Impact Of Synthetic Extracellular Matrices In Combination Therapy With Amniotic Allografting In The Treatment Of Diabetic Foot Wounds: A Case Series.

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Abstract

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, such as Amnio Technology, LLC PalinGen® Flow and PalinGen® XPlus Hydromembrane contain growth factors, cytokines, amino acids, extracellular matrix proteins, and hyaluronic acid which are recognized as intrinsic to the wound healing process. Synthetic extracellular matrices and amnio allografts have large bodies of evidence which demonstrate their effectiveness in the treatment of wounds. Presently, no prior studies have been performed to assess what impact these therapies may have on wound healing when used concurrently. The aim of this investigation was to assess whether a synergistic effect is produced with combination therapy using synthetic extracellular matrix and amniotic allografting. In this article we present three cases of diabetic foot ulcerations treated with combination therapy. PalinGen® Flow and/or PalinGen® XPlus Hydromembrane was implanted with Anthem™ Wound Matrix placed overtop the grafts at weekly intervals. All wounds demonstrated greater than 80% decrease in wound size within four applications and went on to complete closure after six applications.

Introduction

PalinGen® XPlus Hydromembrane is chemically cross-linked with extracellular matrix fibers to give it strength, shape, and slower resorption in vivo [48]. PalinGen® Flow is cryopreserved liquid amniotic allograft [48]. PalinGen® 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 [48]. PalinGen® 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) [48]. Amniotic tissues have been proven to be multipotent and capable of differentiating into adaptogenic, osteogenic, myogenic, endothelial, and neurogenic cell lineages [41-47]. They are derived exclusively from the amnion and are chorion-free [41-47]. Placental tissue is donated by healthy mothers at the time of scheduled cesarean section [41-47]. 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 [23-36].

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 [1]. 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 [1,2]. Delayed wound healing can result due to insufficient extracellular matrix deposition, inadequate perfusion, inhospitable wound pH, bioburden, or excessive immune response [15]. Many healing processes are affected by changes in pH, including angiogenesis, collagen formation, and macrophage activity [1, 3-6]. The pH of a wound has also been shown to affect the toxicity of bacterial end products as well as enzyme activity [1,7]. The pH of a wound has been shown to affect the performance of antimicrobials [21, 38]. This has a direct effect of matrix metalloproteinases (MMPs), which are critical to wound healing and extracellular matrix remodeling [1, 8-12]. Wound pH also affects wound closure, graft take, microbial infection rates, bacterial virulence, and biofilm formation [1, 13-14, 37]. It has been demonstrated that wound healing occurs most effectively at low pH, whereas alkaline wound environments are correlated with chronic wounds [22]. Lactate is a product of aerobic glycolysis and its levels increase during hypoxia which signals macrophages to stimulate VEGF to be released [17-20, 37, 39]. Lactate also enhances collage prolyl hydroxylase activity and procollagen synthesis [17-20, 37]. When oxygen is re-introduced to the wound, collagen deposition is enhanced [17-20, 37]. Lactate has been shown to affect gene expression and cell differentiation [40]. We propose that when used in combination therapy, Anthem™ Wound Matrix and PalinGen® amniotic allografts complement each other and work synergistically to facilitate faster wound healing.

Methods

During the initial visit a thorough history was obtained which was comprised of their 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 of the feet 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. Chlorhexadine 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. PalinGen® Flow and/or PalinGen® XPlus Hydromembrane was implanted directly to the wound bed. Anthem™ Wound Matrix 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. This was followed by a secondary dressing which included an inner absorptive and outer protective layer and appropriate offloading.

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. Wound size measurements were record at each visit. Would size was calculated as the product of the length, width, and depth in cubic centimeters (cm^3). Dynamic wound sizes were compared to the initial wound size and expressed as a percentage of wound closure. Complete wound closure was considered to be when at least 95% of the initial wound size had closed [16].

Foot scrubbed with Provodone-Iodine, rinsed with sterile saline, and allowed to air dry. Sharp debridement performed. Wound measurements and characteristics obtained.	Chlorhexadine applied with applicator to the periwound skin and allowed to air dry, followed by application of benzoin tincture. PalinGen Flow and/or PalinGen XPlus Hydromembrane implanted.	Anthem Wound Dressing was then applied directly overtop the grafts and soaked in sterile saline.	Coaptive film and a non-adherent dressing used to secure the grafts and synthetic extracellular matrix in place.

Case Study #1

History and Physical Exam

Initial visit occurred July 27, 2023 with an 87 year-old male with past medical history of adult onset diabetes mellitus, peripheral neuropathy, end stage renal disease on hemodialysis, hypertension, hyperlipidemia, and history of prostate cancer. Patient has no known allergies and is currently on long-term anticoagulation therapy. No pertinent surgical or social history. Physical examination demonstrated palpable pedal pulses, complete loss of protective sensation, and a posterior heel ulceration of the right heel. No gross deformity of the foot was identified and the patient ambulated without the aid of assistive devices.

Initial Wound Presentation

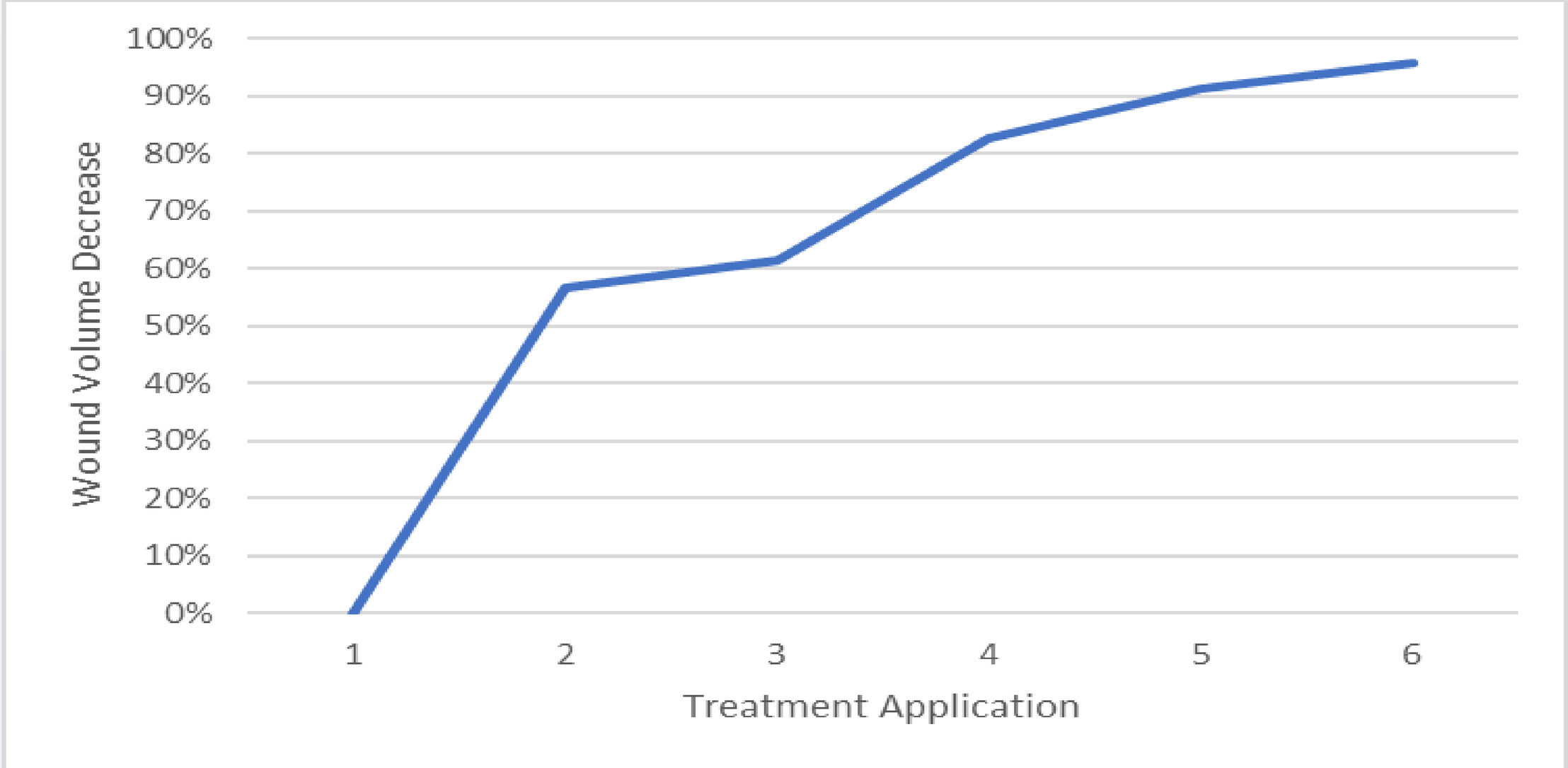
Location: RIGHT Posterior Heel
Wound Age: Present since October 2022
Type: Diabetic Foot Ulcer, Wagner Grade II
Pain: No reported pain.
Measurement: 2.3cm x 1.8cm x 0.2cm
No tunneling, undermining, or sinus tract present.
No drainage or odor noted.
Wound bed 100% granular.
No periwound calor, erythema, or edema.

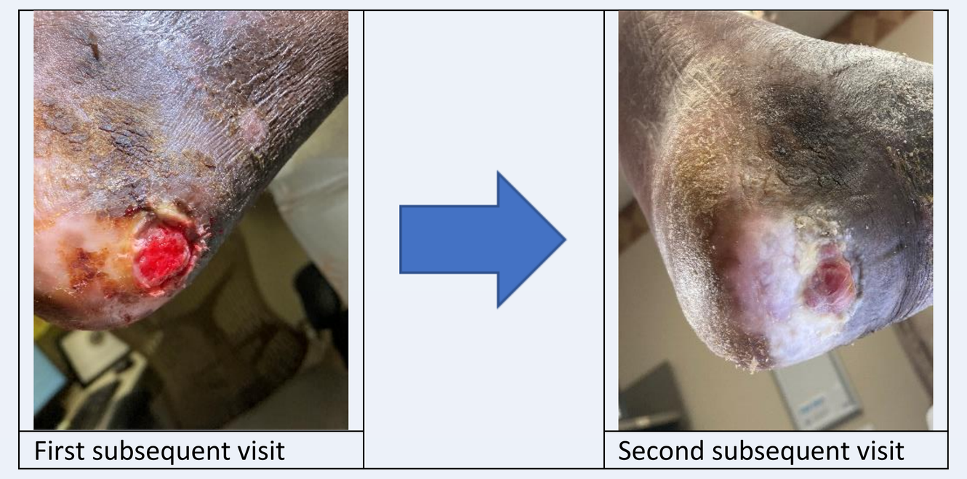
Ancillary Testing

Ankle Brachial Index (ABI) was found to be 1.09 with a toe pressure of 111 mmHg. Radiographs revealed no concerning findings for calcaneal osteomyelitis. Glycosylated hemoglobin had a reported value of 4.5. Wound swab was obtained and sent for culture and sensitivity testing which showed mixed flora, predominately gram positive cocci. Patient was started on oral antibiotic therapy which was continued throughout the duration of treatment. The therapy protocol was initiated with PalinGen® XPlus Hydromembrane and Anthem Wound Matrix.

Dynamic Wound Healing

At the first subsequent visit the wound decreased in volume by 57% and PalinGen® XPlus Hydromembrane and Anthem Wound Matrix were reapplied. The second, third, and fourth subsequent visits saw the wound volume decrease by 61%, 83%, and 91% respectively and each time PalinGen® XPlus Hydromembrane and Anthem Wound Matrix were applied. At the fifth subsequent visit, the wound volume was unchanged and the wound bed was found to have developed hypergranular "proud flesh" which was treated with chemical and thermal cautery, after which PalinGen® XPlus Hydromembrane and Anthem Wound Matrix were applied. On the sixth subsequent visit, which was August 31, 2023, the wound achieved complete closure at 96% decreased wound volume.





First subsequent visit — Second subsequent visit

Case Study #2

History and Physical Exam

Initial visit occurred July 21, 2023 with a 68 year-old male with past medical history of adult onset diabetes mellitus, peripheral neuropathy, peripheral vascular disease, human immunodeficiency virus disease on Bitkarvy, nicotine dependence (50 pack-year history), hypertension, and stage IV non-small cell lung cancer on current treatment with chemotherapy (C3 Fosaprepitant, Carboplatin, Pemetrexed infusion therapy). Patient has a past history of amputation of the right hallux and second digit. Patient has no known allergies and is currently on long-term anticoagulation therapy. Physical examination demonstrated non-palpable pedal pulses, bilateral lower extremity pitting edema (Grade 2-3+), complete loss of protective sensation, and a plantar ulceration of the right 1st metatarsal head. The patient ambulated without the aid of assistive devices.

Initial Wound Presentation

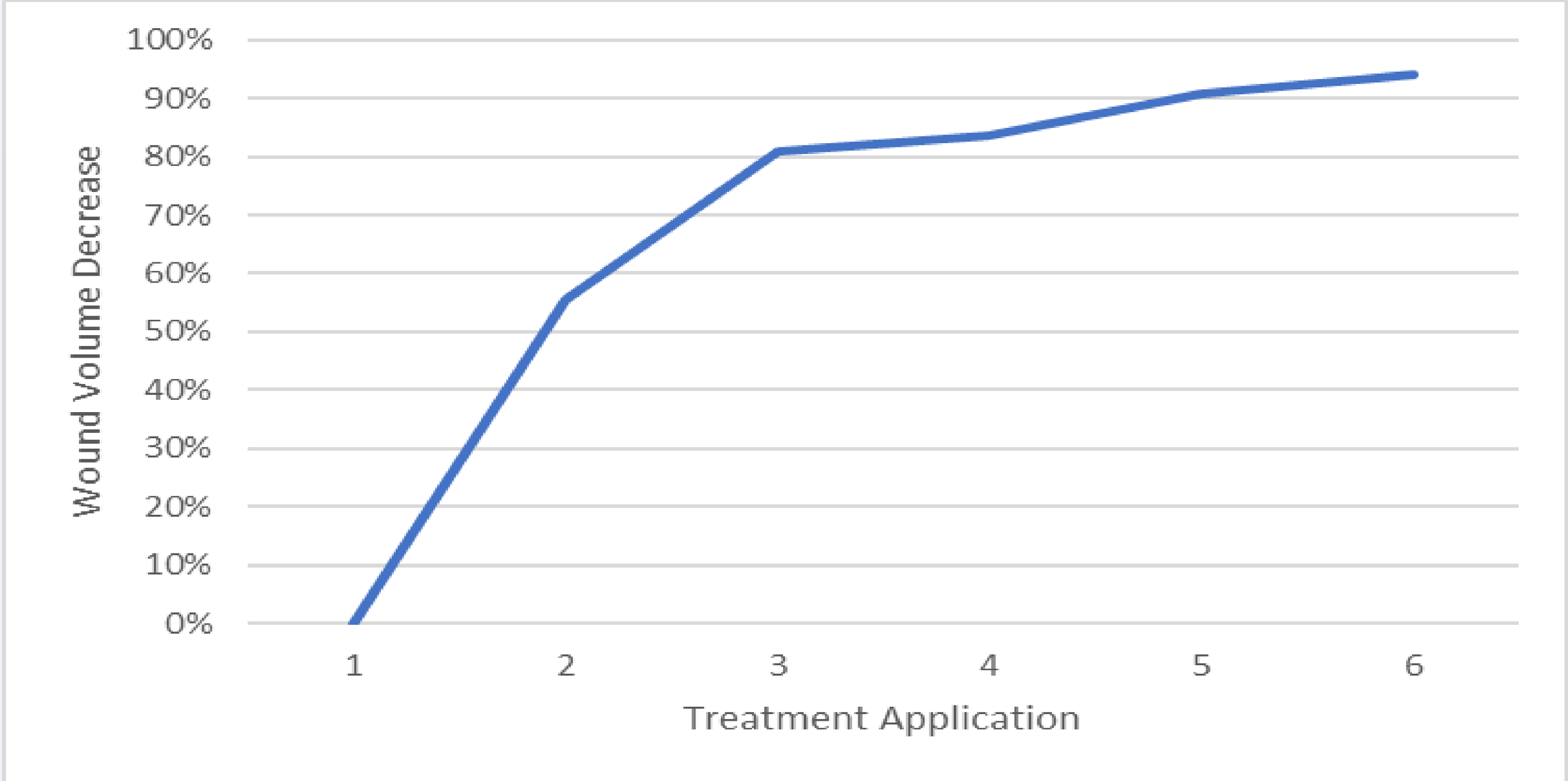
Location: RIGHT Plantar First Metatarsal Head
Wound Age: Present since May 2023
Type: Diabetic Foot Ulcer, Wagner Grade III
Pain: No reported pain.
Measurement: 2.2cm x 1.9cm x 1.8cm , exposed bone
No tunneling, undermining, or sinus tract present.
No drainage or odor noted.
Wound bed 90% granular 5% fibrotic slough.
Periwound calor and edema present. No periwound erythema noted.

Ancillary Testing

Ankle Brachial Index (ABI) was found to be 1.14 and the dorsalis pedis and posterior tibial arteries had monophasic waveforms of doppler examination. Radiographs demonstrated erosive osseous changes of the right first metatarsal head. All infected bone was excised during the initial visit with a clean margin and sent for pathology examination and culture and sensitivity testing which confirmed osteomyelitis of the right first metatarsal head and cultured mixed flora. Glycosylated hemoglobin had a reported value of 6.0. Wound swab was obtained and sent for culture and sensitivity testing which again showed mixed flora. Patient was started on oral antibiotic therapy which was continued throughout the duration of treatment. The therapy protocol was initiated with PalinGen® Flow and Anthem Wound Matrix.

Dynamic Wound Healing

At the first subsequent visit the wound decreased in volume by 56% and PalinGen® XPlus Hydromembrane and Anthem Wound Matrix were applied. The second, third, and fourth subsequent visits saw the wound volume decrease by 81%, 84%, and 91% respectively and each time PalinGen® XPlus Hydromembrane and Anthem Wound Matrix were applied. At the fifth subsequent visit, the wound volume decreased by 94%, PalinGen® Flow, PalinGen® XPlus Hydromembrane and Anthem Wound Matrix were then applied. On the sixth subsequent visit, which was August 30, 2023, the wound achieved complete closure at 99% decreased wound volume.



Initial Visit — Third Subsequent Visit

Case Study #3

History and Physical Exam

Initial visit occurred May 10, 2023 with a 75 year-old male with past medical history of adult onset diabetes mellitus, peripheral neuropathy, peripheral vascular disease, hypertension, hyperlipidemia, chronic obstructive lung disease, malnutrition, failure to thrive, history of former heavy tobacco use, and history of colorectal cancer . Patient has known allergy to codeine, hydroxyzine, Lantus, NovoLog, bupropion, buspirone, and horse serum proteins and is currently on long-term anticoagulation therapy. Physical examination demonstrated non-palpable pedal pulses, complete loss of protective sensation, and dorsal ulceration of the right hallux interphalangeal joint. The patient presented to clinic laying supine on a stretcher and had been bedridden since October 2022.

Initial Wound Presentation

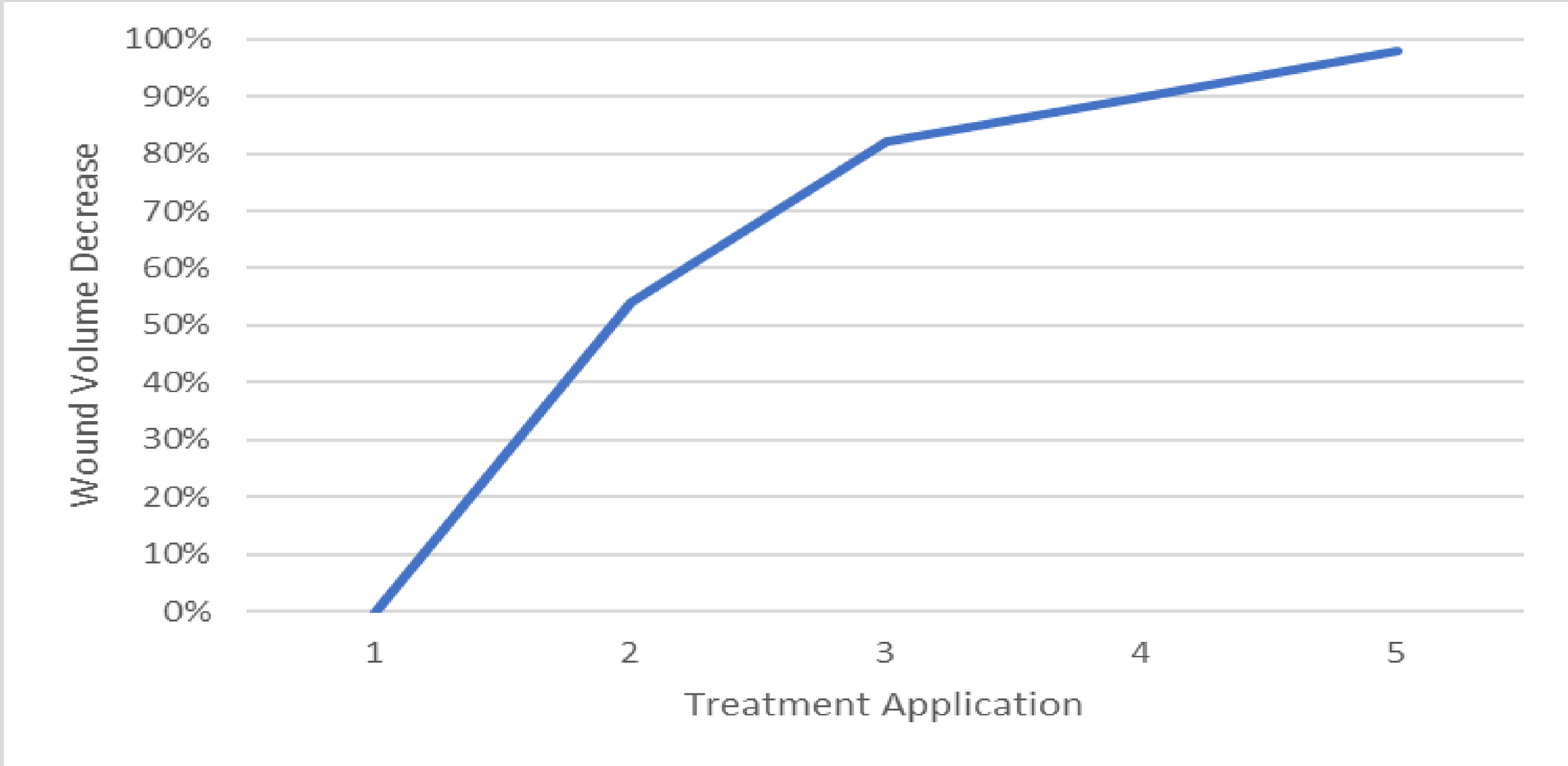
Location: RIGHT Dorsal Hallux IPJ
Wound Age: Present since January 2023
Type: Diabetic Foot Ulcer, Wagner Grade III
Pain: No reported pain.
Measurement: 2.6cm x 1.9cm x 1.7cm, exposed bone.
No tunneling, undermining, or sinus tract present.
No drainage or odor noted.
Wound bed 60% granular 40% fibrotic slough.
No periwound calor, erythema, or edema.

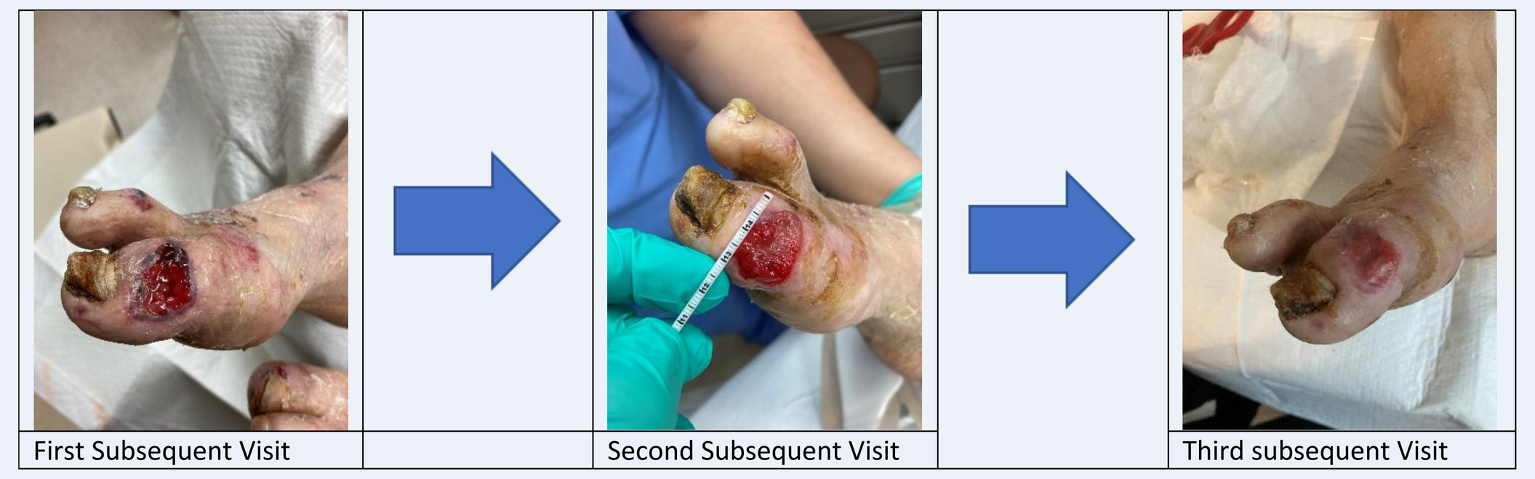
Ancillary Testing

Ankle Brachial Index (ABI) was found to be 0.86 with a toe pressure of 15 mmHg. Radiographs and magnetic resonance imaging demonstrated erosive osseous changes of the right hallux proximal phalanx consistent with osteomyelitis. All infected bone was excised during the initial visit with a clean margin and sent for pathology examination and culture and sensitivity testing which confirmed osteomyelitis of the right hallux and cultured methicillin-resistant Staphylococcus aureus. Vascular surgery was consulted and determined that the patient would need a below-knee-amputation, which the patient declined. Glycosylated hemoglobin had a reported value of 13.0. Wound swab was obtained and sent for culture and sensitivity testing and cultured cultured methicillin-resistant Staphylococcus aureus. Patient was started on oral antibiotic therapy which was continued throughout the duration of treatment. The therapy protocol was initiated with PalinGen® XPlus Hydromembrane and Anthem Wound Matrix.

Dynamic Wound Healing

At the first subsequent visit the wound decreased in volume by 54% and PalinGen® XPlus Hydromembrane and Anthem Wound Matrix were applied. The second and third subsequent visits saw the wound volume decrease by 82% and 90% respectively and each time PalinGen® XPlus Hydromembrane and Anthem Wound Matrix were applied. On the fourth subsequent visit, which was June 7, 2023, the wound achieved complete closure at 98% decreased wound volume.





First Subsequent Visit — Second Subsequent Visit — Third Subsequent Visit

Results

All wounds achieved complete wound closure (decrease in wound volume >95%) by six weeks from initiating the treatment protocol. By the fourth application, all wounds saw an 80% or greater decrease in wound volume. The average time to wound closure was 5.6 weeks. 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.

Discussion

While all the wounds treated in the case series were diabetic ulcerations of similar severity, (Wagner Grades II and III), the patient population was heterogenous and had significant comorbidities and therefore may not represent the average diabetic patient with less severe comorbidities. The implication of the positive outcomes we observed with these patients however suggests that the combination therapy would have equal to or greater impact on healthier diabetic patients with foot ulcerations.

Currently our research team is conducting a prospective case series involving a larger sampling of diabetic patients with foot ulcerations being treated with three separate therapy protocols; Anthem™ Wound Matrix monotherapy, PalinGen® amniotic allografting monotherapy, and combination therapy with Anthem™ Wound Matrix and PalinGen® amniotic allografting. Our aim is to better compare the therapies and ascertain a better understanding of the impact of combination therapy. This future publication will include one year follow up of the patients in this case series.

During the course of our investigate we found that by the third or fourth therapy application hypergranular "proud flesh" would develop in the wound which necessitated cauterization to allow normal healing to take place. We also noted on visits that occurred between weekly treatment applications that the Anthem™ Wound Matrix hardened, creating a physical barrier, which we believe helped protect the wound from contamination by external microorganism and keep the amniotic allografts firmly adhered. At the weekly treatment application visits, the Anthem™ Wound Matrix was always found to be fully dissolved.

Previous studies have reported reduction in wound pain and sensitivity with application of amniotic allografts [49] and with synthetic extracellular matrices [50]. All patients in this case series had peripheral neuropathy with complete loss of protective sensation so an assessment of pain improvement was not obtainable. An avenue of future research would be to assess the lessening of pain and sensitivity with combination therapy in sensate patients.

For the purposes of this case series, wound closure was reported to be when the total wound volume decreased by greater than 95% [16]. It is important to note that while all the wounds examined in this case series achieved complete closure by this metric, they did require continued treatment beyond the period of this study to achieve 100% closure.

Conclusion

The outcome of this case series supports the use of amniotic allografting in combination with synthetic extracellular matrices as a therapeutic option for the management of diabetic ulcerations of the foot.

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.

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