# AXOLOTL BIOLOGIX

## Research and Clinical Publications

# TABLE OF CONTENTS

# Who is Axolotl Biologix and what are Axolotl Allografts

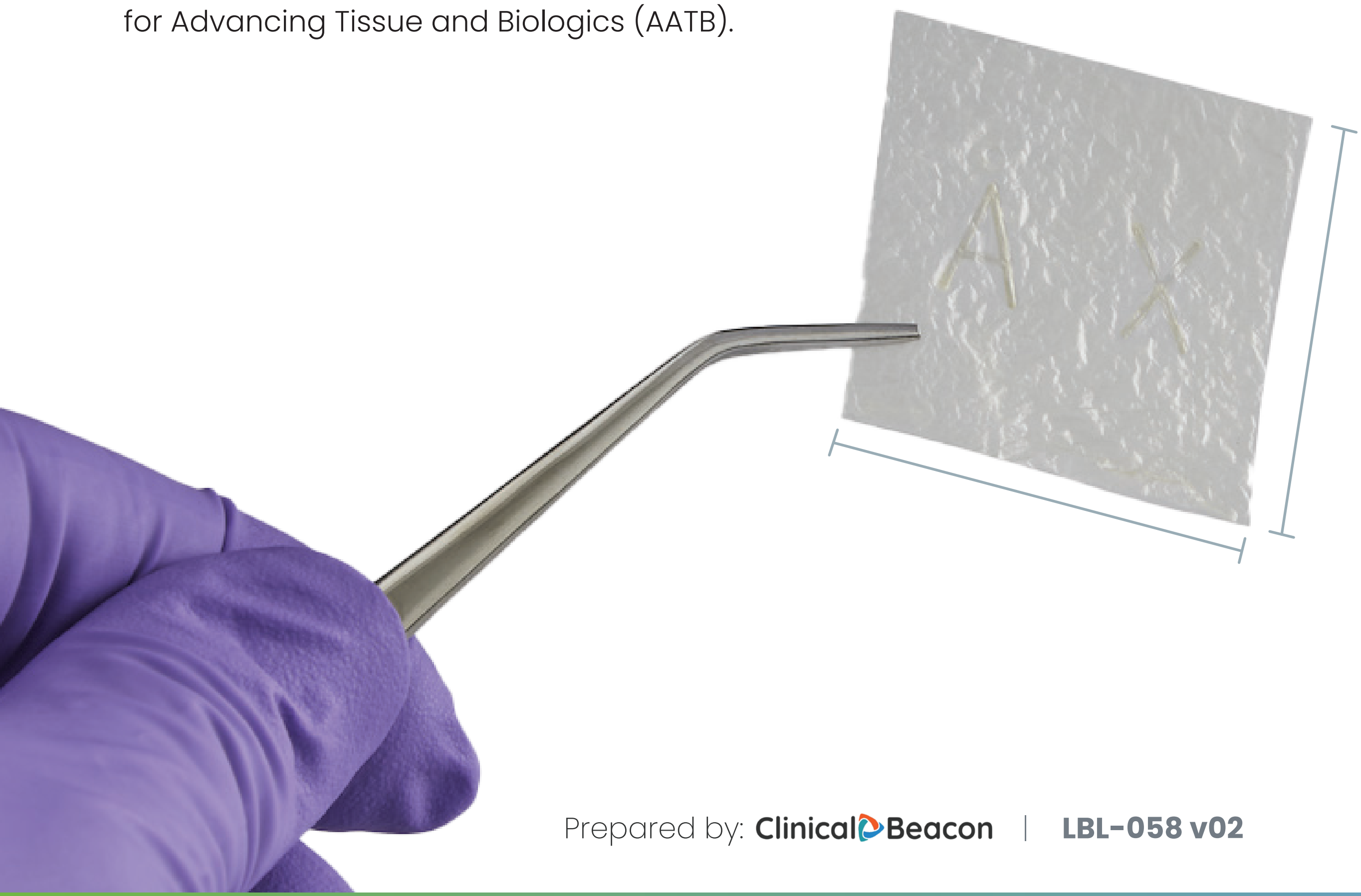
Axolotl Biologix headquartered in Scottsdale, Arizona develops innovative human amnion graft products that enhance and aid in wound and tissue care. The products are validated by strict regulatory standards, peer-reviewed research and widely accessible through strategic market initiatives.

Axolotl Graft™ and Axolotl DualGraft™ are either a single or bi-layered dehydrated human amnion membrane allografts derived from the amniotic lining of the placenta. Axolotl amnion allografts are indicated as a barrier and structural membrane designed to cover acute and chronic wounds and tissues. Axolotl amniotic allografts are marketed under Section 361 of the PHS act and regulated solely under 21 CFR Part 1271. Axolotl Biologix has FDA Tissue Reference Group (TRG) letters on file and products are manufactured in Association for Advancing Tissue and Biologics (AATB).

The consented donor tissue is recovered and processed under sterile conditions, in accordance with all Federal regulations and quality assurance standards in a controlled environment. The allografts are processed through minimally manipulated techniques. This type of processing retains the qualities of the native extracellular matrix (ECM). Axolotl allograft tissue products are terminally irradiated in the final package.

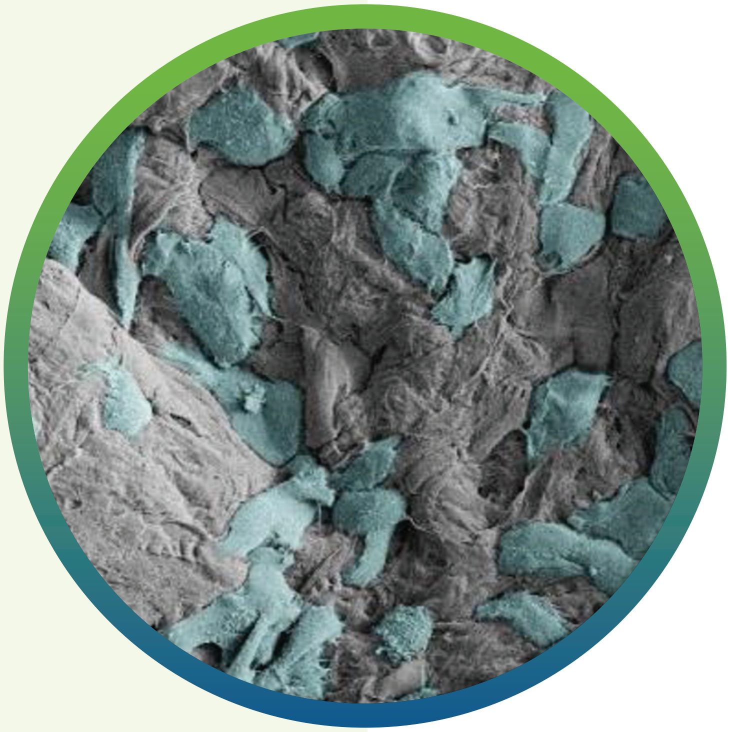
Product offering occurs in the following configurations:

| | |
|---|---|
| **1 x 1 cm** | **4 x 4 cm** |
| **1 x 2 cm** | **4 x 6 cm** |
| **2 x 2 cm** | **4 x 8 cm** |
| **3 x 3 cm** | **10 x 10 cm** |
| **2 x 4 cm** | |



# Axolotl Amniotic Applications

Axolotl Amniotic Allograft provides a protective barrier from the surrounding environment for acute and chronic wounds including partial and full thickness wounds examples include diabetic ulcers, venous ulcers, pressure sores/ulcers, chronic vascular ulcers, tunneled/undermined wounds, non-pressure ulcers, surgical wounds (e.g., donor site/grafts, post-laser surgery, post-Mohs surgery, podiatric wounds, wound dehiscence), trauma wounds (e.g., abrasions, lacerations, partial thickness burns, skin tears), and draining wounds.



Axolotl Graft™ and Axolotl DualGraft™ are human amniotic membrane allografts intended to serve as structural barriers or coverings. The grafts are flexible and conformable, allowing placement across varied anatomic contours, tissue planes, and surgical sites, including over exposed native tissues or surgical hardware where a homologous structural covering is desired. These grafts may be used in open, minimally invasive, or robotic-assisted surgical workflows.

## **Research Publication One:** "The Role of the Extracellular Matrix (ECM) in Wound Healing: A Review"

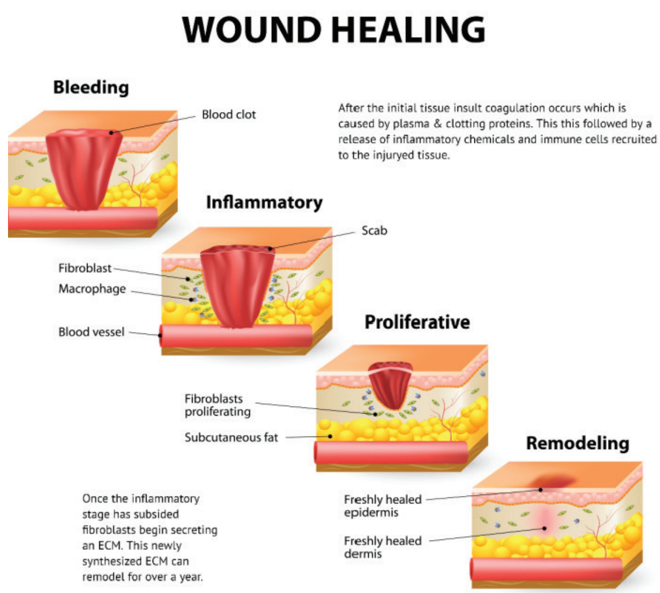
**Provides a naturally occurring structural matrix:**
The extracellular matrix (ECM) is a 3-dimensional environment made up of collagens, elastin, laminin, fibronectin, and other proteins that give tissues their native architecture and organization

**Acts as a protective covering layer, similar to its native role:**
Human amniotic membrane is biologically designed to serve as a protective barrier during development—an inherent characteristic that makes it useful in clinical settings requiring a natural covering tissue

**Contains structural proteins commonly found in native tissue ECM:**
Amniotic membrane includes many ECM components such as collagen types I, II, IV, V, VI, fibronectin, proteoglycans, and laminin—proteins widely recognized for supporting tissue environments

**Supports a physiologic environment during tissue management:**
The ECM plays a natural role in maintaining a supportive environment for cells by providing adhesion sites and scaffolding structures that are intrinsic to normal tissue organization

**Historically used in medical disciplines for its inherent structural properties:**
Amniotic Allografts contain ECM-based materials and contain the same intrinsic, naturally occurring structure components of intact human tissue




**Link:** Diller, R.B., & Tabor, A.J. (2022). The Role of the Extracellular Matrix (ECM) in Wound Healing: A Review. Biomimetics, 7(3), 87. https://pubmed.ncbi.nlm.nih.gov/35892357/

## **Research Publication Two:** “The Preparation and Clinical Efficacy of Amnion-Derived Membranes: A Review.”

**Processing method influences final graft characteristics:**
Preservation and processing techniques (e.g., cryopreservation, dehydration, decellularization) affect membrane thickness, structural integrity, and retained biological components, underscoring the importance of product selection based on clinical handling preferences and application needs.

**Mechanical conformity and handling versatility:**
The thin, elastic, and tensile properties of amniotic membrane allow it to conform to complex anatomical surfaces, supporting its use across a wide range of surgical and wound-covering applications.

**Source of structural and regulatory proteins:**
The tissue contains a diverse array of endogenous growth factors, cytokines, and regulatory proteins naturally embedded within the matrix, which may contribute to a favorable local tissue environment during healing.

**Biologically active yet non-immunogenic tissue:**
Amniotic membrane demonstrates low antigenicity due to minimal expression of HLA Class I and absence of HLA Class II antigens, supporting its use as an allogeneic tissue without routine immune rejection concerns.

**Broad clinical adoption as a surgical covering and barrier:**
Amniotic membrane allografts have been widely utilized by clinicians across multiple surgical and wound-care settings as a protective covering or biological barrier, supporting tissue management in applications where maintenance of a moist, protected environment is desired.

| Properties | Contributing Factors |
|---|---|
| Anti-inflammatory | AM suppress the pro-inflammatory cytokines such as TNFx, IL-1, IL-6 and IL-8 and produce anti-inflammatory factors: IL-10, IL-4, TGF-B, HGF, PGE-2, HLA-G, and IDO. |
| Anti-microbial | AM serves as physical barrier against the external environment with close adherence to wound surface and producing anti-microbial peptides such as beta defensis, elafin. |
| Anti-scarring | AM reduces MMP and other proteases via the secretion of TIMPs, and downregulation of TGF-B. |
| Non-immunogenic / low antigenicity | Low expression of histocompability (HLA Class II) antigens A, B, C, DR or B2. Presence of HLA-G and Fas ligand. |
| Analgesic properties | Pain relief is proposed due to efficient covering of the nerve endings. Anti-inflammatory growth factors such as IL-10, IL-1RA proposed to contribute to pain relief. |
| Angiogenic | Pro-angiogenic factors observed: VEGF-A, angiopoietin-1, HGF, and FGF-2, PEDF, MMPs. Anti-angiogenic factors: TSP-1, endostatin, TIMPs 1, 2, 3, and 4. |
| Promote cellular differentiation and adhesion | Contains the structural proteins: Collagen types I through VI and VII, laminin, fibronection, and vitronectin. |
| Supporting epithelialization | Basement membrane is a substrate for cell migration, proliferation differentiation, and epithelialization with growth factors such as: KGF, b-FGF, and TGF-B. |

**Link:** Ingraldi, A. L., Audet, R. G., & Tabor, A. J. (2023). The Preparation and Clinical Efficacy of Amnion-Derived Membranes: A Review. Journal of Functional Biomaterials, 14(10), 531. https://doi.org/10.3390/jfb14100531
https://www.mdpi.com/2079-4983/14/10/531

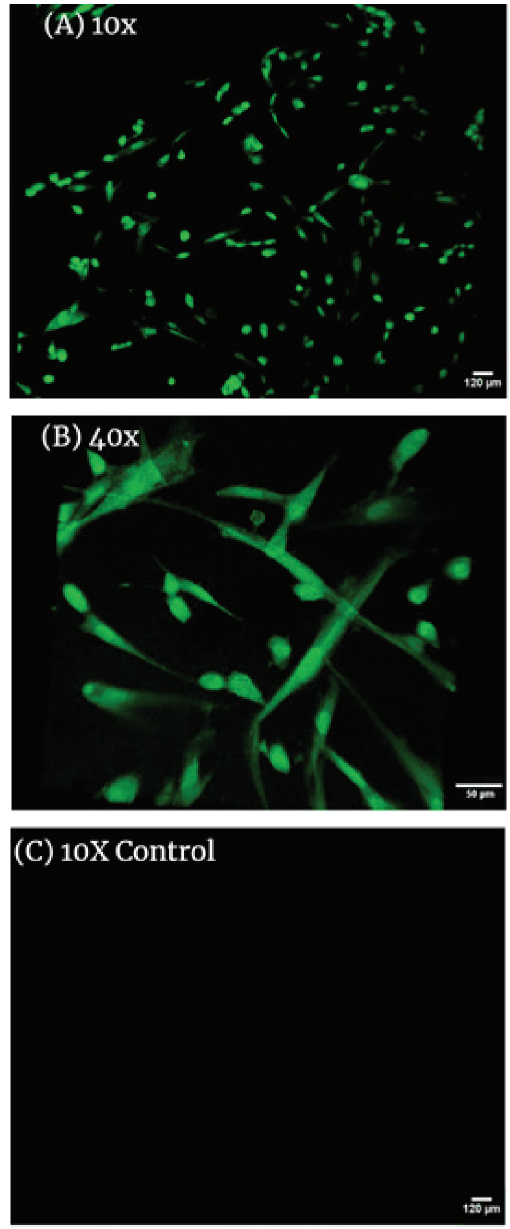
## Research Publication Three:
## "Characterization of Amnion-Derived Membrane for Clinical Wound Applications."

**Cellular attachment and viability observed in vitro:**
Human dermal fibroblasts seeded onto the amniotic membrane demonstrated attachment, normal morphology, and metabolic activity, as visualized by confocal microscopy and quantified using ATP-based viability assays.

**Increased metabolic activity compared to cell-only controls:**
Quantitative CellTiter-Glo assays showed higher luminescence signals for fibroblasts cultured on amniotic membrane compared to fibroblasts cultured without membrane, indicating a higher number of metabolically active cells in membrane-associated cultures.

**Extensive proteomic profile identified:**
Mass spectrometry identified an average of approximately 7,600 unique proteins, over 82,000 peptides, and nearly 97,000 peptide ion variants across tested membrane lots, including core matrisome and ECM-associated proteins.

**Hydration-dependent mechanical behavior documented:**
Tensile testing showed that hydrated membranes exhibited lower stiffness and greater elasticity compared to dry samples, supporting conformability and manipulation during clinical use without tearing.




Confocal images of adult HDF cells on Axolotl Biologix DualGraft™: (A) 10x magnification in confocal microscopy (Leica TCS SPE II) with 488 nm laser shows many cells present within the membrane; this image was taken using the Z-stack feature of the confocal micropscope, outlining the 3D placement of cells within the membrane; (B) 40x magnification presents hDF with normal morphology with elongated arms and rounded cell bodies either recently attached or preparing for division; (C) 10x magnification of unseeded membranes treated with fluorescent probe (control sample); no cells are highlighted, demonstrating that the membrane is decellularized and cells viewed in seeded samples are true positives. We used BioRender.com 2024 to combine images into one unit; images are from Ingraldi, A. (2023) Cell Tracker Green HDF on Membrane Report. Internal Axolotl Biologix report: unpublished.


**Link:** Ingraldi AL, Allen T, Tinghitella JN, Merritt WC, Becker T, Tabor AJ. Characterization of Amnion-Derived Membrane for Clinical Wound Applications. Bioengineering (Basel). 2024 Sep 24;11(10):953. doi: 10.3390/bioengineering11100953. PMID: 39451330; PMCID: PMC11504399. https://pubmed.ncbi.nlm.nih.gov/39451330/

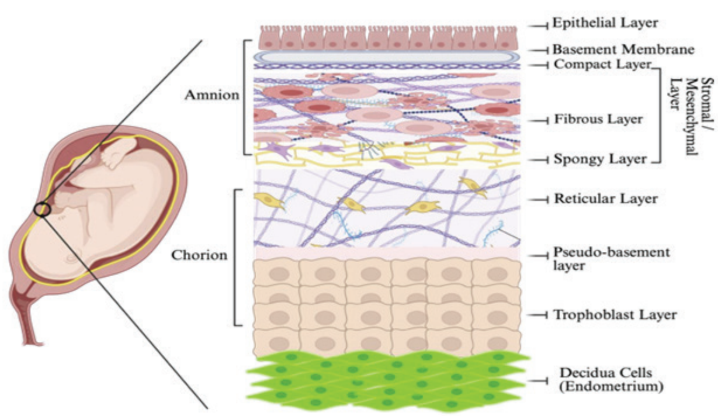
## Research Publication Four:
## "Amnion vs. Amnion/Chorion Placental Allografts: A Comparative Review of Composition, Mechanisms, and Clinical Use."

**Placental membranes are not interchangeable tissues:**
Scientific review of placental membrane biology demonstrates that the amnion and chorion possess distinct native structures and biological characteristics. Understanding these differences may help clinicians better interpret allograft composition and application considerations.

**Placental membranes naturally function as protective covering tissues:**
The review highlights that placental membranes natively serve as protective fetal barrier tissues with tensile strength, flexibility, and conformability. These intrinsic structural characteristics may contribute to their widespread clinical use as covering and barrier allografts across wound and surgical applications.

**Preservation and processing techniques may alter membrane composition:**
The publication emphasizes that preservation, decellularization, sterilization, and dehydration methods can influence structural integrity, retained proteins, and membrane biomechanics. Understanding these processing variables is important when interpreting differences between placental-derived allografts and published clinical literature.

**The amniotic membrane contains naturally occurring regulatory components associated with tissue homeostasis:**
The review summarizes literature demonstrating that amniotic membrane contains extracellular matrix components, cytokines, and regulatory proteins associated with modulation of inflammatory signaling and organized tissue remodeling. Native amnion tissue has been reported to contain anti-inflammatory mediators, protease inhibitors, and structural matrix proteins that contribute to its biologically protective environment.




**Link:** David Cajthaml, Alison Ingraldi and Aaron Tabor, Amnion vs. Amnion/Chorion Placental Allografts: A Comparative Review of Composition, Mechanisms, and Clinical Use, J Clinical and Medical Research and Studies, V (5)I(2), DOI: 10.59468/2836-8525/154

# Case Study One:
## "Amniotic Membrane and Amnion-Conditioned Media Promote Chronic Wound Healing – A Case Report."

**Progressive changes in wound appearance over time:**
Following clinician-directed application of decellularized, dehydrated amniotic membrane as part of an integrated wound management approach, serial observations documented visible changes in wound appearance over successive follow-up visits.

**Formation of granulation tissue noted during early follow-up:**
At the first documented follow-up visit (approximately 7 days post-initial application), the treating physician observed increased granulation tissue within previously open wound areas.

**Restoration of local tissue responsiveness during treatment course:**
During subsequent visits, the clinician noted increased bleeding during debridement and a return of pain sensation, suggesting changes in local tissue responsiveness over the course of treatment.

**Documented wound closure over extended follow-up:**
The case report documents near-complete wound closure at approximately four months following initiation of treatment, with complete closure observed at a later follow-up visit approximately eleven months after initial intervention.


Healing progression in chronic wounds treated with CM and dHAM. All days listed are post-initial treatment (Day 0). A: Post-surgery to remove infected hardware 34 days before the start of treatment. B: Day 0 start of treatment with CM and dHAM, following surgical incision. C: Day 7, showing notable granulation tissue in all open areas. D: Day 35, following 3 treatments with CM adn dHAM. E: Day 53, following surgical suturing. F: Day 88, approximately 5 weeks from recent surgery. G: Day 128, a closed structural barrier (scab), four months from first treatment. H: Final image demonstrating a fully closed wound, Day 352.


**Link:** Audet RG, Tabor AJ, Diller RB, Kellar RS (2023) Amniotic Membrane and Amnion-Conditioned Media Promote Chronic Wound Healing – A Case Report. Med Case Rep Case Series 4(15):
https://doi.org/10.38207/JMCRCS/2023/SEP04150599
https://49p.84f.myftpupload.com/wp-content/uploads/2024/07/Audet-et-al-2023.pdf

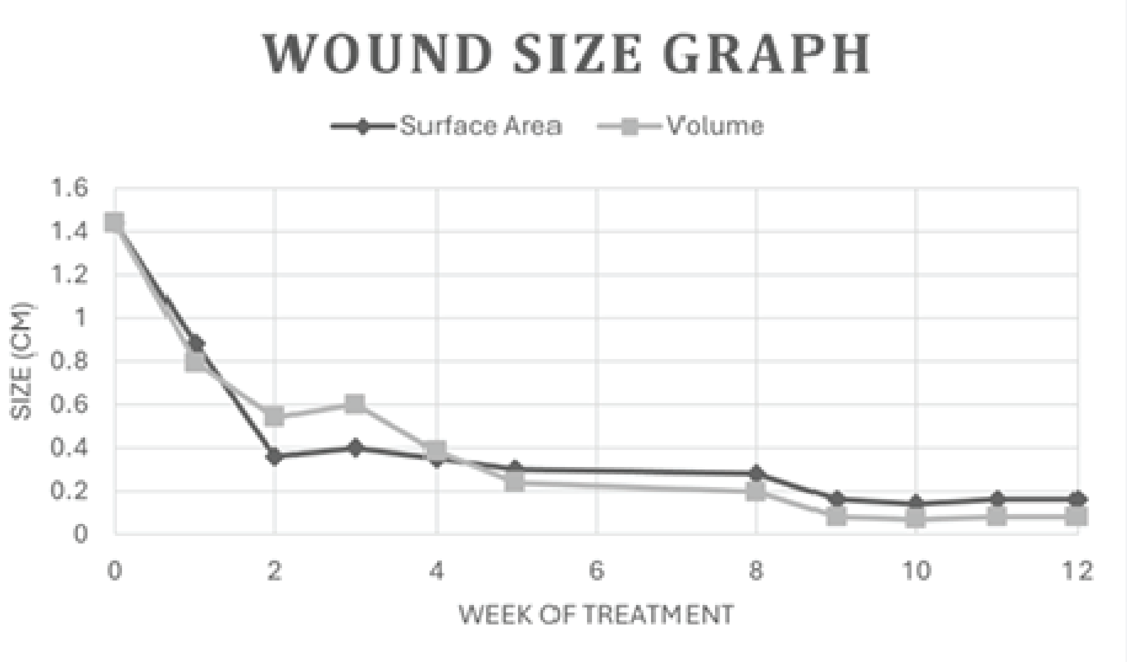
## Case Study Two:
## "Utility of Amniotic Membrane in Treating a Geriatric Diabetic Patient with a Chronic Pressure Ulcer."

**Use as a biological covering in a chronic pressure ulcer setting:**
In this case report, a dual-layer dehydrated human amniotic membrane allograft was applied to a chronic sacral pressure ulcer as part of an advanced wound management strategy following failure of multiple prior standard and biological interventions.

**Progressive reduction in wound size documented over time:**
Serial weekly measurements demonstrated a consistent downward trend in wound surface area and volume following initiation of amniotic membrane treatment

**Calculated surface area reduction of approximately 89%:**
Based on clinician-recorded measurements, the wound exhibited an overall calculated surface area reduction rate of 89% across the treatment period.

**Patient tolerance and comfort during treatment course:**
The patient experienced minimal discomfort during membrane application and follow-up care, and no moisture-associated skin damage or dressing-related complications were reported.



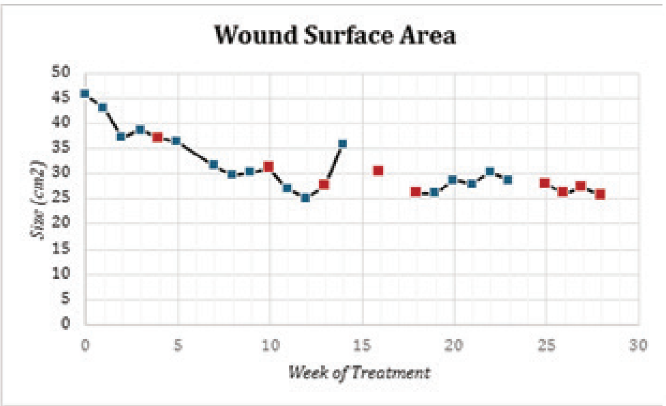

**Link:** Ingraldi AL, Galbreath K, Jones D, Lee D, Tabor A. A Clinical Case Report: Utility of Amniotic Membrane in Treating a Geriatric Diabetic Patient with a Chronic Pressure Ulcer. J Diabetes Clin Res. 2024;6(1):8-14. https://49p.84f.myftpupload.com/wp-content/uploads/2024/07/article-pdf-1714199584-1165.pdf

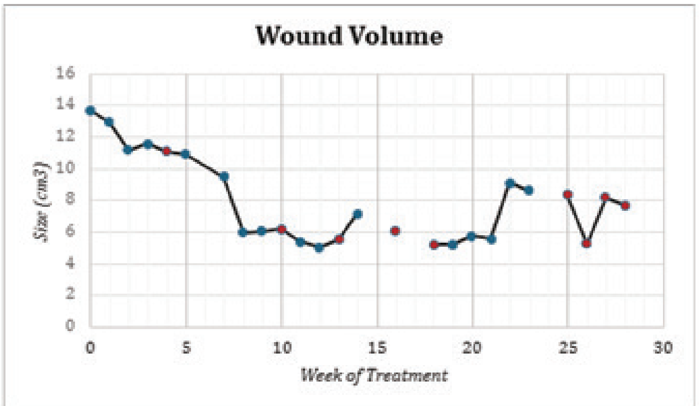
# Case Study Three:
## "Stimulation of the Wound Healing Process of an Electrocution-Associated Ulceration Using Amniotic Membrane: A Case Study."

**Use as a biological covering in a chronic, electrically induced ulcer:**
In this case study, a commercially available dehydrated human amniotic membrane (dHAM) allograft was applied to a long-standing neuropathic ankle ulcer secondary to a high-voltage electrical injury, following failure of multiple prior conservative and advanced wound therapies.

**Approximately 44% reduction in wound surface area and volume:**
Based on clinician-recorded measurements, the neuropathic ankle ulcer exhibited an approximate 44% reduction in wound surface area and volume from baseline over the documented treatment course.

**Observed changes in wound bed and peri-wound tissue:**
Follow-up evaluations documented visible changes in tissue quality, including granulation tissue development, improved peri-wound edge appearance, and maintained wound moisture without purulence or odor.




**Link:** Alison Ingraldi, Daniel Davis, Darlene Lee, Aaron J. Tabor. Stimulation of the Wound Healing Process of an Electrocution-Associated Ulceration Using Amniotic Membrane: A Case Study. Innov Insights Case Rep Rev. 2025; 1(1): 1-10. https://axobio.com/wp-content/uploads/2025/07/stimulation-of-the-wound-healing-process-of-an-electrocution-associated-ulceration-using-amniotic-membrane-a-case-study-11.pdf

# Case Series One:

## "Post-Mohs Surgical Defect Repair with Dehydrated Human Amnion-Amnion Membrane: A Retrospective Clinical Case Study."

**Use as a biological covering for secondary-intention post-Mohs wounds:**
In this retrospective case series, dehydrated human amnion–amnion membrane allografts were applied to post-Mohs surgical defects and managed by secondary intention healing, serving as a protective biological covering within standard dermatologic and reconstructive workflows.

**Integration into routine post-Mohs wound management:**
Application of the amniotic membrane was performed following standard wound assessment and minimal debridement, without fixation, and covered with non-adherent dressings, demonstrating compatibility with common post-Mohs care protocols.

**Clinician-directed, repeat application based on wound presentation:**
Reapplication frequency and duration were determined by physician discretion, reflecting real-world clinical use across varying wound sizes, locations, and patient comorbidities.

**Observed progression of granulation tissue formation:**
Across cases, clinicians documented the appearance and progression of granulation tissue during follow-up visits, often beginning at wound margins and advancing inward over time.

**Complete epidermal covering observed in most cases:**
Five of six patients demonstrated complete epidermal covering during the documented follow-up period, while one patient exhibited partial closure over an extended timeframe.

**Table 2:** Patient Healing Time

| | Patient #1 | Patient #2 | Patient #3 | Patient #4 | Patient #5 | Patient #6 |
|---|---|---|---|---|---|---|
| **Surgical MMS location** | SCC Scalp Mohs | BCC Left forehead Mohs | Right neck Mohs | SCC Left Cheek Mohs | BCC Right forehead & Nose Mohs | SCC left hand Mohs |
| **Re-epithelization** | ☒ | ☒ | ☒ | ☒ | ☒ | ☒ |
| **Complete Epidermal covering** | ☐ | ☒ | ☒ | ☒ | ☒ | ☒ |
| **Average Healing Time (in weeks)** | >16 weeks | 5 weeks | 5 weeks | 6 weeks | 10 weeks | 4 weeks |

**Link:** Ingraldi AL, Lee D, Tabor AJ (2023) Post-Mohs Surgical Defect Repair with Dehydrated Human Amnion-Amnion Membrane: A Retrospective Clinical Case Study. J Med Case Rep Case Series 4(17): https://doi.org/10.38207/JMCRCS/2023/SEP041701115

# Society Poster Presentations

### Characterization of Human Amniotic Membranes for Clinical Wound and Wrap Applications.

This was presented at the Symposium on Advanced Wound Care in Las Vegas, NV, 2022. The study highlights the unique properties of human amniotic membranes, which are considered important scaffolding materials due to their 3D structural architecture and function, as well as their source of growth factors and cytokines. The research emphasizes the need for standardized processing protocols to ensure consistency across amniotic membrane products and their potential in various clinical applications, including wound and tissue treatments.

Audet, R., Ingraldi, A., Tabor, A. Characterization of Human Amniotic Membranes for Clinical Wound and Wrap Applications, Fall Symposium on Advanced Wound Care 2022. https://axobio.com/publications/

### Assessment of Cellular Biocompatibility of an Allograft Membrane: Viability and Migration of Distinct Cell Types

This was presented at the Symposium on Advanced Wound Care in Las Vegas, NV, 2025. Researchers evaluated human dermal fibroblasts, osteoblasts, and skeletal muscle cells cultured on the amniotic membrane to assess basic biocompatibility, cell attachment, viability, and migration. Using live-cell imaging, metabolic viability assays (WST-1), and confocal microscopy, the study demonstrated that all three cell types were able to attach to, remain viable on, and interact with the membrane structure over time.

In addition to cell-based testing, the poster includes an advanced proteomic analysis of multiple membrane lots performed by an independent third-party laboratory. This analysis identified thousands of proteins and peptides, including extracellular matrix components, growth factors, and regulatory proteins commonly associated with tissue structure and cellular activity.

Overall, the findings support that the dehydrated amniotic membrane functions as a biocompatible extracellular matrix scaffold capable of supporting multiple clinically relevant cell types. The work is intended to provide foundational scientific characterization of the membrane's properties and does not make clinical efficacy or treatment claims.

Ingraldi, A. & Tabor, A. Assessment of Cell Responses and Biocompatibility of an Allograft Membrane: Viability and Migration of Distinct Cell Types, Symposium on Advanced Wound Care, 2025. https://axobio.com/publications/

# RCT Publication and Upcoming Publications

## Randomized Controlled Trial Update

Axolotl Biologix is conducting a Level I, prospective, randomized controlled trial titled "Dehydrated Human Amnion Membrane and Standard of Care Versus Standard of Care Alone in Nonhealing Diabetic Foot Ulcers" (NCT06550596).
This RCT is managed by the Serena Group CRO, under the direction of Dr. Thomas Serena. 84 participants have been enrolled and randomized, No series adverse events (SAEs) have been reported; and interim analysis of data shows trends that the use of Axolotl Amniotic Allograft increase Percent Area Reduction (PAR) compared to standard of care.

## Mega–Meta-Analysis Update #1

"Axolotl Biologix participated in a large mega–meta-analysis comparing allograft skin substitutes to standard of care. The analysis was conducted, presented, and published by the CRO, Serena Group, and included multiple participating organizations, several of which currently have CMS-approved LCD products. Initial preliminary data demonstrated a statistically significant difference in wound closure when allograft skin substitutes were compared to standard of care. Axolotl Biologix anticipates continued publication and dissemination of its own RCT in 2026.

## Upcoming Peer Reviewed Publications:

Axolotl Biologix believes in a string evidence-based approach as such the following upcoming publications are in process:
Axolotl Allografts in surgical applications use- varying case reports and series.
Axolotl Allografts in use with surgical robotics- varying clinical reports and series.