A Retrospective Comparison of Standalone and Combination Therapy Using Synthetic Extracellular Matrix Dressings and Amniotic Allografting in the Treatment of Lower Extremity Wounds.

Arthur Evensen, DPM, PGY-III; Jodi Walters, DPM; Nicklett Johnston, DNP; Amanda Sepulveda, NP; Valarie Samoy, DPM; James Dancho, DPM; David Jolley, DPM, Lorrie Mills, CCRP
Department of Veteran Affairs - Southern Arizona Veteran Affairs Healthcare System

SAVAHCS

VA | U.S. Department of Veterans Affairs

Abstract

The use of monotherapy with synthetic extracellular matrix dressings, monotherapy with amniotic allografts, and combination therapy utilizing synthetic extracellular matrix dressings with amniotic allografting has been documented in scientific literature however currently no studies exist that compare these three treatment modalities against one another in the treatment of lower extremity wounds. Synthetic extracellular matrices are artificial polymers that are elongated and deposited as a matrix of nanofibers which mimic the native extracellular matrix. RenovoDerm® Anthem™ Wound Matrix is a comprised of polyglycolic acid and poly (L-lactide-co-caprolactone) which degrade by hydrolysis into a-hydroxy and fatty acids, lowering the pH and promoting regenerative cellular activity including angiogenesis. Amniotic allografts contain growth factors, cytokines, amino acids, extracellular matrix proteins, and hyaluronic acid which are recognized as intrinsic to the wound healing process. PalinGen® XPlus Hydromembrane is an amniotic allograft that is chemically cross-linked with extracellular matrix fibers to give it strength, shape, and slower resorption in vivo. The use of these two advanced wound care modalities in combination with one another has been has limited research however the existing studies suggest a possible synergistic relationship when they are combined. This multispecialty retrospective study of twenty-one patients at the Southern Arizona Veteran's Affairs Health Care System between 2021 and 2024 aims to highlight the differences between treatments with regard for rate of reduction in wound volume and time to achieving a predefined treatment goal of 95% wound volume reduction.

Introduction

PalinGen® XPlus Hydromembrane is chemically cross-linked with extracellular matrix fibers to give it strength, shape, and slower resorption in vivo.[1] PalinGen® Flow is cryopreserved liquid amniotic allograft.[1] These amniotic allografts contain collagen types I, III, IV, V, and VII, cytokines, hyaluronic acid, fibronectin, laminin, fibrinogen, amino acids, proteoglycans, tissue inhibitors of metalloproteinases (TIMPs), extracellular matrix proteins and mesenchymal stem cells which are all recognized as part of the complex wound healing process.[2]

Amniotic allografts also include key growth factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor beta (TGF-β).[3] Amniotic tissues have been proven to be multipotent and capable of differentiating into adaptogenic, osteogenic, myogenic, endothelial, and neurogenic cell lineages.[3-9] They are derived exclusively from the amnion and are chorion-free.[3-9] Placental tissue is donated by healthy mothers at the time of scheduled cesarean section.[3-9]

When applied to a wound however, the amniotic stem cells contained in amniotic allografts can experience rapid cell death and clearance. The ultrafine fibers of electrospun synthetic extracellular matrices have been shown to promote stem cell survival and proliferation.[10-22]

Anthem™ Wound Matrix is a three-dimensional electrospun synthetic polymer matrix which mimics the native extracellular matrix which facilitates pro-regenerative cellular adhesion, infiltration, and proliferation.[23-24] It is made of two bioresorbable synthetic polymers, Polyglycolic Acid and Poly(L-lactide-co-caprolactone) and acts as a protective barrier which promotes a pro-healing microenvironment by lowering the pH of the wound and through lactate mediated effects.[24-25]

Delayed wound healing can result due to insufficient extracellular matrix deposition, inadequate perfusion, inhospitable wound pH, bioburden, or excessive immune response.[23] Many healing processes are affected by changes in pH, including angiogenesis, collagen formation, and macrophage activity.[24,25-29] The pH of a wound has also been shown to affect the toxicity of bacterial end products as well as enzyme activity.[24,30] The pH of a wound has been shown to affect the performance of antimicrobials.[31-32] This has a direct effect of matrix metalloproteinases (MMPs), which are critical to wound healing and extracellular matrix remodeling.[24,33-37]

Wound pH also affects wound closure, graft take, microbial infection rates, bacterial virulence, and biofilm formation.[24,38-40] It has been demonstrated that wound healing occurs most effectively at low pH, whereas alkaline wound environments are correlated with chronic wounds. [41] Lactate is a product of aerobic glycolysis, and its levels increase during hypoxia which signals macrophages to stimulate VEGF to be released.[40,42-43] Lactate also enhances collage prolyl hydroxylase activity and procollagen synthesis.[40,42] When oxygen is re-introduced to the wound, collagen deposition is enhanced.[40-41] Lactate has been shown to affect gene expression and cell differentiation.[44]

We propose that when used in combination therapy, synthetic extracellular matrix dressings and amniotic allografts complement each other and work synergistically to facilitate faster wound healing.

Methodology

This study describes a retrospective comparison of monotherapy and combination therapy using synthetic extracellular matrix dressings and amniotic allografts in lower extremity wound care at the Southern Arizona Veterans Affairs Health Care System using basic, anonymized data derived from patient records. Patients with lower extremity wounds treated with either synthetic extracellular matrix dressings, amniotic allografts, or a combination of both between July 2021 and October 2024 were included in the analysis in accordance with the principles outlined in the Declaration of Helsinki. This study meets Institutional Review Board exemption under 38CFR16.104 and qualified for exemption under category 4(iiii). The waiver of HIPAA authorization was approved as outlined and described in VA Central IRB Form 103 signed by the designated VA Central IRB representative. All criteria for granting the waiver as specified in 45 CFR 164.512 was met. This study utilized patients from multiple clinics and providers within the Southern Arizona Veteran's Affairs Health Care System including the Saguaro Wound Clinic and Podiatric Surgery Clinic.

Decisions over several treatment variables (outer dressings, offloading, antibiotic therapy), was determined by the individual practitioners. Local protocol and clinical judgment determined the use of any additional adjunctive therapies.

The study was divided into three treatment arms: monotherapy with synthetic extracellular matrix dressings, monotherapy with amniotic allografts, and combination therapy utilizing both. Each treatment arm was comprised of seven patients, totaling twenty-one patients enrolled in the study. To be eligible for the study, patients had to be older than eighteen, have served in the United States military, and received wound care for a lower extremity wound at the Southern Arizona Veterans Affairs Healthcare System between July 2021 and October 2024 using either monotherapy with synthetic extracellular matrix dressings, monotherapy with amniotic allografts, or combination therapy with greater than or equal to six consecutive weeks of documented treatment applications.

Protocol

During the initial visit, a thorough history was obtained which was comprised of the patient's past medical history, current medications, allergies, surgical history, and social history. A complete review of systems was performed, and a lower extremity focused exam was conducted which included vascular, dermatologic, neurologic, and musculoskeletal. Radiographs were obtained and blood tests were performed including glycosylated hemoglobin levels. A wound swab was then collected from the wound and sent for culture/sensitivity testing. If radiographs demonstrated findings concerning for osteomyelitis, or if there was exposed bone present in the wound, bone samples would be obtained and sent to for pathology examination and culture/sensitivity testing.

The initial visit and all subsequent visits always followed the treatment protocol below:

The foot would be scrubbed with Provodone-Iodine using a sponge brush, rinsed clean with sterile saline, and allowed to air dry. Sharp debridement was performed removing all non-viable tissue. Wound characteristics including location, size measurements, and descriptions of the wound bed, edges, and peri-wound skin were obtained. Any drainage, odor, pain, or signs of infection were also noted. Chlorhexidine was then applied with an applicator to the peri-wound skin and allowed to air dry. This was followed by application of benzoin tincture to the periwound skin.

In the synthetic extracellular matrix dressing monotherapy arm of the study, Anthem™ Wound Matrix was then applied directly to the wound bed after being cut to size and soaked in sterile saline. Utilizing coaptive film and a non-adherent dressing, the synthetic extracellular matrix dressing was secured in place.

In the amniotic allografting monotherapy arm of the study, liquid or membrane amniotic allograft was implanted directly to the wound bed. Liquid amniotic allograft was applied if there were any deep tissues exposed. Once granulation tissue covered the deep tissue, membrane amniotic allograft would be used instead. Utilizing coaptive film and a non-adherent dressing, the synthetic extracellular matrix dressing was secured in place.

In the combination therapy arm of the study, liquid or membrane amniotic allograft was implanted directly to the wound bed. Liquid amniotic allograft was applied if there were any deep tissues exposed. Once granulation tissue covered the deep tissue, membrane amniotic allograft would be used instead. Synthetic extracellular matrix dressing was then applied directly overtop the grafts and soaked in sterile saline.. Utilizing coaptive film and a non-adherent dressing, the grafts and synthetic extracellular matrix were secured in place. See Figure 1.

Regardless of treatment modality, all wounds received a secondary dressing which included an inner absorptive and outer protective layer, and each patient was offloaded according to the location of their wound, their ambulatory status, and the requirements of their activities of daily living. Patients were seen on a weekly basis. All dressings of the foot were kept dry and intact for the duration of the week and were only removed at the next weekly visit when the treatment protocol would then be repeated.

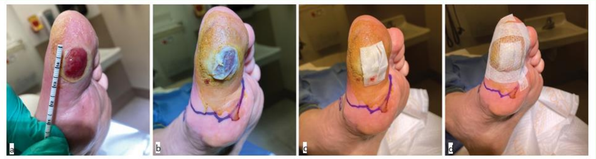

Figure 1 Foot scrubbed with povidone-iodine, rinsed with sterile saline, and allowed to air dry. Sharp debridement performed. Wound measurements and characteristics obtained. b) Chlorhexidine applied with applicator to the periwound skin and allowed to air dry, followed by application of benzoin tincture. Amniotic allografts are applied. c) Synthetic extracellular matrix dressing was then applied directly over top of the grafts and soaked in sterile saline. d) Coaptive film and a non-adherent dressing was used to secure the grafts and synthetic extracellular matrix in place.

Data Analysis

Initial wound volume measurements were obtained and calculated as the product of the length, width, and depth in cubic centimeters (cm^3). The wound was designated with a Wagner Grade [45] and each participant was given an ASA classification [46]. A wound severity score was assigned to each participant which was the sum of the total cm^3 of the initial wound size rounded to the nearest integer, the Wagner Grade, and the ASA classification. See figure 2.

Wound size measurements, calculated as the product of the length, width, and depth in cubic centimeters (cm^3), were recorded at baseline and at each of the six subsequent applications of the selected therapy. Each wound trajectory was graphed using a line chart in Microsoft Excel for each of the three treatment modalities. An average was obtained for the number of weeks it took to achieve 95% or greater wound volume closure [47] for each treatment arm of the study. See Figures 2, 3, 4, and 5.

Results

	Wound Size	Wagner Grade	ASA Classification	Wound Severity Index
AWD1	1	3	3	7
AWD2	1	3	3	7
AWD3	1	3	3	7
AWD4	2	2	2	6
AWD5	1	2	3	6
AWD6	1	2	2	5
AWD7	1	2	2	5
				43
PG1	1	2	2	5
PG2	4	2	2	8
PG3	4	2	2	8
PG4	2	2	2	6
PG5	2	2	2	6
PG6	1	1	3	5
PG7	1	2	2	5
				43
CT1	11	3	3	17
CT2	1	3	3	7
CT3	1	2	2	5
CT4	3	3	3	14
CT5	17	3	2	22
CT6	2	3	3	8
CT7	1	2	2	5
				78

Figure 2 Wound Severity Index

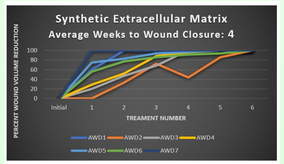

Figure 3 Synthetic Extracellular Matrix Monotherapy

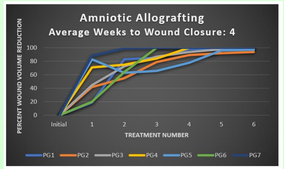

Figure 4 Amniotic Allografting Monotherapy

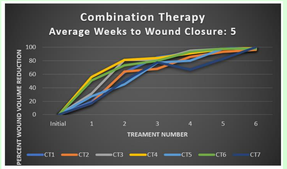

Figure 5 Combination Therapy

All wounds achieved greater than 95% decrease in wound volume by six weeks from initiating the treatment protocol. Radiographs obtained at the end of therapeutic period demonstrated no new osseous erosions concerning for osteomyelitis and throughout the course of the therapy no new soft tissue infections occurred. None of the wounds went on to amputation. The synthetic extracellular matrix monotherapy achieved the therapy goal of 95% decrease in wound volume by an average of four weeks with the treatment group having a wound severity index of 43. Similarly, the amniotic allografting monotherapy group achieved the therapy goal of 95% decrease in wound volume by an average of four weeks and had the same calculated wound severity index of 43. The combination therapy group achieved the therapy goal of 95% decrease in wound volume by an average of five weeks, however its calculated wound severity index was 78.

Discussion

The results of this study demonstrated that rate of wound volume reduction between the synthetic extracellular matrix monotherapy and amniotic allografting monotherapy are the same. This finding is further supported by considering that the wound severity index for both these treatment arms was identical which allows for an "apples to apples" comparison. This unexpected finding has interesting implications for providers when making clinical decisions about what advanced wound care products to use as the two therapies significantly differ in terms of cost and ease of use.

While the results of this study showed that the rate of wound volume reduction using combination therapy lagged by a week on average to the monotherapies, the wound severity index for the combination group was nearly double at 1.8 times greater for that set of patients. Taking this into account, the study supports the use of amniotic allografting in combination with synthetic extracellular matrices as a superior wound care modality over monotherapy alone.

It is important to note that this case was performed at the Southern Arizona Veteran Affairs Health Care System, therefore cost and reimbursement were not factors in determining treatment. Anthem Wound Matrix (RenovoDerm, Columbus, Ohio) is branded specifically for the Department of Veteran Affairs. In private and commercial facilities, it is branded as the Phoenix Wound Matrix® (RenovoDerm, Columbus, Ohio). Amniotic allografts, and synthetic extracellular matrices are all reimbursable through Medicare and private insurance and have their own designated HCPCS codes. The cost to benefit ratio would need to be assessed on an individual provider and patient basis. All pricing of the wound care products used in this case are available to the public through the Department of Veteran Affairs Federal Supply Schedule.

Limitations

This retrospective study utilized a convenience sample in its procurement of data and as such, is subject to investigator bias. The wounds examined in this study were heterogenous with respect to etiology and severity of the wound. The patients involved in this study were diverse in terms of their respective comorbidities and physical status. It is also important to note that this was a multidisciplinary, multi-clinic study and there were multiple providers involved in the care of patients which may have influenced outcomes. Care was taken to avoid bias wherever possible and follow consistent treatment protocols and obtain uniform wound characteristics, but inconsistency was impossible to entirely avoid. The significant difference of the combination therapy group having nearly double the wound severity index limits the comparisons that can be made.

Future Research

Previous studies have reported reduction in wound pain and sensitivity with application of amniotic allografts [48] and with synthetic extracellular matrices [49]. An avenue of future research would be to assess the lessening of pain and sensitivity with combination therapy in sensate patients. The authors of this study plan to perform a randomized, controlled, prospective study using combination therapy to further elucidate the synergistic benefits we've observed in the previous case reports, case series, and now this retrospective study.

Conclusion

The outcome of this retrospective comparison of standalone and combination therapy using synthetic extracellular matrix dressings and amniotic allografting in the treatment of lower extremity wounds supports the use of amniotic allografting in combination with synthetic extracellular matrices as a therapeutic option for the management of complex lower extremity wounds.

Conflict of Interest Statement

The authors of this article declare no conflict of interest. The companies involved had no role in the design of the study; in the collection, analyses, or interpretation of date; in the writing of the manuscript, or in the decision to publish the results.

Acknowledgements

This material is based upon work supported by the Department of Veterans Affairs, Veterans Health Administration, and Office of Research and Development. The authors gratefully acknowledge the Southern Arizona VA Health Care System which provided facilities and materials for this research.

References – Scan QR Code