

**Data Collection:** Data points to be collected throughout this study include but are not limited to participant demographics, medical history, use of concomitant medications and therapy, infection rates, wound dehiscence, pain scales, quality of life assessments, ulcer sizes and ulcer images.

**Data Analysis/Statistical Procedures:** Proposed primary outcome measures will include average percent change in wound size and proportion of ulcers completely healed (skin re-epithelialization without drainage or dressing requirements confirmed at two consecutive study visits) in the two study groups. Secondary outcomes will include wound pain scores at weekly visits. Statistical differences in the average percent change in wound size, proportion of wounds completely healed, and pain scores will be determined via parametric and non-parametric statistical analysis as appropriate, respectively. Significance will be set at $p<0.05$.

**Costs:** This study is not expected to yield any additional costs to participants. Participants will not be compensated to participate in this study. Costs for all study related activities will be covered by the Transformative Research Seed Grant awarded previously awarded to this study.

**Plans for Submission of an Extramural Research Grant Application:** Following completion of the proposed study, we plan to utilize the results obtained as preliminary data in support of applying for NIH Phase I & II Small Business Technology Transfer (STTR) grants in order to fund a larger randomized clinical trial. The targeted submission date of the NIH application will be January 5, 2017. Upon considering the potential broader impacts of the proposed research; if proven efficacious it is our hope to initiate additional clinical trials which focus on evaluating the efficacy of AmnioExCel™ in conjunction with the standard of care treatment on patients with 1) chronic sternal wounds resulting from procedures requiring sternotomy, and 2) quadriplegia or paraplegia patients who suffer from decubitus (pressure) ulcers in an attempt to improve healing and reduce the time spent in long-term acute care (L-TAC) facilities. The latter study/patient population may be of particular interest to the U.S. Department of Defense and thus may serve as an additional targeted future funding agency. All future funding applications and manuscripts stemming from this work will be co-authored in collaboration between the PI and Co- I.

**References:**


1. Sándor, T. Pathomechanism of chronic venous insufficiency and leg ulcer. *Acta Physiol. Hung.* 91, 131–45 (2004).
2. Stojadinovic, O. *et al.* Deregulation of epidermal stem cell niche contributes to pathogenesis of nonhealing venous ulcers. *Wound Repair Regen.* 22, 220–7
3. Hu, X., Sun, H., Han, C., Wang, X. & Yu, W. Topically applied rhGM-CSF for the wound healing: a systematic review. *Burns* 37, 729–41 (2011).
4. Jones, J. E. & Nelson, E. A. Skin grafting for venous leg ulcers. *Cochrane database Syst. Rev.* CD001737 (2007). doi:10.1002/14651858.CD001737.pub3
5. Akle, C., Welsh, K., Adinolfi, M., Leibowitz, S. Immunogenicity of hAECs after transplant into volunteers. *Lancet* 1002–1005 (1981).
6. Koob, T. J., Lim, J. J., Massee, M., Zabek, N. & Denozière, G. Properties of dehydrated human amnion/chorion composite grafts: Implications for wound repair and soft tissue regeneration. J. Biomed. Mater. Res. B. Appl. Biomater. (2014). doi:10.1002/jbm.b.33141
7. Fetterolf, D., Snyder, R. Scientific and clinical support for the use of dehydrated amniotic membrane in wound management. Wounds 24, 299–307 (2012).
8. Suri, K. *et al.* Sutureless amniotic membrane ProKera for ocular surface disorders: short-term results. *Eye Contact Lens* 39, 341–7 (2013).
9. Volkov, M. V & Podkolzin, V. A. [Use of the amniotic tissue of human placenta in arthroplasty]. *Ortop. Travmatol. Protez.* 26, 20–3 (1965).
10. Tao, H. & Fan, H. Implantation of amniotic membrane to reduce postlaminectomy epidural adhesions. *Eur. Spine J.* 18, 1202–12 (2009).
11. Silini, A., Parolini, O., Huppertz, B. & Lang, I. Soluble factors of amnion-derived cells in treatment of inflammatory and fibrotic pathologies. *Curr. Stem Cell Res. Ther.* 8, 6–14 (2013).
12. Shay, E., He, H., Sakurai, S. & Tseng, S. C. G. Inhibition of angiogenesis by HC·HA, a complex of hyaluronan and the heavy chain of inter-α-inhibitor, purified from human amniotic membrane. *Invest. Ophthalmol. Vis. Sci.* 52, 2669–78 (2011).
13. Robson, M. C. & Krizek, T.-J. The Effect of Human Amniotic Membranes on the Bacterial Population of Infected Rat Burns. *Ann. Surg.* 177, 144–149 (1973).

14. Hori, J., Wang, M., Kamiya, K., Takahashi, H., Sakuragawa, N. Immunological characteristics of amniotic epithelium. *Cornea* 25, S53–S58 (2006).
15. Kang, J. W. *et al.* Immunomodulatory effects of human amniotic membrane-derived mesenchymal stem cells. *J. Vet. Sci.* 13, 23 (2012).
16. Zhang, S., He, H., Day, A. J. & Tseng, S. C. G. Constitutive expression of inter-α-inhibitor (IαI) family proteins and tumor necrosis factor-stimulated gene-6 (TSG-6) by human amniotic membrane epithelial and stromal cells supporting formation of the heavy chain- hyaluronan (HC-HA) complex. *J. Biol. Chem.* 287, 12433–44 (2012).
17. Werber, B. & Martin, E. A prospective study of 20 foot and ankle wounds treated with cryopreserved amniotic membrane and fluid allograft. *J. Foot Ankle Surg.* 52, 615–621 (2013).
18. Basso, T. Use of dehydrated amniotic membrane allograft on recalcitrant lower extremity wounds. In *The Symposium of Advanced Wound Care* (2014).
19. Rosenblum, B. Case series demonstrating the healing capability of diabetic foot ulcers using dehydrated amniotic membrane allograft. in *The Symposium of Advanced Wound Care* (2014).
20. Peters, W. Clinical efficacy of dehydrated amniotic membrane allograft and off-loading on diabetic foot ulcers. In *The Symposium of Advanced Wound Care* (2014).
21. Beless, D., Marcus, B. Dehydrated amniotic membrane allograft therapy for complicated non-healing wound: A promising therapy where other treatments have failed. In *The Symposium of Advanced Wound Care* (2014).
22. Lintzeris, D., Yarrow, K., Johnson, L., White, A., Hampton, A., Strickland, A., Albert, K., Cook, A. Case series demonstrating the impact of dehydrated human amniotic membrane allograft on wound healing in acute and chronic wounds. In *The Symposium of Advanced Wound Care* (2014).
23. Hori, J., Wang, M., Kamiya, K., Takahashi, H., Sakuragawa, N. Immunological characteristics of amniotic epithelium. *Cornea* 25, S53–S58 (2006).
24. Kang, J. W. *et al.* Immunomodulatory effects of human amniotic membrane-derived mesenchymal stem cells. *J. Vet. Sci.* 13, 23 (2012).
25. Zhang, S., He, H., Day, A. J. & Tseng, S. C. G. Constitutive expression of inter-α-inhibitor (IαI) family proteins and tumor necrosis factor-stimulated gene-6 (TSG-6) by human amniotic membrane epithelial and stromal cells supporting formation of the heavy chain- hyaluronan (HC-HA) complex. *J. Biol. Chem.* 287, 12433–44 (2012).
26. Werber, B. & Martin, E. A prospective study of 20 foot and ankle wounds treated with cryopreserved amniotic membrane and fluid allograft. *J. Foot Ankle Surg.* 52, 615–621 (2013).
27. Basso, T. Use of dehydrated amniotic membrane allograft on recalcitrant lower extremity wounds. In *The Symposium of Advanced Wound Care* (2014).
28. Rosenblum, B. Case series demonstrating the healing capability of diabetic foot ulcers using dehydrated amniotic membrane allograft. in *The Symposium of Advanced Wound* Care (2014)
29. Peters, W. Clinical efficacy of dehydrated amniotic membrane allograft and off-loading on diabetic foot ulcers. In *The Symposium of Advanced Wound Care* (2014).
30. Beless, D., Marcus, B. Dehydrated amniotic membrane allograft therapy for complicated non-healing wound: A promising therapy where other treatments have failed. In *The Symposium of Advanced Wound Care* (2014).

31. Lintzeris, D., Yarrow, K., Johnson, L., White, A., Hampton, A., Strickland, A., Albert, K., Cook, A. Case series demonstrating the impact of dehydrated human amniotic membrane allograft on wound healing in acute and chronic wounds. In *The Symposium of Advanced Wound Care* (2014).