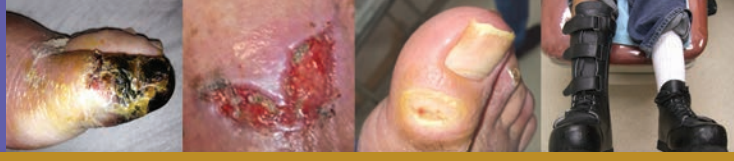


Amniotic Membranes for Diabetic Foot Ulcerations

Here's an update on their use.

BY ALISON MIGONIS, DPM AND JOHN M. GIURINI, DPM

The management of chronic diabetic foot wounds poses a significant economic burden on the healthcare budget of the United States as they and their sequelae are a major source of morbidity and resource consumption for patients with diabetes. In 2007, an estimated $116 billion in direct costs were spent in the treatment of diabetes and its complications. At least one-third of these costs were attributed to the treatment of foot ulcers.[1] The attributable cost for two years following newly diagnosed foot ulceration has been reported to be $28,000.[2] This would explain the significant interest and activity in the development of wound care products that may accelerate wound healing and potentially decrease cost of care.

Wound healing has classically been described as including three phases: inflammatory phase, proliferative phase, and the remodeling or maturation phase. Today, we understand wound healing to be a much more dynamic process achieved by a coordination of cytokines and growth factors. Acute pedal wounds typically proceed through a timely reparative process, resulting in the restoration of anatomic and functional integrity of the foot. Chronic wounds, on the other hand, become stuck in the inflammatory phase, leading to a delay in the formation of granulation tissue. This in turn decreases the tensile strength of a wound due to a decreased level of collagen, extracellular matrix proteins, and fibroblast proliferation. Chronic wounds have also been found to have decreased levels of growth factors, a high bioburden, and increased matrix metalloproteases (MMPs). This results in an imbalance between proteolytic enzymes and their inhibitors and leads to the presence of senescent cells.

Other factors that contribute to delayed wound healing include local factors such as the presence of foreign bodies, ischemia, or infection, as well as systemic factors such as advanced age, malnutrition, tobacco use, uncontrolled diabetes, or renal disease. It has been determined that if a wound does not achieve a 50 percent reduction in wound size after four weeks of standard treatment, the wound should be reassessed and the use of advanced therapeutic agents considered.[3]

Cellular and tissue-based products have been developed with the intent to expedite wound healing in chronic wounds. They have been developed to either provide definitive wound coverage, as an alternative to a split-thickness skin graft, or as part of a staged wound closure process. Cellular tissue products, most commonly Apligraf® and Dermagraft,® have been shown to be effective modalities to improve healing rates in chronic diabetic foot wounds.[4]

Continued on page 90

Today, we understand wound healing to be a much more dynamic process achieved by a coordination of cytokines and growth factors.

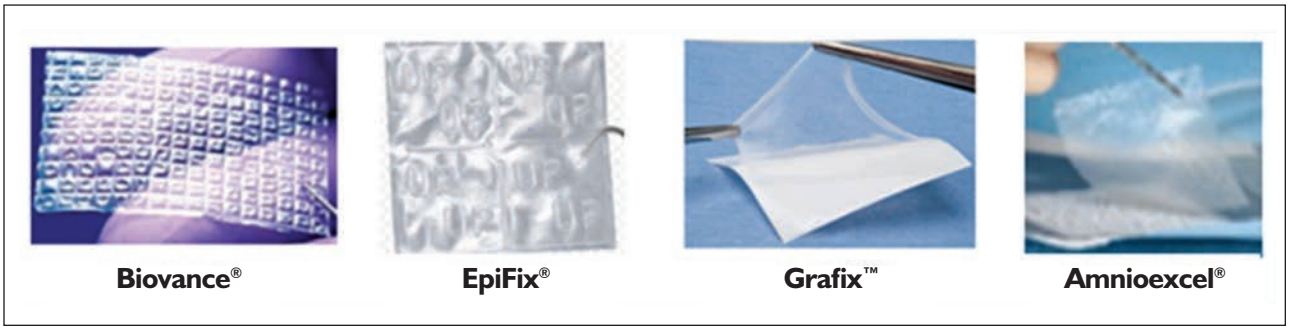
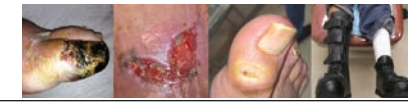


Figure 1: Some available amniotic membrane products



Amniotic Membranes *(from page 89)*

Apligraf® is a bilayered skin equivalent, consisting of both epidermal and dermal components. The outer layer contains allogeneic human keratinocytes while the inner or undersurface contains the dermal layer consisting of human fibroblasts on type 1 collagen dispersed in a protein matrix. In diabetic foot ulcers, Apligraf® was shown to significantly increase the wound healing rate as well as decrease the median time to complete wound closure.[5]

Dermagraft® consists of neonatal dermal fibroblasts cultured in vitro onto a bio-absorbable polyglactin mesh, producing a living, metabolically active tissue containing the normal dermal matrix proteins and cytokines. Dermagraft® has been shown to incorporate quickly into the wound with good vascularization and with no adverse side effects.[6] More recently, amniotic membrane is a cellular tissue product that shows great potential in assisting in wound healing of diabetic foot wounds.

What Is Amniotic Membrane?

The amniotic membrane surrounds and protects the developing fetus in utero. There are two distinct layers: the amnion layer and chorion layer, both derived from the inner layer of the placenta. The amnion lies closest to the fetal side while the chorion is on the maternal side. The amnion consists of a layer of epithelial cells anchored to a basement membrane that underlies compact, collagen-rich tissue. The chorion, which is three to four times thicker than the amnion layer, is composed primarily of dense collagen fibers in an interfibrillar matrix containing proteoglycans and elastic fibers.

The amniotic membrane is a metabolically active tissue that continuously remodels and grows to accommodate the growing fetus. Collagen provides the structural matrix/strength to the amniotic membrane, while the remodeling of this tissue is regulated by growth factors, cytokines, chemokines, and other regulatory molecules produced by the endogenous cells in the amniotic membrane. The amniotic membrane is an avascular structure made up of many structures including collagen, extracellular matrix, and biologically active cells (stem cells). It also contains regenerative molecules including fibroblast growth factor (FGF), platelet derived growth factor (PDGF), keratinocyte growth factor (KGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF) and nerve growth factor (NGF).[7]

An in vitro study comparing growth factor levels between the single layer amnion and the multilayered amnion/chorion showed that the bilayered tissue contained more growth factors than the single layer amnion graft.[8] While the amnion and chorion contain a similar array of growth factors, cytokines, and regulatory factors, the chorion contributed 75% of the overall growth factor content in the amnion/chorion graft. This is likely due to the fact that the chorion is three to four times thicker than the amnion and contains substantially more factors per square centimeter than the amnion.

Amniotic Tissue Processing

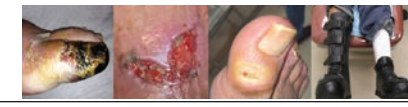
The source of amniotic tissue for use in wound care is donor placental tissue. Due to ethical issues in obtaining, preparing, and storing ma-

Continued on page 92

TABLE 1:

Processing and Preservation Properties of Several Commercially Available Products

Product Name	Company	Layers	Processing	Shelf Life
Biovance®	Alliqua BioMedical	Amnion Layer	Dehydrated	5 years @ Room Temperature
EpiFix®	MiMedx®	Amnion Layer Chorion Layer	Dehydrated	5 years @ Room Temperature
Amnioexcel®	Derma Sciences	Amnion Layer	Dehydrated	5 years @ Room Temperature
Grafix™ Prime	Osiris Therapeutics, Inc.	Amnion Layer	Cryopreserved	2 years in Freezer @ (-75° → -85°)
Grafix™ Core	Osiris Therapeutics, Inc.	Chorion Layer	Cryopreserved	2 years in Freezer @ (-75° → -85°)
Neox® 100 Wound Allograft	Amniox Medical, Inc.	Amnion Layer	Cryopreserved	2 years in Freezer @ (-75° → -85°)



Amniotic Membranes (from page 90)

terial, coupled with the potential for infectious disease transmission, fresh amniotic tissue is no longer utilized in wound care centers. When prepared for use as an allograft, the membrane is harvested immediately after birth, cleansed, sterilized, and processed to remove the cellular components of the graft. Commercially available amniotic membrane products undergo a range of processing and preservation techniques, including dehydration and cryopreservation to guarantee a continuous supply in a clinical setting.[9] There are also a range of membrane configurations, from amnion alone to amnion and chorion. Table 1 represents the processing and preservation properties of several commercially available products.

In addition to the cellular products amniotic tissue provides, there is evidence that it maintains its anti-inflammatory, anti-fibroblastic and antimicrobial properties after cryopreservation or dehydration, resulting in a readily available tissue with regenerative potential.[10] Every method of processing and preservation affects the properties of the biologic material, and there are no studies that directly compare the specific processing techniques. The ideal preservation method not only supports the integrity of structures, but maintains the biological activity of matrix cell signaling factors that are essential as a stimulus for host cells to migrate and infiltrate the graft. The most commonly used method is cryopreservation of human amniotic membrane at—800C in glycerol. Storage in glycerol dehydrates the tissue by replacing most of the intracellular water without altering the osmotic concentration, thus preserving cellular integrity. Amniotic membrane can be safely stored for up to two years.

Cryopreservation is a process that has been shown to not significantly alter tissue structure, collagen, or glycosaminoglycan content. Cryopreservation technique contains high molecular weight hyaluronic acid and the HC-HA complex that has been demonstrated to impart anti-inflammatory and anti-scarring properties of amnion tissue due to the reduction in pro-inflammatory cytokines.[11]

A dehydration process safely and gently separates placental tissues, cleans and re-assembles various layers, then dehydrates the tissue. During this time, the tissue may be freeze-dried or air-dried. This sterilized tissue is then packaged and can be stored at room temperature with a shelf life of five years.[12] The effects of dehydrated human amniotic membrane (dHAM) were evaluated in vitro. It demonstrated adequate cell proliferation and cell migration. Also, following rehydration of the dHAM, ELISA testing revealed that EGF, TGF-b, bFGF and PDGF-BB were found to be released into solution.

It is important to follow the manufacturer's recommendations on application of the material as this differs among products.

TABLE 2:

Characteristics of Amniotic Membrane

- Contains cytokines and essential growth factors
- Bacteriostatic
- Reduces scar tissue
- Provides matrix for migration and proliferation of cells
- Anti-inflammatory
- Non-immunogenic
- Provides a biologic barrier
- Enhances wound healing process
- Decreased pain due to improved hydration of wound bed

Use in Wound Care

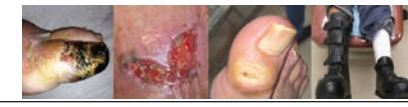
Human amniotic membrane has been used since the latter half of the 20th century for the treatment of wounds, particularly in the field of ophthalmology, including corneal ulcerations, covering large conjunctival lesions and acute chemical burns of the eye.

Amniotic membrane has several characteristics that make it suited for use in wound healing. How amniotic membrane products assist in the reparative process of wounds has not been entirely characterized, but data appears to suggest that the high abundance of growth factors that result from combining amnion and chorion may contribute to the tissues' bioactivity and clinical efficacy of the graft for wound repair and tissue regeneration.[13] The anti-inflammatory properties are thought to be secondary to the expression of TGF-beta and pro-inflammatory cytokines, such as interleukin.[10,14]

What Does the Research Say?

Literature supporting the use of amniotic membrane in diabetic foot wounds is still in the early phases, with few large clinical trials available. In a level 4 case series of 3 patients with recalcitrant diabetic foot ulcers, all patients had a greater than 50% reduction at the end of 4 weeks.[15] In another larger but still level 4 case series of five diabetic foot ulcers, all exhibited complete closure at an average of 7.3 weeks.[16] In a level 3 retrospective case series of 20 patients using cryopreserved amniotic membrane, 18 patients exhibited complete wound healing at

Continued on page 94



Amniotic Membranes *(from page 92)*

12 weeks.[17] Abrams treated 20 patients with dehydrated amnion/chorion membrane (dHACM), all having 100% full closure, with a 42% reduction in cost and 50% reduction in the time to closure.[18]

The efficacy of amniotic tissue products has also been demonstrated in clinical trials. In a prospective, randomized single center study, there were 25 patients with a diabetic foot ulcer of at least 4 weeks duration. One group was treated with dehydrated human amnion/chorion membrane (dHACM) and standard wound care, the control group with standard wound care alone. At 4 and 6 weeks, the dHACM group had an overall healing rate of 77% and 92%, respectively, as compared to the standard of care, which had overall healing rates of 0% and 8%.[19]

In a multicenter randomized controlled trial of weekly dHACM as an adjunctive therapy to standard of care, 40 patients were randomized to the dHACM or standard of care (SOC) alone. At 12 weeks, 85% of DFU healed compared to 25% in the SOC. Mean time to healing was 36 days vs. 70 days in the SOC group.[20]

Zelen, et.al. conducted a prospective randomized controlled multi-center comparative study comparing dHACM vs. biological skin substitute (BSS) vs. standard of care. Average healing time for wounds with dHACM was 23.6 days, 47.9 days for the biologic skin substitute (BSS), and 49 days for the standard of care. Complete healing of wounds at 6 weeks for DHACM was 97%, 45% for BSS, and 35% with standard care. Also, this demonstrated the cost effectiveness of DHACM vs. BSS, as fewer grafts were required to achieve complete healing.[13]

Lavery, et al. performed a multicenter, randomized, controlled clinical trial evaluating the use of cryopreserved amniotic membrane on chronic diabetic foot ulcers. Fifty patients received cryopreserved amniotic membrane and 47 received the standard of care. At 12 weeks, 62% healed in the cryopreserved amniotic membrane group, while 21% healed in the control group.[21] Thus, their effectiveness in the long-term, including limb salvage and recurrence rates, and cost-effectiveness is currently lacking.

Application Process

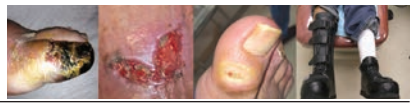
The wound bed is prepared as with any advanced wound care product through adequate debridement. The wound should be free of all necrotic and fibrous tissue and there should be no signs of infection. Debridement has been shown to create an inflammatory response which will signal the migration and proliferation of stem cells and growth factors provided within the patient's body.[18]

Amniotic membrane products come in a variety of sizes, ranging from 1 cm2 to greater than 100 cm2. Amniotic membrane tissue is packaged in a sterile container. It is laid on the wound, ensuring that the stromal collagen layer is facing the wound. It is important to follow the manufacturer's recommendations on application of the material as this differs among products. There is no need to suture the material in place, but Steri-strips may be used to hold the graft in place. A secondary non-adherent dressing is then applied to maintain moist wound healing. Within 1–2 weeks, the amniotic membrane is typically incorporated into the wound. Improvement in the size and depth occurs typically within 2–3 weeks, sometimes sooner. There currently is no evidence to say whether weekly applications are more efficacious than biweekly applications.

Conclusion

Chronic wounds continue to be both a major health burden and economic burden on the healthcare system worldwide. Despite the increased knowledge and the many technological advances in wound care products, the fundamental principles of wound care—debridement, off-loading, infection, and reperfusion—remain the standard of care. Amniotic membrane products are the newest proactive wound care products available for chronic, non-healing diabetic wounds and their use is on the rise. Although there currently is limited data regarding most amniotic membrane-based products, there is substantial clinical evidence supporting the rationale and effectiveness of amniotic membrane allograft as an adjunct to standard wound care regimens for chronic diabetic foot ulcerations. Ongoing and future studies will further define

Continued on page 95



Amniotic Membranes *(from page 94)*

and establish the value of amniotic membrane, and its cost-effectiveness. **PM**

References

[1] Driver, Vickie R., et al. "The costs of diabetic foot: the economic case for the limb salvage team." Journal of vascular surgery 52.3 (2010): 17S-22S.

[2] Ramsey, Scott D., et al. "Incidence, outcomes, and cost of foot ulcers in patients with diabetes." Diabetes care 22.3 (1999): 382-387.

[3] Sheehan, Peter, et al. "Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial." Diabetes care 26.6 (2003): 1879-1882.

[4] Garwood, Caitlin S., John S. Steinberg, and Paul J. Kim. "Bioengineered alternative tissues in diabetic wound healing." Clinics in podiatric medicine and surgery 32.1 (2015): 121-133.

[5] Veves, Aristidis, et al. "Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers." Diabetes care 24.2 (2001): 290-295.

[6] Hansbrough, John F., et al. "Evaluation of a biodegradable matrix containing cultured human fibroblasts as a dermal replacement beneath meshed skin grafts on athymic mice." Surgery 111.4 (1992): 438-446.

[7] Garwood, Caitlin S., and John S. Steinberg. "What's new in wound treatment: a critical appraisal." Diabetes/metabolism research and reviews 32.S1 (2016): 268-274.

[8] Koob, Thomas J., et al. "Cytokines in single layer amnion allografts compared to multilayer amnion/chorion allografts for wound healing." Journal of Biomedical Materials Research Part B: Applied Biomaterials 103.5 (2015): 1133-1140.

[9] Ilic, Dusko, et al. "Human amniotic membrane grafts in therapy of chronic non-healing wounds." British medical bulletin 117.1 (2016): 59-67.

[10] Laranjo, Mafalda. "Preservation of Amniotic Membrane." Amniotic Membrane. Springer Netherlands, 2015. 209-230.

[11] Arnold, Y., Leroux JD, Williams M., Danilkovitch. A., A Comparison study of anti-inflammatory effecs of cellular versus acellular human repair matrices. In: Symposium on Advanced Wound Care; 2013 May 2-5; Las Vegas (NV).

[12] Koob, Thomas J., et al. "Properties of dehydrated human amnion/chorion composite grafts: implications for wound repair and soft tissue regeneration." Journal of Biomedical Materials Research Part B: Applied Biomaterials 102.6 (2014): 1353-1362.

[13] Sheikh, Emran S., Ednan S. Sheikh, and Donald E. Fetterolf. "Use of dehydrated human amniotic membrane allografts to promote healing in patients with refractory non healing wounds." International wound journal 11.6 (2014): 711-717.

[14] ElHeneidy, Hossam, et al. "Amniotic membrane can be a valid source for wound healing." International journal of women's health 8 (2016): 225.

[15] Shah, Alap P. "Using amniotic membrane allografts in the treatment of neuropathic foot ulcers." Journal of the American Podiatric Medical Association 104.2 (2014): 198-202.

[16] Penny, H., et al. "Dehydrated human amnion/chorion tissue in difficult-to-heal DFUs: a case series." J Wound Care 3.104 (2015): 106-109.

[17] Werber, Bruce, and Erin Martin. "A prospective study of 20 foot and ankle wounds treated with cryopreserved amniotic membrane and fluid allograft." The Journal of Foot and Ankle Surgery 52.5 (2013): 615-621.

[18] Abrams, M. "Our experience utilizing advanced wound therapy combined with an evidence-based approach to threatening wounds reduces amputations in the Caribbean healthcare system." Poster presentation, Desert Foot (2012).

[19] Zelen, Charles M., et al. "A prospective randomized comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers." International wound journal 10.5 (2013): 502-507.

[20] DiDomenico, Lawrence A., et al. "Aseptically Processed Placental Membrane Improves Healing of Diabetic Foot Ulcerations: Prospective, Randomized Clinical Trial." Plastic and Reconstructive Surgery Global Open 4.10 (2016).

[21] Lavery, Lawrence A., et al. "The efficacy and safety of Grafix® for the treatment of chronic diabetic foot ulcers: results of a multi-centre, controlled, randomised, blinded, clinical trial." International wound journal 11.5 (2014): 554-560.

Dr. Migonis is Chief Resident, Division of Podiatric Surgery at Beth Israel Deaconess Medical Center, and a Clinical Fellow at Harvard Medical School.

Dr. Giurini is Chief, Division of Podiatric Surgery at Beth Israel Deaconess Medical School and an Associate Professor in Surgery at Harvard Medical School.