EPIFIX® Dehydrated Human Amnion/Chorion Membrane (dHACM) Therapy: The New Standard in Bioactive Wound Healing
PRESENTERS

William W. Li, MD, is President, Medical Director, and Co-Founder of the Angiogenesis Foundation. He has been actively engaged in angiogenesis research and clinical development for three decades. He has held appointments on the clinical faculties of Harvard Medical School, Tufts University, and Dartmouth Medical School. Dr. Li is a Founding Director of the American College of Wound Healing and Tissue Repair, an Honorary Fellow of the American College of Wound Care Specialists, and has served as advisor and consultant to leading global public and private companies. Dr. Li is involved in national and international efforts to advance the applications of angiogenesis-based therapeutics across diverse medical fields, including oncology/hematology, cardiology, ophthalmology, vascular surgery, dermatology, and wound care. Dr. Li has been published in many leading peer-reviewed medical journals. Dr. Li has disclosed that he is a consultant to MiMedx, Novadaq and Smith & Nephew.

Lisa J. Gould, MD, PhD, FACS, is an Affiliate Professor in the Department of Molecular Pharmacology and Physiology at the University of South Florida. She has served on the executive board of the Wound Healing Society for more than 10 years and is an active participant in multiple academic societies. She is also the Director of the Wound Recovery and Hyperbaric Medicine Center at Kent Hospital, Warwick, Rhode Island. Dr. Gould is board certified by the American Board of Plastic Surgery, achieved a Certificate of Added Qualifications in Hand Surgery, and is a Fellow of the American College of Surgeons. She has been practicing plastic and reconstructive surgery with an emphasis on difficult wound problems since 1999, serving as Chief of Plastic Surgery at the James A. Haley Veterans Hospital (2007-2012), where she provided wound and surgical care for our nation’s wounded warriors, including treatment of pressure sores in the 100-bed spinal cord unit. Dr. Gould has disclosed that she is a consultant to MiMedx.

Vickie R. Driver, DPM, MS, FACFAS, is a Professor of Orthopedic Surgery (Clinical) at the Brown University School of Medicine, the Division Chief of Podiatric Surgery for the New England VA Healthcare Medical Center in Providence, R.I., and the Director of Research for Hyperbarics and the Wound Care Center at Rhode Island Hospital. She is also the President of the Board of Directors for the Association for the Advancement of Wound Care (AAWC), and has served on the AAWC Board of Directors for seven years. She is the Fellow of the American Board of Foot and Ankle Surgery. A well-known national and international educator, researcher and surgeon in the fields of limb preservation and wound healing, Dr. Driver has published over 100 peer-reviewed journal articles and abstracts, and participated as an investigator in over 60 clinical research trials. She has directed post-doctoral fellowship training in both wound healing and limb preservation for 15 years. Dr. Driver has disclosed that she is a scientific advisor to MiMedx.

Gary Gibbons, MD, is the Medical Director of the South Shore Hospital Center for Wound Healing in Weymouth, Massachusetts, and a vascular surgeon by training. Dr. Gibbons was the first director of the Deaconess/Joslin Diabetic Wound Center, dedicating his career to diabetic patients with wounds complicated by peripheral arterial disease who are in need of revascularization. Dr. Gibbons is a Professor of Surgery at the Boston University School of Medicine, and has been in practice for over 40 years. Dr. Gibbons has disclosed that he is a clinical consultant to MiMedx, Spiracur, Celleration and Osiris Therapeutics.

Paul Glat, MD, is the Director of the Division of Plastic Surgery, Director of the Burn Unit, and Director of Cleft Palate and Craniofacial programs at St. Christopher’s Hospital for Children in Philadelphia. Dr. Glat is consistently in demand as a guest lecturer at plastic and reconstructive surgery meetings in the United States and abroad. His writings and research have appeared in several major plastic surgery textbooks and over 90 plastic surgery publications. Dr. Glat has no potential conflicts of interest to disclose within the context of this supplement.
EPIFIX® Dehydrated Human Amnion/Chorion Membrane (dHACM) Therapy:
THE NEW STANDARD IN BIOACTIVE WOUND HEALING

INTRODUCTION
A multidisciplinary expert advisory panel of five leading physicians was convened in October, 2014, in Boston, Massachusetts to determine the appropriate use of amniotic tissue products for the treatment of acute and chronic wounds. Primary objectives of the expert advisory panel were to discuss how preserved cytokines and chemokines are helping define the multifaceted roles of EpiFix®, a dehydrated human amnion/chorion membrane (dHACM) allograft (MiMedx Group Inc., Marietta, GA), in the treatment of diabetic foot ulcers and venous leg ulcers; to review the growing body of scientific and clinical evidence associated with EpiFix®; and to exchange practical information regarding the use of EpiFix® in addressing challenges in plastic, cosmetic and reconstructive surgery.

This supplement was created to provide an overview of the discussions and emerging data supporting the clinical use of EpiFix®. Topics of this supplement include current issues and advances in treating acute and chronic wounds in the United States; the evolution and emergence of amniotic membrane tissues in wound care; different processing techniques and their effects on amniotic membrane grafts in facilitating healing; the unique structure of EpiFix®; and functional differences between EpiFix® and other amniotic membrane tissue products.

THE PROBLEM OF CHRONIC WOUNDS AND AMNIOTIC MEMBRANE AS A TREATMENT OPTION
Acute and chronic wounds are prevalent and burdensome to patients and the U.S. health care system. It has been estimated that 1 to 2% of the population will experience a chronic wound during their lifetime. In the U.S. alone, chronic wounds affect 6.5 million patients. Effective treatments for chronic wounds such as diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs) are urgently needed to help reduce time to healing, associated treatment costs, and the risk of further complications. It is estimated that up to 25% of all people with diabetes will develop a diabetic foot ulcer, while venous ulcers account for 70%-90% of ulcers found on the lower leg.

“The chorion membrane from the amniotic sac is not directly associated with maternal tissue and therefore would not promote an immune response if transplanted.”
— Dr. Gould

Amniotic membrane tissue is a treatment option that has been shown to facilitate healing of these difficult wounds. However, to make good clinical decisions regarding the use of amniotic membrane tissue, it is important to understand the nature of the tissue, the characterization of different tissue layers as well as the different processing techniques for various amniotic membrane products and the associated healing rates.
AMNIOTIC TISSUE: ORIGIN OF AMNION AND CHORION

Amniotic membrane, or the amniotic sac, is comprised of an inner layer called the amnion and an outer layer called the chorion. The amniotic sac is formed after conception and during the fetal maturation process. The chorionic plate is intertwined with the placenta and is often confused with the chorion membrane, which is part of the amniotic sac and does not come in contact with maternal tissue (Figure 1). As the embryo continues to grow, the amnion layer surrounding the fetus expands and conjoins with the chorion within the first trimester of development to become the amniotic sac. Amniotic membranes are composed principally of three types of material: structural collagen and extracellular matrix, biologically active cells and a large number of important regenerative molecules.

The use of amniotic membrane in the clinical setting has a history spanning over 100 years. At present, amniotic products are delivered in multiple configurations, including single and multi-layer formats, and they are processed with varying techniques. Since all amniotic tissues are not processed in the same way, the preserved tissues cannot be viewed as equal with respect to their healing properties.

PURION® PROCESSING OF EPIFIX® — THE POWER OF AMNION AND CHORION

When the amniotic sac is being processed for use in the clinical setting, the specific processing technique can affect the preservation of proteins like growth factors, cytokines, and chemokines, thus affecting the stimulus for host cells to migrate, infiltrate and engraft into the tissue. EpiFix® features both the amnion and chorion layers and, due to the proprietary PURION® Process utilized, retains a substantial amount of growth factors to enhance wound healing.

The PURION® Process for EpiFix® includes gentle separation of the placen-
tal tissues, removal of the spongy layer from between the amnion and chorion layers (Figure 2), cleansing, reassembling of the amnion and chorion layers, and gentle tissue dehydration. The PURION® Process removes blood components while protecting the intricate extracellular matrix (ECM) of the amnion and chorion membrane, leaving intact cells and the extracellular matrix. When dehydration is completed, the graft is cut to multiple sizes, packaged, and sterilized, yielding an EpiFix® allograft that can be stored at ambient conditions for up to 5 years.

“The EpiFix® graft has more cytokines and chemokines than amnion alone and as a result of combining both the amnion and chorion layers, it delivers a meaningful clinical difference we can see in the office.”  
— Dr. Glat

As mentioned earlier, different processing techniques yield varied quantities of preserved growth factors, cytokines, and chemokines. It is known that some amniotic products use harsh chemicals and detergents during the processing phase that may remove the majority of cellular materials, including cells, blood, and soluble growth factors. In comparison, as described above, the PURION® Processed EpiFix® uses a gentle cleansing wash that removes the maternal blood while preserving the growth factors, cytokines, chemokines and extracellular matrix without damaging the cells. The PURION® Process retains the native cells and pericellular matter in the tissue after processing.

EPIFIX® AND SCIENTIFIC RIGOR

To further outline the unique benefits of the processing technique utilized for EpiFix®, a series of published scientific papers in collaboration with the Georgia Institute of Technology and the Stanford University Medical School characterizing PURION® Processed EpiFix® was discussed by the panel members. They reviewed...
proposed mechanisms of action reported in each of the papers. The panelists also discussed published scientific papers comparing processed amnion-only grafts versus two MiMedx® grafts, AmbioDry2™, a single layer amnion opthalmic graft, and EpiFix®, an amnion and chorion layered graft for the preservation of wound healing and inflammatory regulators (Figures 3A and 3B). An additional animal study demonstrated that the PURION® Process was more effective in preserving wound healing and inflammatory regulators than other processed tissues tested.9

DIFFERENCES IN TYPES OF SKIN SUBSTITUTES

The effectiveness of cellular versus bio-preserved cellular materials in treating wounds was debated between panel members. Amniotic membrane tissues are ideal for transplantation as they have been found to be immunologically privileged and do not require decellularization. This differentiates amniotic tissue from a xenograft, which is derived from animal tissues and needs to be decellularized to reduce a patient’s immunological response to the implanted material. Cellular products can contain living or non-living cells. Living cell grafts can contain keratinocytes, fibroblasts and/or other cells that secrete growth factors to help induce a patient’s healing response. The cells act as simple delivery systems for introducing growth factors into the wound. It has been reported, however, that the cells only survive up to 72 hours in the wound environment.10

EPIFIX®: A BIOACTIVE TISSUE MATRIX ALLOGRAFT

EpiFix® is a bioactive tissue matrix that contains bio-preserved intact, non-living cells (Figure 4). There are a multitude of growth factors and cytokines contained within the EpiFix® allografts even though the cells are not viable. It has been demonstrated that EpiFix® induces fibroblasts to proliferate, migrate, and upregulates basic fibroblast growth factor (bFGF), granulocyte stimulating factor (GCSF) and placental growth factor (PIGF) biosynthesis.7

<table>
<thead>
<tr>
<th>Regulators of Wound Healing in EpiFix®</th>
<th>Regulators of Inflammation in EpiFix®</th>
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<tbody>
<tr>
<td>CYTOKINES</td>
<td>CYTOKINES</td>
</tr>
<tr>
<td>Ang</td>
<td>GCSF</td>
</tr>
<tr>
<td>ANG-2</td>
<td>GM-CSF</td>
</tr>
<tr>
<td>bFGF</td>
<td>GDF-15</td>
</tr>
<tr>
<td>BMP-5</td>
<td>IFNγ</td>
</tr>
<tr>
<td>BDNF</td>
<td>IL-1α</td>
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<tr>
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<td>IL-1β</td>
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<tr>
<td>EGF</td>
<td>IL-1ra</td>
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<tr>
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<td>IL-12p40</td>
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<tr>
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<td>TIMP-2</td>
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<td>TIMP-4</td>
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Figure 4. EpiFix® contains intact cells and different amounts of various growth factors.

“...not all amniotic products are identical, and clinicians should understand the differences in regard to membrane layers, processing, preservation of growth factors, and clinical outcomes.”

— Dr. Li

Figure 5. There is a long list of growth factor cytokines and chemokines included in dHACM, some of which are known to regulate inflammation and wound healing.4-8
EpiFix® has been shown to contain more than 50 cytokines and growth factors (Figure 5), and to promote fibroblast and human microvascular endothelial cell proliferation in vitro.5-8 The cellular signals contained in the tissue upregulate the production of angiogenic factors, and they recruit and promote engraftment of endogenous progenitor cells, including hematopoietic cells, likely via stromal cell-derived factor-1 (SDF-1) and other growth factors. This indicates that angiogenesis and postnatal vasculogenesis could be contributing modes of action for EpiFix® activity, and that the bioactive nature causes the surrounding cells to respond by upregulating biosynthesis of growth factors to promote healing.9

An important consideration is how the growth factors interact with each other. Amnion and chorion contain different amounts and types of the various growth factors. The chorion layer is four to five times thicker and contains more growth factors as a result (Figure 6). EpiFix® contains about 20 times more chemokines and cytokines than competitive products comprised of amnion alone (Figure 7).5 In addition, dermal fibroblasts have demonstrated an increase in production of bFGF, GCSF, and PI GF when cultured in the presence of 5 and 10 mg/mL EpiFix® extract. These are distinguishing factors that set EpiFix® apart from amnion-only grafts.

**A DEEPER LOOK AT ANGIOGENESIS AND WOUND HEALING**

When a wound is present, it is vital to be able to restore the perfusion provided by the tissue capillaries. Microcirculation plays a significant role in angiogenesis because the complex branching patterns that occur are specialized depending on the tissue in which they reside. Endothelial cells form channels in various ways depend-
on the tissue, and if the goal is to restore capillaries, there must be an understanding of what control signals are required to rebuild blood vessels within the tissue in which they are growing. Authors of this study proposed that the quick response may result from the ability of EpiFix® to stimulate fibroblasts to secrete other important growth factors that in turn stimulate other cell types present in a wound to release their own set of growth factors. After appropriate sharp debridement and moist wound care, EpiFix® modulates the chronic wound environment, resulting in normalization of many of the dysfunctional cellular responses. During this process, growth factors and cytokines from EpiFix® amplify secretion of additional growth factors from endothelial cells to help accele-
ate wound healing. Additional growth factor signaling is important and not limited to just a single growth factor, but the multitude of growth factors that are contained in EpiFix® and may be secreted by responding host cells at the wound site. These same factors can be produced by endogenous platelets, neutrophils, monocytes, macrophages, and stem cells that are helping the healing process.

Stem cell participation occurs fairly early within the wound-healing cascade. When stem cells, a stem cell magnet such as EpiFix®, or growth factors like SDF-1 are added into this process, the biology of wound healing is presumably being boosted. The optimal time to get stem cells into a wound space is unclear.

When there is a stem cell magnet recruiting endogenous stem cells, these native stem cells would migrate from the surrounding tissue or through the circulation to the local injured tissue as opposed to being injected or placed topically in a wound as would be the case with a living cellular therapy. Although inflammation is usually considered destructive, in this case, some inflammatory immune cells are important to provide additional angiogenic stimulus. The stem cells do not stay long in the wound, but as soon as they are incorporated, they release their own plethora of growth factors, cytokines, and paracrine factors to be-

**Figure 9.** Results from a study involving a parabiosis model showed that EpiFix® induced greater recruitment of engrafted GFP cells inside the implant when compared to the sham implant, and an acellular bovine dermis (Primatrix®, TEI Biosciences, Boston, MA) control. (Adapted from Maan, et al.)

**Figure 10.** The investigation supported the hypothesis that EpiFix® could serve as a stem cell magnet that, when applied to a wound, could attract endogenous stem cells to engraft into the area of pathology.
EpiFix® Dehydrated Human Amnion/Chorion Membrane (dHACM) Therapy

Gin healing. In order for angiogenesis and regeneration to occur, adequate wound bed preparation is imperative to remove any infection, necrotic tissue, or pressure that may combat engraftment of the patient's stem cells.

As a healing wound returns local tissue to physiological perfusion, the need for pro-angiogenic stimulus is profoundly reduced. The healing tissue actively down-regulates the production of growth factors, and inflammation also subsides. Healed tissues also up-regulate angiogenesis inhibitory factors that help prune the microcirculation to baseline levels required for proper tissue oxygenation. The expert panel was intrigued that the cytokine profile in EpiFix® shows such multiplicity, and that the release kinetics are also different. While more research is needed to determine how this fits into the physiological model, clearly when wounds heal normally, they actually rely on this repertoire of coexisting inhibitors and stimulators. Maturation at the very end of angiogenesis to create good stable blood vessels involves recruitment of MSCs. In this case, MSCs are the precursors to pericytes and smooth muscle cells that support the newly formed vessel and stabilize that circulation.

DIABETIC FOOT ULCERS – A GROWING EPIDEMIC

Amidst much new dialogue and guidelines about treating diabetic foot ulcers (DFUs), it is apparent that DFUs are a growing epidemic. In 2005, 80,000 people with diabetes had a lower extremity amputation, 85% of which were preceded by an ulcer.13 A second lower extremity amputation was required in 30% after 3 years, and 51% after 5 years.14 In addition, it has been reported that within 3 years of surgery, there is an approximate 50% mortality rate in these patients.15 Likewise, Armstrong showed that 50% of patients receiving a diabetes-related amputation will die within 5 years and suggested that the impacts of diabetes-related wounds and amputation are a greater burden on our healthcare system than cancer.16

GOOD STANDARD OF CARE TECHNIQUES IMPERATIVE FOR QUALITY HEALING

Preventing amputations in patients with diabetes should always begin with good ulcer care, including but not limited to assessment of the patient, his or her comorbidities and the wound, surgical debridement and control of infection, vascular evaluation and treatment, off-loading/pressure relief, and appropriate dressings. While some diabetic ulcers may be superficial and can heal with conservative stan-

Figure 11. This graph represents the mean percentage of reduction of ulcer surface area by week for patients treated with EpiFix® (n=13) or standard of care (n=12). An 80%+ reduction in wound area was seen in EpiFix® patients at week 1 versus 20% in patients receiving standard of care only. (Adapted from Zelen, et al.)19

Figure 12. Patients treated with EpiFix® showed a 92% healing rate at 6 weeks (P = 0.001) versus 8% healing in patients receiving standard of care. (Adapted from Zelen, et al.)19

<table>
<thead>
<tr>
<th>Ulcers healed</th>
<th>SOC (n=12)</th>
<th>EpiFix® (n=13)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>4 Weeks</td>
<td>0 (0%)</td>
<td>10 (77%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 Weeks</td>
<td>1 (8%)</td>
<td>12 (92%)</td>
<td>&lt;0.001</td>
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<table>
<thead>
<tr>
<th>Ulcers Surface Area Reduction (%</th>
<th>0 1 2 3 4 5 6</th>
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<tbody>
<tr>
<td>SOC n=25</td>
<td>100 80 60 40 20 0</td>
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<tr>
<td>EpiFix n=13</td>
<td>100 80 60 40 20 0</td>
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</table>

Ulcers Surface Area Reduction Over Time

Preventing amputations in patients with diabetes should always begin with good ulcer care, including but not limited to assessment of the patient, his or her comorbidities and the wound, surgical debridement and control of infection, vascular evaluation and treatment, off-loading/pressure relief, and appropriate dressings. While some diabetic ulcers may be superficial and can heal with conservative stan-
standard of care treatment, they are often notoriously slow to resolve, taking up to several months to heal. Indeed, one meta-analysis showed that less than 25% of DFUs heal with conservative care within 3 months.17 Wound Healing Society guidelines recommend consideration of advanced wound therapies if a diabetic ulcer does not reduce in size by 50% or more after 4 weeks of standard therapy.18

CLINICAL EVIDENCE SUPPORTS EARLY INTERVENTION WITH EPIFIX® FOR TREATING DFUS

Results of four peer-reviewed clinical studies of EpiFix® for the treatment of Wagner grade I and II DFUs were reviewed by the panel. These studies included a 25-patient randomized, controlled clinical trial (RCT)19 as well as a retrospective crossover20 and long-term follow-up study for this patient population.21 A prospective 40-patient randomized comparative study22 of weekly versus biweekly application of EpiFix® for the treatment of DFUs was also reviewed. It is important to note that a fifth clinical trial was published after the roundtable convened and has been included in order to convey the growing clinical body of evidence for EpiFix®. This latest RCT is a 60-patient, multicenter comparative effectiveness study of EpiFix® vs. Apligraf® (Organogenesis, Canton, MA) vs. the standard of care for the treatment of DFUs.23 The initial RCT compared 4- and 6-week healing rates of 13 patients with 13 wounds treated with EpiFix® versus 12 patients with 12 wounds treated with moist wound healing (control).19 Wound treatment for both groups included surgical debridement, weekly moist wound healing dressing changes, and offloading. In addition, patients in the EpiFix® arm of the study received an EpiFix® graft applied every 2 weeks under a nonadherent dressing. The results showed 77% of EpiFix®-treated wounds healed in 4 weeks and 92% healed within 6 weeks compared to 0% and 8% of control wounds, respectively (P<0.001).19 The EpiFix® group received an average of 2.5 grafts to closure (Figures 11 and 12). This low rate of healing for control is similar to rates reported with standard of care in other studies of advanced wound care products ranging from 2-5% at 4 weeks and 4-10% after 6 weeks.24, 25 Based on published healing rates of other advanced wound healing modalities, the initial protocol for this EpiFix® RCT included enrollment of 80 patients. However, once the data showed that 90% of patients treated with EpiFix® were healed at 6 weeks, it was recommended by independent adjudicators that the trial be concluded.

A follow-up DFU retrospective crossover study20 included control patients from the initial RCT who did not achieve greater than 50% closure after 6 weeks and elected to crossover to receive the EpiFix® treatment. The study patients served as their own control based on data collected during the initial 6 weeks of the RCT. Ninety-one percent of these patients healed within 10 weeks.20 A long-term follow-up study of DFUs healed with EpiFix® in the initial RCT and crossover studies was also conducted with 18 of the 22 eligible patients returning for follow-up at 9-12 months.

9-12 Month Follow-up

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<tr>
<td>%</td>
<td>%</td>
<td></td>
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<tr>
<td>Remained Healed</td>
<td>94.4%</td>
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<tr>
<td>Reopened</td>
<td>5.6%</td>
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Figure 13. Long-term follow-up showed 94.4% of patients remained healed at 9-12 months. (Adapted from Zelen, et al.)21

“As MiMedx® continues to invest in additional clinical studies, one must look at the amount of current and future clinical, scientific and economical data on EpiFix® to help with clinical decision making today.”

— Dr. Driver
months. Follow-up examinations showed 5.6% (1/18) of patients had a recurrent DFU and 94.4% remained fully healed (Figure 13).

Weekly versus biweekly application (every other week) of EpiFix® for treating DFUs has also been investigated in a prospective, randomized single-center clinical trial of 40 patients treated with EpiFix®. The mean time to complete healing was 4.1 ± 2.9 versus 2.4 ± 1.8 weeks ($P=0.039$) in the biweekly versus weekly groups, respectively. Complete healing occurred in 50% versus 90% by 4 weeks in the biweekly and weekly groups, respectively ($P=0.014$). The total percent healed was 92.5% within the 12-week study period. All but one patient (39/40, 97.5%) had >50% reduction in wound size within 4 weeks and 37/40 patients (92.5%) treated with EpiFix® had complete healing within 12 weeks.

While a similar number of grafts were used on each healed wound (biweekly group 2.4 ± 1.5, vs. 2.3 ± 1.8 in the weekly group, $P=0.841$), those wounds receiving weekly EpiFix® healed 41.5% faster than those treated with EpiFix® biweekly, despite a greater mean HbA1c in the patients who received weekly applications (Figure 14). The results of these studies validate the initial RCT data showing that EpiFix® was effective in healing 90+% of DFUs.

Results of a landmark multicenter RTC of clinical and economic comparative effectiveness of EpiFix® vs. Apligraf® vs. the standard of care for the treatment of DFUs were not yet available at the time of this roundtable panel discussion, but are included in this supplement. The RCT included 60 patients divided into 3 study arms.

<table>
<thead>
<tr>
<th>Duration</th>
<th>EpiFix® (n=20)</th>
<th>Apligraf® (n=20)</th>
<th>Standard Care (n=20)</th>
<th>EpiFix® Vs. Apligraf®</th>
<th>EpiFix® Vs. Standard Care</th>
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<tbody>
<tr>
<td>Median Healing Time</td>
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<td>49 Days</td>
<td>49 Days</td>
<td>$P=0.0001$</td>
<td>$P=0.0001$</td>
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Figure 14. Rates of complete healing at 2 week intervals for patients treated with weekly vs. biweekly application of dHACM. (Adapted from Zelen, et al.)

Figure 15. Comparison of complete healing rates at weeks 4 and 6 (n=60). (Adapted from Zelen, et al.)

Figure 16. Comparison of speed of healing. (Adapted from Zelen, et al.)
All patients received weekly sharp debridement and offloading with a removable cast walker. Twenty patients in the control group received daily collagen-alginate dressing changes with a moisture-retentive dressing. Twenty patients in the EpiFix® arm received weekly applications of EpiFix® and a moisture-retentive dressing, and 20 patients received weekly applications of Apligraf®. The endpoints for this study were the percentage of patients who achieved complete healing, time to heal, and cost of treatment. Study ulcers achieving ≤20% healing with standard care treatment during the two-week run-in period remained in the trial.

The study results are presented in Figures 15, 16, 17 and 18. These results show the use of EpiFix® for Wagner grade I and II DFUs healed twice as many wounds, almost four times faster, at one-fifth the cost, and had far less graft wastage (one-sixtieth) compared to Apligraf®. The results also suggest that EpiFix® use may well decrease the direct and indirect costs of DFU treatment, and prevent longer-term medical complications such as amputation. Results from this trial not only reconfirmed the 90% plus healing rates...
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of EpiFix® in the treatment of Wagner grade I and II DFUs, the trial also demonstrated the superiority of EpiFix® over both Apligraf® and the standard of care in complete healing, speed to healing, and cost of treatment.

A LOOK AT VENOUS LEG ULCERS

Venous leg ulcers (VLUs) develop in an estimated 2.5 million patients per year in the U.S., costing up to $18 billion annually.26 Patients with VLUs have been shown to utilize more medical resources with two times the cost for private payers and 50% more for Medicare than patients without VLUs.27 The prevalence of VLUs increases with age and they are 2 to 3 times more common in females.28 VLU recurrence rates range from 54%-78%29 and the many risk factors include venous insufficiency, obesity, immobility, phlebitis, deep vein thrombosis, and family history.30 VLUs have a major negative impact on the quality of life in affected patients due to pain, physical impairments (immobility), social impairments, and psychological effects.31, 32 The average direct cost of treating a patient with a VLU is $9,685 per year.33 Many patients suffer from less tangible costs such as a decreased capacity to work and time lost from work.32

When there is impaired venous return in any part of the system, which can include deep, superficial, or mixed, red and white blood cells stick to the vessel walls and red blood cells (RBCs) break down. This leaves iron deposits in the walls, which leak out into the tissues. Normally, venous return brings blood back to the heart, but if the valves become dysfunctional or there is an obstruction, the blood flows back down as far as it can, even into the feet. This dysfunctional process creates a chemical response, an inflammatory response with a resultant buildup of tissue breakdown products within these tissues. Activated white blood cells enter the tissues and release their chemicals, resulting in further tissue damage, leading to auto-digestion and increased buildup of degradation products. This impedes diffusion of oxygen and other nutrients into affected areas. The result of this impediment can be death of the tissues surrounding the veins, which leads to a venous ulcer. Venous ulcers typically occur on the lower leg near the ankle where vein pressures are highest and are typically surrounded by skin with a rusty brown color from extravasated iron deposits.

“It is important to remember that the approach to the VLU problem isn’t just about treating wounds, but rather treating patients with wounds who have clinical evidence of venous insufficiency/reflux, and commonly have other comorbidities such as obesity and diabetes contributing to the wound.”

— Dr. Gibbons
PRINCIPLES OF STANDARD OF CARE FOR VLUS

Standard therapy for venous disease incorporates leg compression to reduce the diameter of the vein, increasing flow velocity, decreasing the chance of thrombosis and reducing edema. It activates fibrinolytic activity in the blood and reduces the filtration of fluid out of the intravascular space, improving lymphatic flow and reducing edema. Graduated compression reduces reflux and improves venous outflow for the calf muscle pump to get blood back to the heart, decreasing venous pressure at rest and with ambulation (Figure 19).

When there is mixed venous/arterial disease, the venous disease must be treated first by revascularizing patients with peripheral arterial disease, and by protecting high-risk areas, controlling edema, and providing good nutrition, medications, and physical and emotional therapy. Also, when addressing the hostile wound environment, sharp surgical debridement includes dependently draining pus and removing devitalized tissue, bone, bacteria proteolytic enzymes and senescent cells.

EPIFIX® FOR THE TREATMENT OF VLUS

EpiFix® has demonstrated effectiveness in treating VLUs. In a multicenter RCT, effectiveness of EpiFix® and multilayer compression versus multilayer compression alone was evaluated in treating VLUs. EpiFix® was applied once or twice during a 4-week study period. Reduction in wound size of ≥40% occurred in a significantly greater numbers of patients receiving EpiFix® versus those receiving multilayer compression therapy only (33/53 [62%] vs 10/31 [32%]; P = 0.005) (Figure 20). Over the 4-week study period, VLUs treated with EpiFix® were significantly reduced in size compared to those treated with multilayer compression only (Figure 21). Based on the surrogate endpoint of ≥40% wound area reduction within 4 weeks as a predictor of overall healing, these data from the first controlled trial of EpiFix® VLU treatment indicate that EpiFix® is effective in treating VLUs and serves as a defining point for further clinical studies.
EpiFix® Delivers New Clinical Option in Plastic Surgery

The mechanisms behind the beneficial effects of EpiFix® in treating chronic extremity wounds may translate to the treatment of other types of wounds, including burns, radiation-induced wounds, surgical wounds, breast reconstruction, and pressure ulcers. Other clinical indications for consideration include Mohs surgery, laser resurfacing and hair restoration. The potential advantages of using EpiFix® for burns and other plastic surgery cases versus skin grafts include improved mobility with the applied membrane and reduction in scarring in the healed area. EpiFix® helps heal a tissue defect while reducing inflammation. It reduces scarring and causes no clinical signs of rejection. While there are no RCT data yet describing the use of EpiFix® in acute wounds, multiple cases were presented during the panel meeting of effective EpiFix® treatment of acute wounds in plastic surgery. Two acute wound cases are described below.

Case Reports

Case Report 1: Pediatric Partial-Thickness Burn

A toddler presented with a partial-thickness scald burn on the face (Figure 22). EpiFix® was applied, and the patient’s pain resolved quickly after covering the raw nerve endings in the burn. The patient returned home the day after application and returned one week later for follow-up (Figure 23). The pain was managed and the burn was healing well at that point. At 3 to 4 weeks after the application, the patient was getting some pigment back in the skin and showed no signs of future scarring (Figure 24). The clinician found the membrane to be flexible and easy to work with during the treatment.

Case Report 2: Non-Healing Wound in a Cancer Patient

A patient with pancreatic cancer presented with a non-healing wound secondary to abdominal resection (Figure 25). The wound had persisted for about one year despite multiple applications of porcine small intestinal submucosa. A new treatment plan was initiated. The wound was curetted at the bedside and EpiFix® was applied (Figure 26). At two weeks post initial application, the wound was curetted again and given a second application (Figure 27). Four weeks following the first application, the wound was almost completely healed (Figure 28).

Conclusion

Amniotic membrane is an important and complex tissue that is natu-
The New Standard in Bioactive Wound Healing

rally derived and contains a variety of complex cytokines and growth factors that are required in reproduction as well as healing. Essentially, amniotic membrane with amnion and chorion represent different types of amniotic sac tissues. The processing method affects the quality of the growth factor preservation. Unlike other amniotic membrane tissue, EpiFix® is processed with the inclusion of chorion layer, which adds growth factors and cytokines that improve the effectiveness of the matrix and the overall product, particularly when compared to amnion-only grafts. A controlled animal study demonstrated that the PURION® Process used with EpiFix® is more effective in preserving wound healing and inflammatory regulators than another processed tissue tested.

There are no living cells in EpiFix®. This differentiates EpiFix® from other tissues in that EpiFix® contains numerous bioactive components that continue to be present and released over time to promote healing. Mechanisms of action of EpiFix® have been reported in several in vitro and in vivo scientific studies. Growth factors contained within EpiFix® have been identified in peer-reviewed publications as have the effects of the growth factors on various cell types from fibroblasts to endothelial cells to stem cells. Investigators have demonstrated the capability of EpiFix® to mobilize, recruit and serve as a stem cell magnet to enhance healing.

![Figure 25. Case Report: A patient with pancreatic cancer presented with a non-healing wound secondary to abdominal resection.](image)

![Figure 26. Case Report: The wound was curetted at the bedside and dHACM was applied.](image)

![Figure 27. Case Report: At two weeks post-application, it was curetted again and given a second application of dHACM.](image)

![Figure 28. Case Report: After two more weeks, the wound was almost completely healed.](image)

**BENEFITS OF EPIFIX® AMNIOTIC MEMBRANE TISSUE**

- EpiFix® is a dehydrated amniotic membrane tissue comprised of both amnion and chorion membranes.
- EpiFix® helps heal tissue to restore a defect while reducing inflammation.
- EpiFix® reduces scarring and is immunologically privileged.
- There are no living cells in EpiFix®. Unlike other tissues, EpiFix® contains numerous bioactive components that are released over time to enhance healing.
- With in vivo studies, with the application of EpiFix®, native stem cells are attracted from the surrounding tissue or through the circulation to the base of the local injured tissue as opposed to being injected or placed topically in a wound as would be the case with a living cellular therapy.
- With in vivo and in vitro studies, investigators have demonstrated EpiFix’s capability to mobilize, recruit and serve as a stem cell magnet to enhance healing.
to healing and cost of treatment. The results significantly demonstrated a 90% plus healing rate and with long-term follow-up, 94.4% remained healed at one year. Results from a most recent landmark multicenter RCT not only reconfirmed the 90% plus healing rates of EpiFix® in the treatment of DFUs, the study also demonstrated the superiority of EpiFix® over both Apligraf® and standard care in complete healing, speed to healing and cost of treatment. A number of randomized, multicenter trials are being designed or are underway to further validate initial research.

In a surrogate study evaluating the effectiveness of EpiFix® in treating VLUs, 62% of patients achieved ≥40% wound closure at 4 weeks. These study results generate useful data that can guide clinical decision making.

Based on initial scientific data, EpiFix® appears to accelerate healing of both DFUs and VLUs, as evidenced by an increased percentage of wounds healed at all measured time periods, compared to standard of care treatment. It is important to note that in order for angiogenesis and healing to occur with EpiFix® application, adequate wound bed preparation is imperative. EpiFix® application frequency for DFUs appears to make a difference in healing rates, with weekly applications yielding superior outcomes over biweekly applications. Health care providers and payers will continue to welcome data from additional EpiFix® studies involving diabetic and venous leg ulcers.

Finally, in addition to the management of chronic wounds, the panel members discussed other important wound types that may benefit from treatment with EpiFix®, including burns, reconstructive surgery, Mohs surgery, cosmetic or impaired healing situations such as radiation desquamation, non-healing wounds in elderly patients, and all diabetic wounds not healing in a timely sequence.

References

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Today’s wound healing no longer needs to rely on products containing fragile living cells or the freezers and careful handling they require.

EpiFix® amnion/chorion membrane allografts:

• Attract multiple cell types to aid in wound regeneration¹-³
• Demonstrated 90%+ healing rates in three DFU randomized clinical studies and a crossover study; 90%+ remained healed after 9-12 months⁴-⁷
• Healed twice as many DFUs 4 times faster at one-fifth the cost of Apligraf® in a prospective, randomized, controlled, multi-center comparative study⁷
• Achieved ≥40% wound area closure at 4 weeks in 62% of VLU study patients⁸

EpiFix® sets the new standard for wound closure.

Store at ambient conditions  •  Sizes to fit a variety of wounds  •  Reduce graft waste & cost to closure

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