Use of AmnioBand™ Membrane, a Human Amniotic Membrane Allograft, in the Management of Chronic Non-Healing Diabetic Foot Ulcers

Case Studies and Clinical Review

Lawrence DiDomenico, DPM FACFAS[1], Jarrod Kaufman, MD FACS[2] ,Thomas E. Serena MD FACS[3]

1 Director: ACFAS Recognized Reconstructive Surgery Fellowship, Youngstown Ohio
2 Private Practice; General Surgery; Premier Surgical and Vein Center; Brick New Jersey
3 President and CEO; Serena Group; Past-President American Professional Wound Healing Association

INTRODUCTION

Diabetes has become a pandemic in the United States of America with public health estimates showing 26 million people or roughly 8.3% of the population having diabetes.[1,2] Current data has shown that one in three Americans born in 2000 is projected to develop diabetes. In 2003, it was reported that in the age group 20-79 there was an estimated 194 million people worldwide with the condition. Epidemiologic data projects that by the year 2025 this number will increase by 72% to nearly 333 million individuals with diabetes.[1] The cascade of metabolic dysfunction that ensues from hyperglycemia leads to peripheral neuropathy and microvascular disease which are the two most common causes of the most feared complications of diabetes: a pedal ulcer. It is well known that almost 25% of diabetics will develop an ulcer in their lower extremity over their lifetime.[3]

Lower extremity amputations are often the result of ulcerations that become recalcitrant to wound care, and nearly 85% of lower extremity amputations in the diabetic are preceded by a foot ulcer.[4] The ultimate goal of wound therapy is to accelerate the healing process in order to prevent complications of limb and life threatening infections and amputations.[5] The current guidelines published by The Wound Healing Society strongly recommend using advanced wound therapies when a diabetic neuropathic ulceration does not decrease its dimensions by 40% or more after four weeks of standard care dressings with local wound care.[6] Sheehan et al. has also proven that if a wound does not heal by 50% in four weeks, that it has less than a 10% chance of healing by 12 weeks.[7] It is well known that wound healing involves three phases: inflammation, proliferation, and remodeling, and most diabetic wounds tend to become stagnant in the first two phases due to inactive fibroblasts, inflammation and increased bioburden.[8]

The goal in wound healing should be to transform a wound from the chronic to the acute state by creating balance between cell degradation and synthesis, to eradicate fibrosis, as well as promote new granulation and complete epithelialization. This is ultimately accomplished by recruiting growth factors and stimulating angiogenesis.[8-10]

The aim of recruiting growth factors to facilitate wound healing has led researchers to examine the role of stem cells.[11] Amniotic membrane tissue has been shown to attract and recruit stem cells to the area of chronic wounds to help them heal. The high healing rates seen in prospective randomized trials on dehydrated amnion and chorion reflect these findings. Recent studies have shown amniotic membrane allografts consisting of dehydrated amnion/chorion membrane (dHACM) effectively heal greater than 90% of diabetic ulcers over 6 weeks with weekly application.[12]

These amniotic membrane grafts have also been shown to be more cost effective, with less wastage and require a lower number of grafts then bioengineered tissue skin substitutes when applied weekly for wound closure in recalcitrant diabetic wounds. Thus, the data suggests that amniotic membrane is a viable option to expedite diabetic wound closure at a very reasonable cost.[13]

AmnioBand™ Membrane, developed by the Musculoskeletal Transplant Foundation (MTF) is an aseptically processed human allograft placental matrix comprised of the amnion and chorion layers. The allograft maintains the structural properties of the extracellular matrix. It is composed of both human amnion and chorion which serves as the membrane cover. It is already well known that amnion and chorion together provide a large variety of growth factors necessary for wound healing, far more than single layer amnion or chorion only grafts.[12-19] This unique allograft does not require freezing or refrigeration as it can be stored at ambient temperature for up to three years. The allograft can be used in a hydrated or dehydrated state as the membrane composition is hydrophilic. The primary indications for use is to serve as a wound scaffold replacement for damaged integumental tissue such as diabetic foot ulcers, venous leg ulcers, pressure ulcers, or for other homologous use.[14]

THE SCIENCE BEHIND THE MEMBRANE[20]

Quantitative microarray analysis confirmed that the aseptically processed AmnioBand Membrane retained key biological components, VEGF, GAG and HA in similar levels compared to native unprocessed tissue ***(Figure 1)***. Extracts from dehydrated amnion/chorion membranes were presented to Human Umbilical Vein Endothelial Cells (HUVECs) to elucidate their angiogenic capacity ***(Figure 2)***. There was increased tube formation in cultures exposed to the amnion/chorion extracts compared to the control (basal medium) ***(Figure 2)***. Culturing HUVECs on the amnion/chorion allografts highlighted cell friendly matrix with cell attachment, matrix deposition and typical tubular configuration ***(Figure 3)***. Natural Human Dermal Fibroblasts (NHDFs) were also cultured on amnion/chorion membranes and found to adhere, proliferate readily and produce matrix proteins, as shown by SEM imaging ***(Figures 4 & 5)***. Furthermore, IHC analysis revealed cultured NHDFs produced different matrix proteins on top of the amnion layer, as is typical during granulation ***(Figure 6)***.

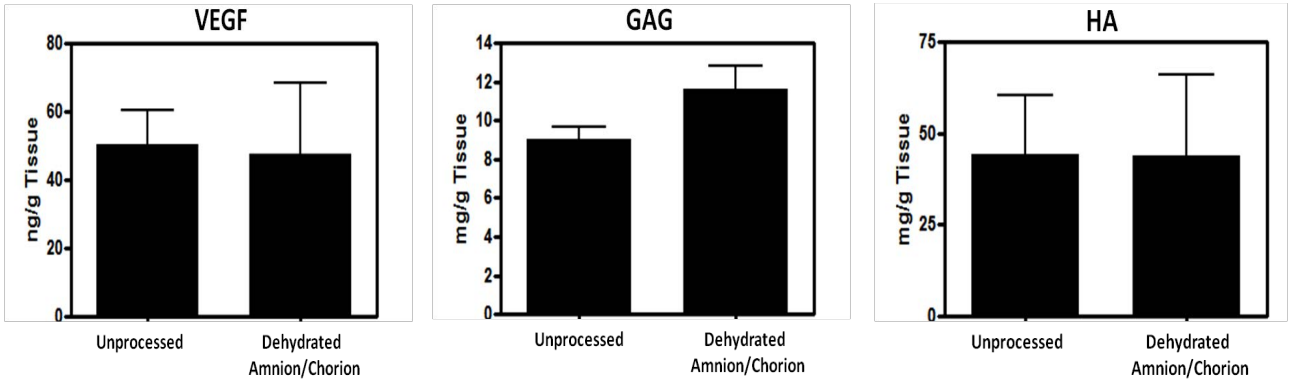

Figure 1: *Quantitative analysis of (a) VEGF; b) GAG; c) Hyaluronic acid (HA) present on dehydrated amnion/chorion tissue. Aseptic processing preserved extracellular matrix components and angiogenic marker, VEGF, in similar levels compared to native unprocessed tissue. GAG and HA support cell migration, attachment and infiltration along with stimulating new matrix deposition. These are critical agents in granulation in normal wound healing.*[8,9]

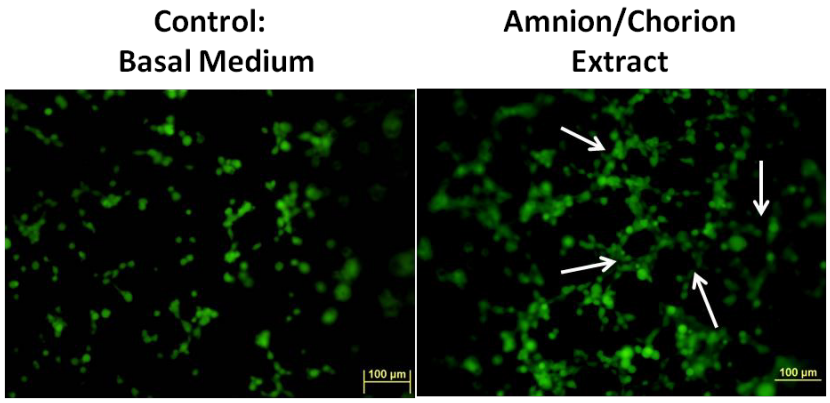

Figure 2: *Angiogenic capacity of HUVECs cultured on Matrigel exposed to amnion/chorion extracts for 7 hours of incubation. Greater tube formation was observed upon exposure to amnion/chorion extracts compared to the negative control.*

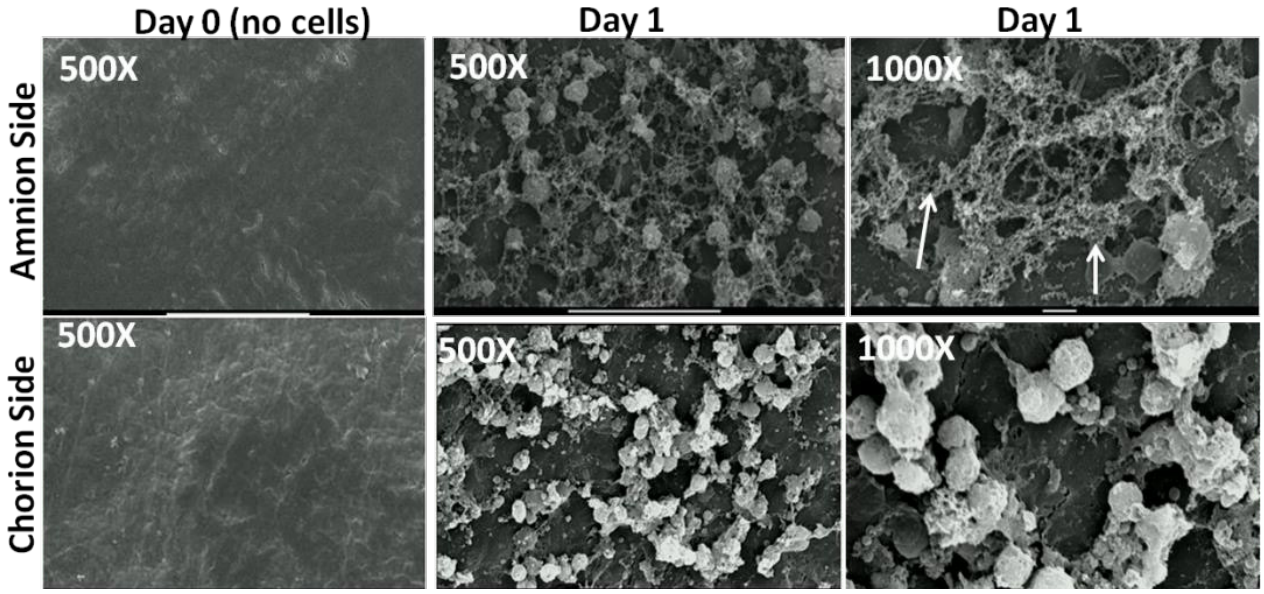

Figure 3: *SEM images of HUVECs cultured on amnion and chorion sides of the allograft. Cells attached within 1 day and secreted matrix in a tubular configuration covering the amnion and chorion surfaces.*

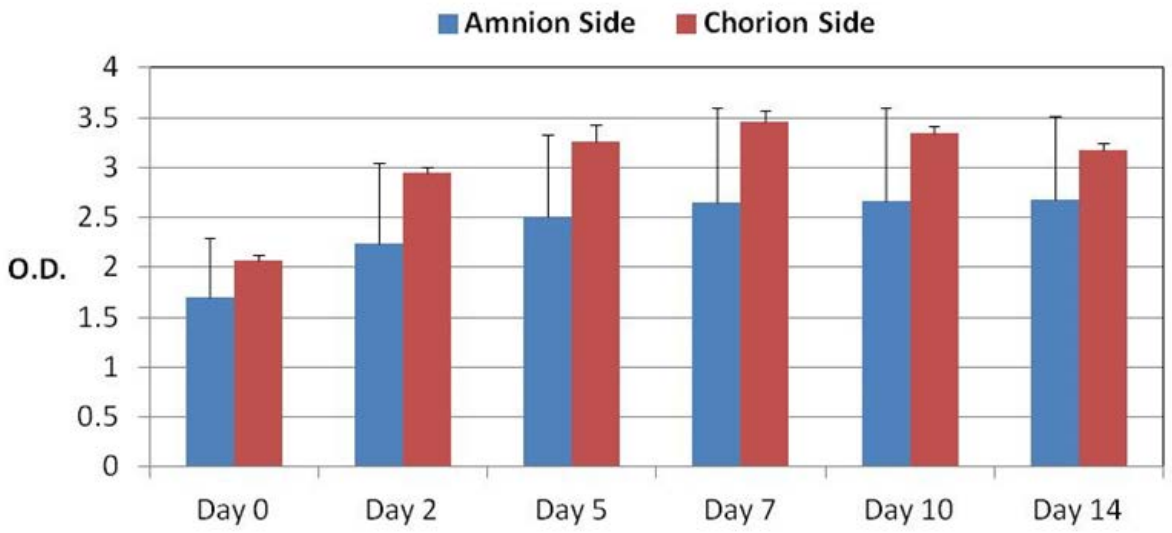

Figure 4: *NHDFs proliferated on both amnion and chorion sides of the allograft, plateauing around day 7. Cell viability was monitored via the CCK-8 assay. The number of living cells is proportional to the amount of formazan dye converted from tetrazolium salts generated by the activity of dehydrogenases in cells.*

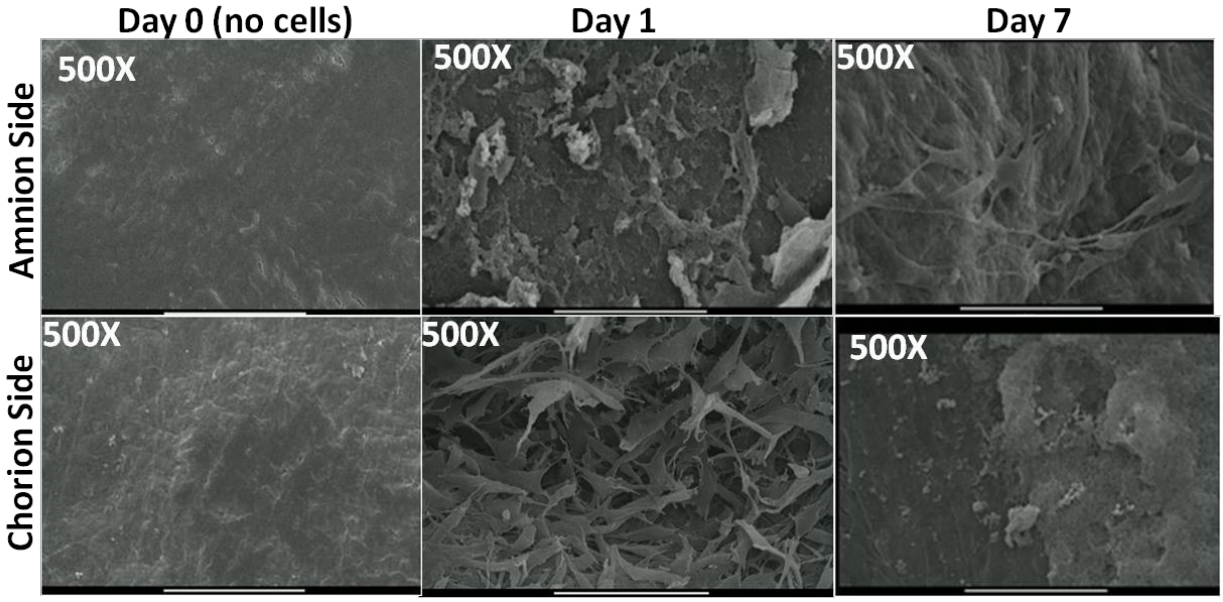

Figure 5: *SEM images of NHDFs cultured on amnion side of graft. Cells adhered and secreted matrix proteins over time covering the amnion and chorion surface.*

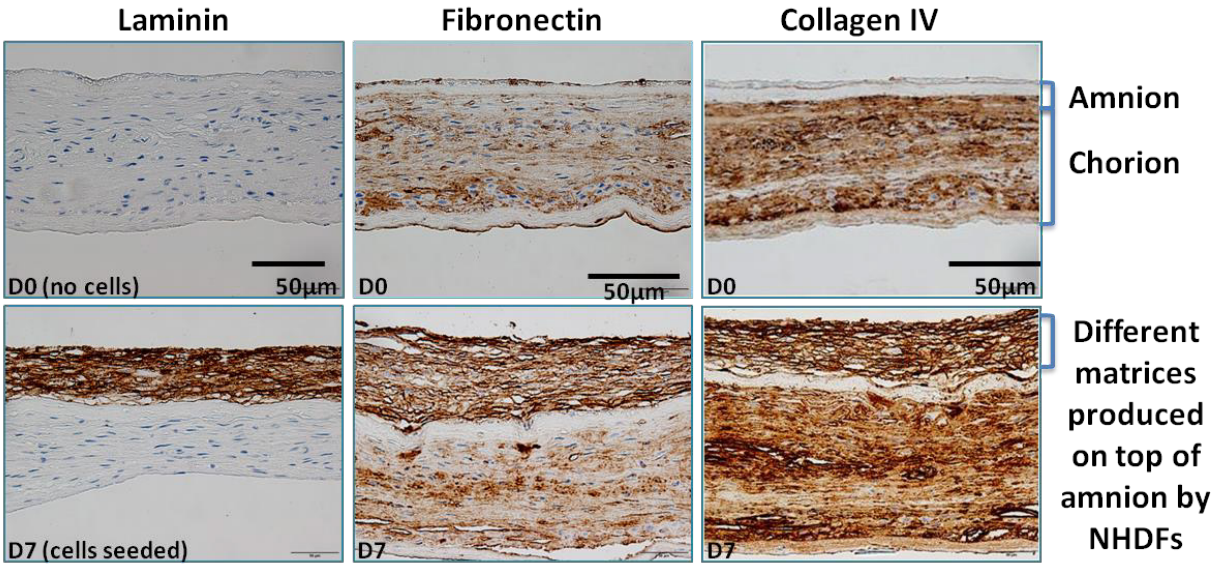

Figure 6: *NHDFs readily adhered on the amnion side of graft and secreted different matrix proteins by day 7. Similar observations were found on the chorion side, with these matrix proteins secreted in a similar fashion by day 7 (magnification 40X). These secreted proteins initiate the granulation process by establishing a new network of matrix proteins, anchoring new matrix to the native tissue, supporting/ organizing cell attachment and migration and facilitating the normal wound healing cascade.*[8,9]

CASE STUDIES

The following case studies highlight the successful use of AmnioBand Membrane in non-healing diabetic foot ulcers.

Case 1

A 61 year-old morbidly obese male presented with a chronic, non-healing right plantar forefoot ulceration present for about 36 weeks, resistant to a variety of conservative wound care modalities including alginates, silvadine and offloading. He is retired from fulltime work and has suffered from diabetic foot ulcers for many years.

Past Medical History: Type two diabetes mellitus with peripheral neuropathy, coronary artery disease, hypertension, hyperlipidemia, and prior ray amputation.

A standard diabetic foot exam was performed that revealed Semmes Weinstein monofilament wire testing, 0/10 points confirming severe peripheral neuropathy. Pulses were 2/4 and a doppler exam was done which revealed biphasic waveforms for both the dorsalis pedis and posterior tibial arteries. An initial hemoglobin A1c level was found to be 10.5%, and serum creatinine was 1.0 mg/dL.

The patient underwent a two week screening period and used a standard of care alginate dressing without improvement. After week two, he received weekly applications of AmnioBand Membrane, and healed the wound successfully at week 6. His final hemoglobin A1c was 8.9%. He was then progressed to diabetic shoes with insoles and a toe filler.

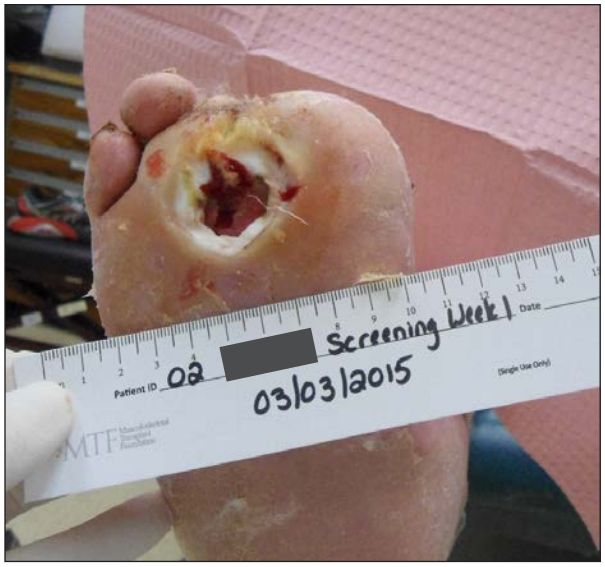

Figure 7a: *Screening Week 1.*
Ulcer History: 36 weeks
HbA1c: 10.5%, Serum Creatinine: 1.0mg/dL
Length: 1.50cm, Width: 2.00cm, Depth: 0.10cm

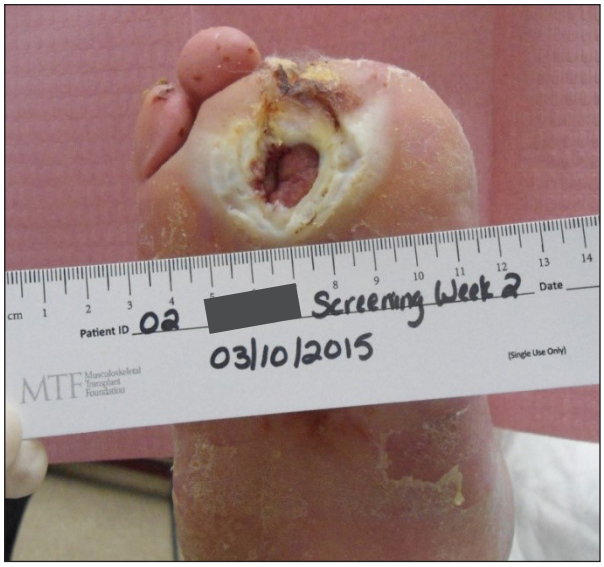

Figure 7b: *Screening Week 2. Ulcer has failed 2 weeks using standard of care with alginate dressings.*
Length: 1.50cm, Width: 2.00cm, Depth: 0.10cm

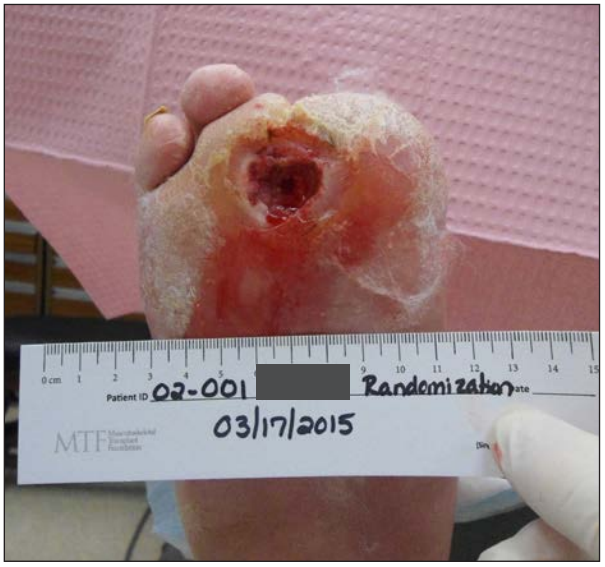

Figure 7c: *Randomization*
Length: 1.50cm, Width: 2.10cm, Depth: 0.10cm

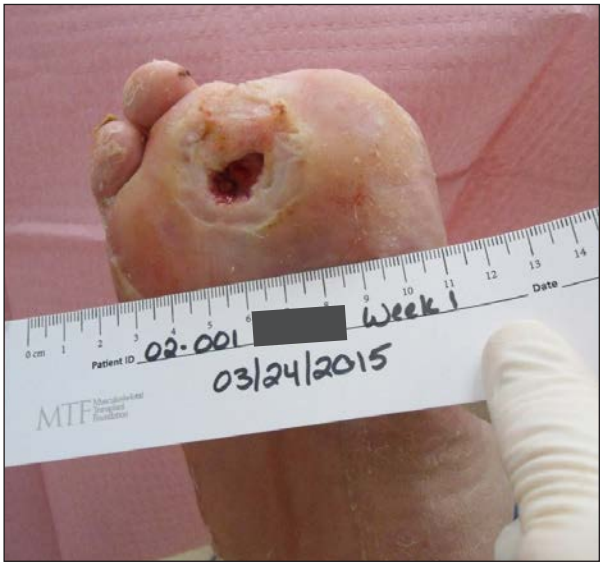

Figure 7d: *Week 1 Amnioband treatment*
Length: 1.00cm, Width: 1.50cm, Depth: 0.10cm

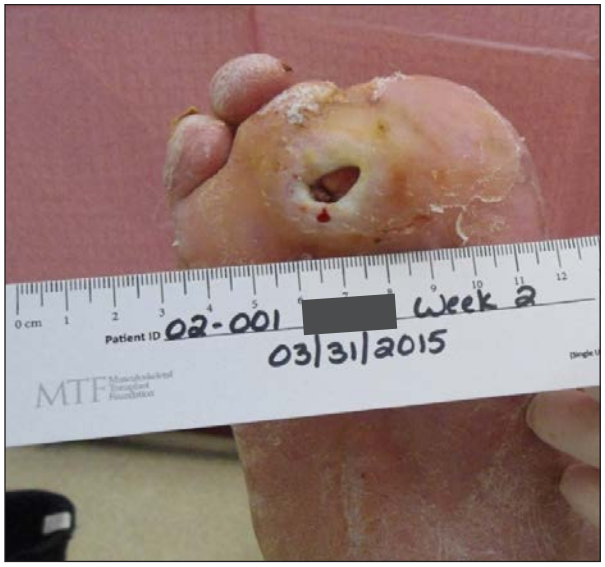

Figure 7e: *Week 2 of AmnioBand treatment.*
Length: 0.50cm, Width: 1.20cm, Depth: 0.10cm

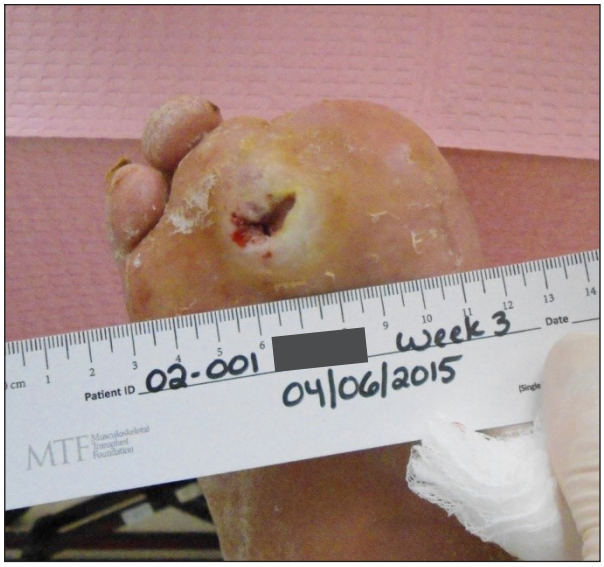

Figure 7f: *Week 3 of AmnioBand treatment*
Length: 0.40cm, Width: 1.20cm, Depth: 0.10cm

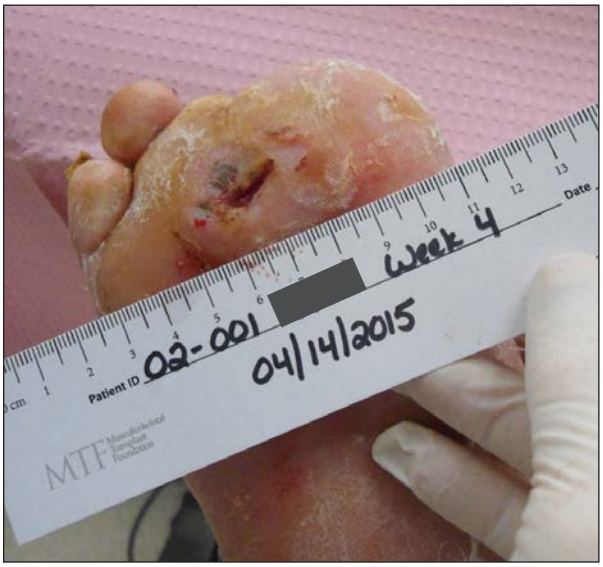

Figure 7g: *Week 4 ofAmnioBand treatment*
Length: 0.30cm, Width: 1.00cm, Depth: 0.10cm

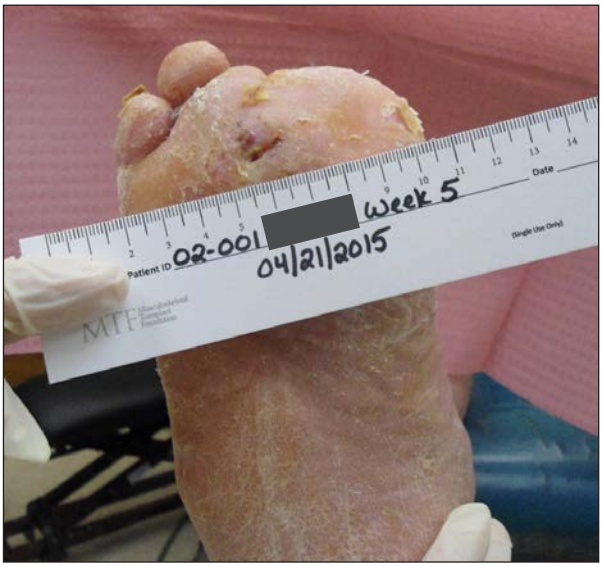

Figure 7h: *Week 5 of AmnioBand treatment*
Length: 0.20cm, Width: 0.50cm, Depth: 0.10cm

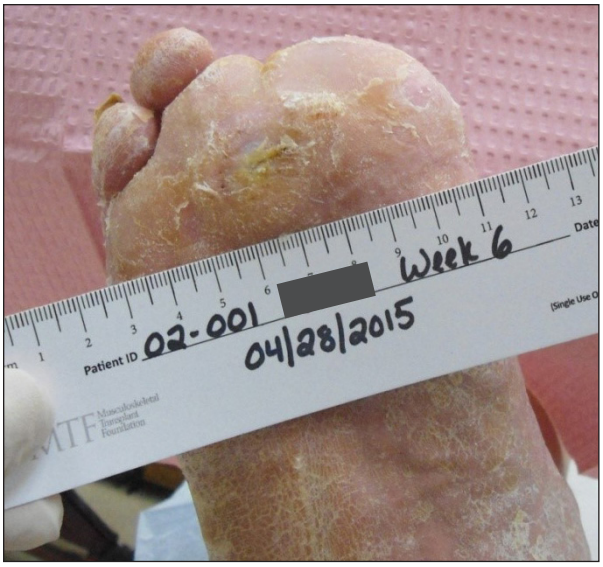

Figure 7i: *After 6 weeks of AmnioBand treatment, the diabetic ulcer has healed.*

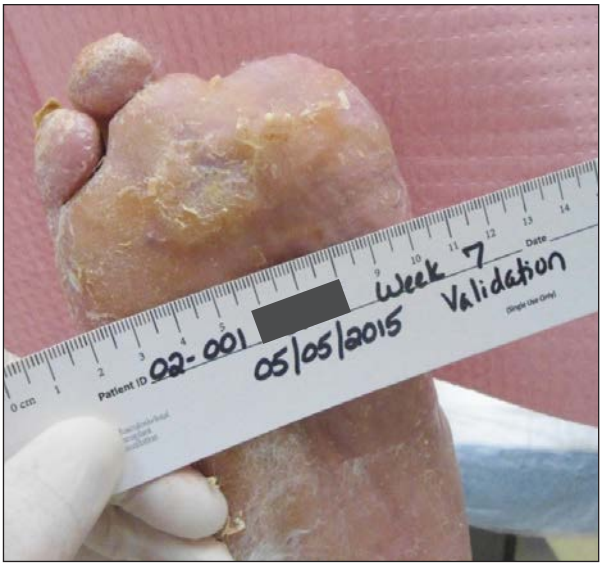

Figure 7j: *Validation Visit 1 week later. Ulcer remains healed with 100% epithelial tissue present.*

Case 2

An 83 year-old caucasian female presented with a non-healing plantar midfoot ulceration to her right foot present for more than 4 weeks, resistant to a variety of wound care treatments including silver alginates and gels as well as multiple offloading devices.

Past medical history: Type two diabetes mellitus with peripheral neuropathy, Addison's Disease, hypothyroidism, GERD, Osteoarthritis, Glaucoma, and stable healed neuropathic fractures to both of her feet.

A standard diabetic foot exam was performed that revealed Semmes Weinstein monofilament wire testing, 0/10 points confirming severe peripheral neuropathy. Pulses were 2/4 and a doppler exam was done which revealed biphasic waveforms for both the dorsalis pedis and posterior tibial arteries. An initial hemoglobin A1c level was found to be 8.2%, and serum creatinine was 0.9 mg/dL.

The patient underwent a two week screening period and used a standard of care alginate dressing without improvement. After week two, she was randomized to receive weekly applications of AmnioBand Membrane. She received a total of 3 treatments and healed without any complications. Her final hemoglobin A1c was: 7.7%. She was progressed to a diabetic shoe with a custom mold.

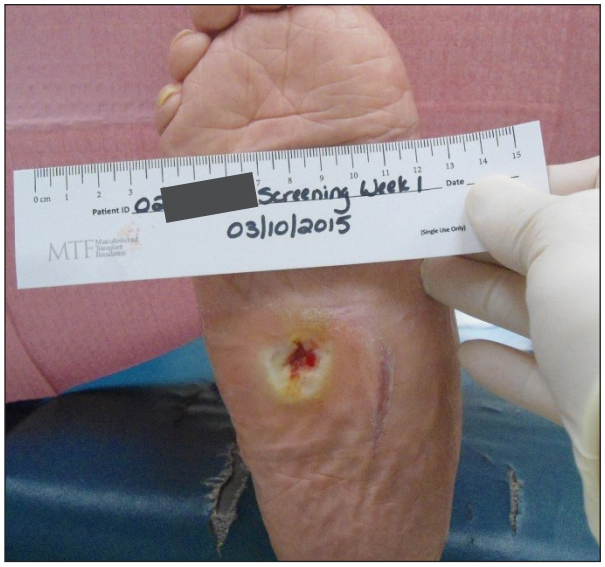

Figure 8a: *Screening Week 1.*
Ulcer History: 4 weeks
HbA1c: 8.2%, Serum Creatinine: 0.9mg/dL
Length: 1.0cm, Width: 1.1cm, Depth: 0.10cm

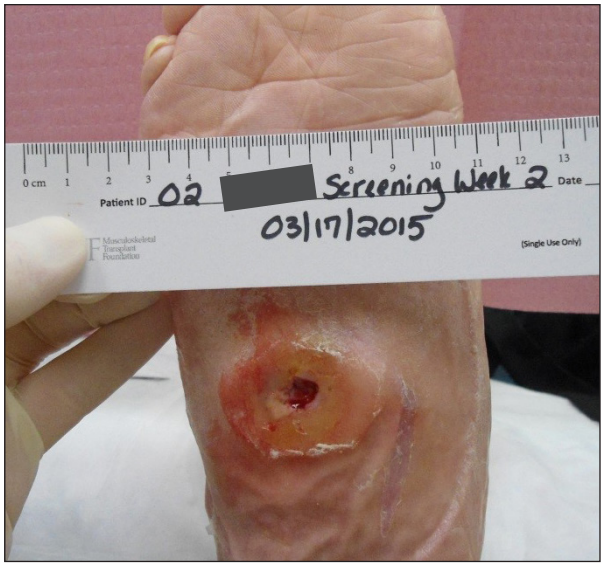

Figure 8b: *Screening Week 2. No change seen using standard of care with alginate dressings.*
Length: 1.0cm, Width: 1.1cm, Depth: 0.10cm

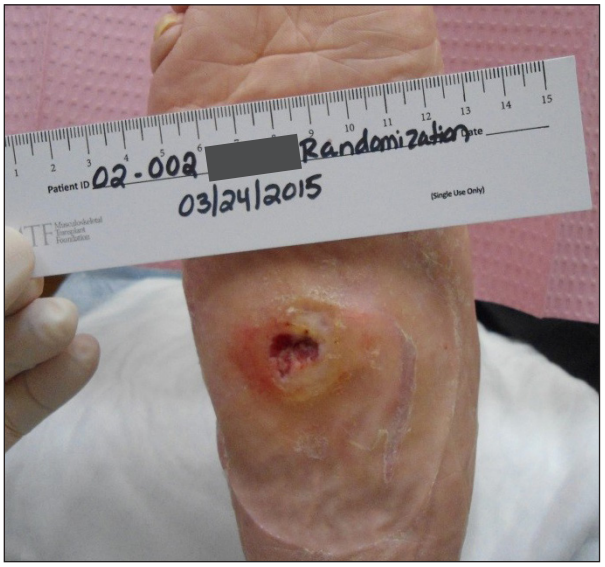

Figure 8c: *Patient randomized to receive AmnioBand treatment.*
Length: 1.0cm, Width: 1.20cm, Depth: 0.10cm

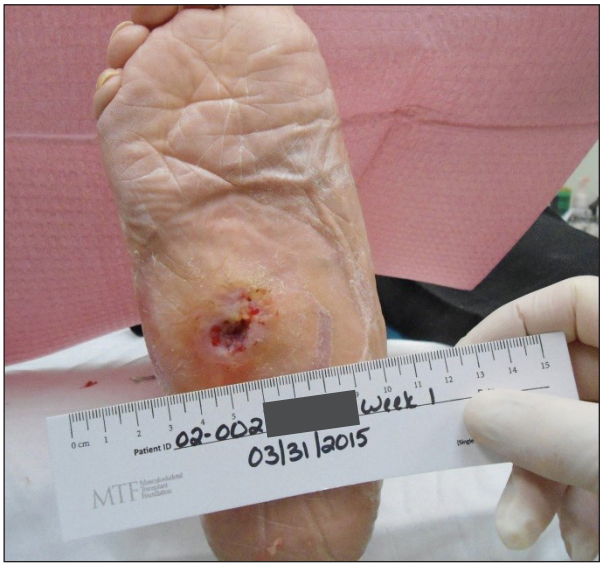

Figure 8d: *After Week 1 of treatment with AmnioBand.*
Length: 0.70cm, Width: 1.00cm, Depth: 0.10cm

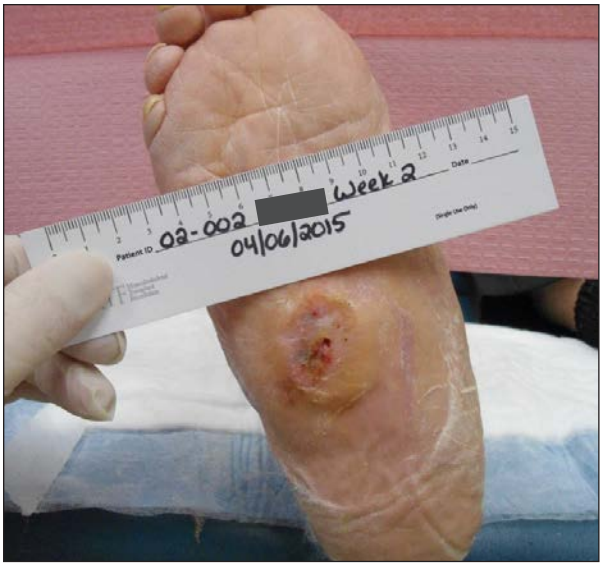

Figure 8e: *Week 2 of treatment with AmnioBand Length: 0.30cm, Width: 0.30cm, Depth: 0.10cm*

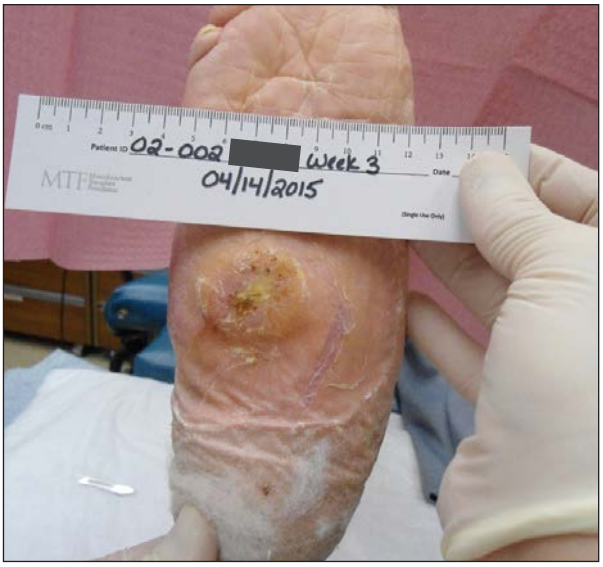

Figure 8f: *Week 3 of treatment. Ulcer has healed.*

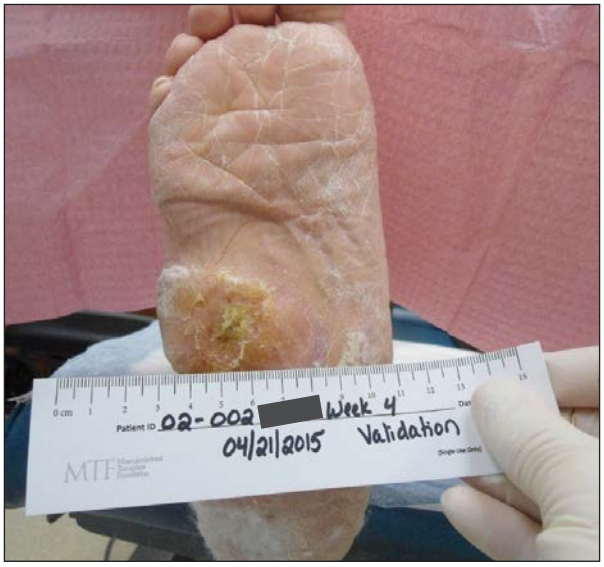

Figure 8g: *Week 4, Validation visit. Ulcer remains healed with 100% epithelial tissue.*

Case 3

A 60 year-old African American male presented with a non-healing plantar lateral left heel ulceration present for about 6 weeks, resistant to a variety of conservative wound care modalities and an off-loading boot.

Past medical history: Type two diabetes mellitus with peripheral neuropathy, cardiovascular disease with previous pacemaker placement, and tobacco use with a greater than 30 packs a year history.

A standard diabetic foot exam was performed to reveal Semmes Weinstein monofilament wire testing at 0/10, confirming peripheral neuropathy. Pedal pulses were noted to be 2/4 and a doppler exam was completed which revealed triphasic waveforms for both the dorsalis pedis and posterior tibial arteries. An initial hemoglobin A1c level was found to be 6.5%, and serum creatinine was 1.3 mg/dL.

The patient underwent a two week screening period and used a standard of care alginate dressing without improvement. After week two, he was randomized to receive weekly AmnioBand Membrane graft, and healed the wound successfully after only one graft application. His final hemoglobin A1c was 5.9%

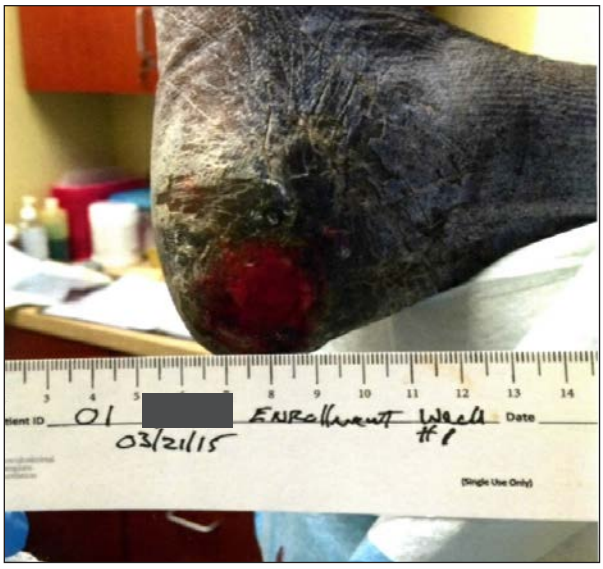

Figure 9a: *Screening Week 1.*
Ulcer History: 6 weeks
HbA1c: 6.5%, Serum Creatinine: 1.3mg/dL
Length: 1.60cm, Width: 2.00cm, Depth: 0.40cm

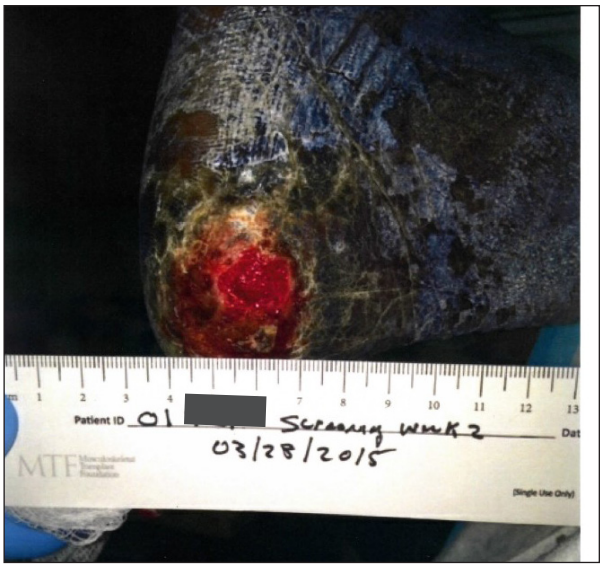

Figure 9b: *Screening Week 2. After 2 weeks of treatment using standard of care with alginate dressings. Length: 1.70cm, Width: 1.80cm, Depth: 0.40cm*

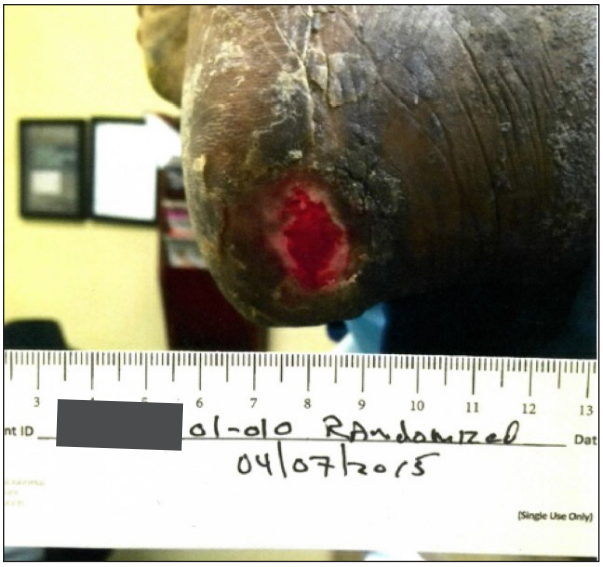

Figure 9c: *Randomization Visit. Patient randomized to receive AmnioBand.*
Length: 1.90cm, Width: 1.80cm, Depth: 0.40cm

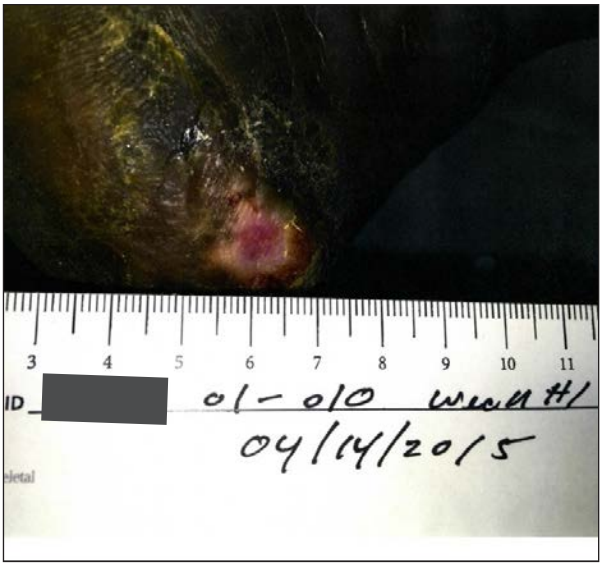

Figure 9d: *First visit after 1 week of AmnioBand treatment.*
Ulcer has healed with 100% epithelial tissue present.

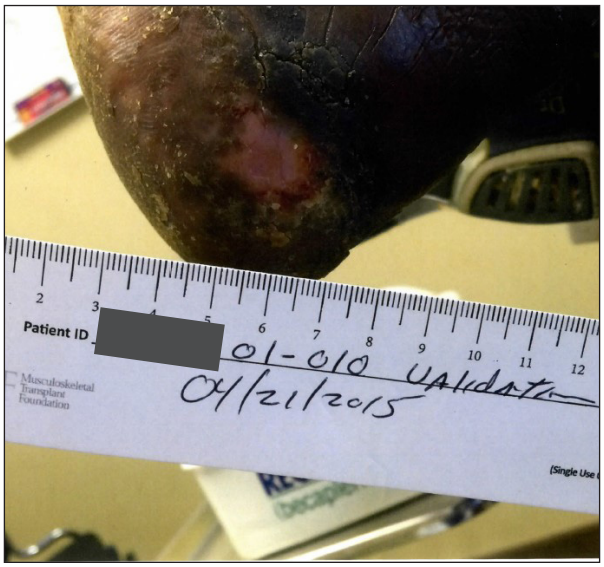

Figure 9e: *Validation Visit. Ulcer remains healed.*

CONCLUSION

Decreasing the time it takes to heal a recalcitrant diabetic wound is critical to prevent future morbidity and allow for limb salvage.[5,11] The majority of non-traumatic lower-leg amputations are in diabetic patients, with 85% of these resulting from non-healing pedal ulcerations that lead to infections.[21]

These case studies presented clearly demonstrate that the abundance of matrix proteins, growth factors, and cytokines present in the AmnioBand Membrane led to great success in each of these wounds. The variety of sizes of AmnioBand Membrane allowed us to start with larger size specific pieces and quickly move to smaller sizes for the final applications, therefore reducing the overall cost to closure and minimizing wastage.

Given all this information the wound care practitioner should give heavy consideration to the use of AmnioBand Membrane as part of their diabetic wound care armamentarium.

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