HUMAN AMNIOTIC MEMBRANE GRAFTS TO ENHANCE HEALING

Frank Burrows, MBA, EMT
David Mason, MD
Good Wound Care

“Treat the whole patient, not just the hole in the patient”

• Ensure adequate perfusion to wound site
• Address metabolic challenges (glucose / nutrition)
• Debride to bleeding tissue – “Cut the wound out of the patient”
• Assess patient’s need for off-loading, compression, and/or negative pressure
• Address bio-burden, biofilms, and infection
• Create a moist healing environment with focus on moisture balance

• Re-balance microscopic wound environment (homeostasis)
  • Wound healing mediators (cytokines and chemokines)
  • Inflammatory mediators
CHRONIC WOUND TREATMENT PARADIGM
Conservative moist wound care will heal the majority of wounds. What is hiding under the surface? When wounds do not achieve >50% area reduction in 4 weeks with conservative care, the wound will remain open!

For years, leaving opportunities along the way for:

- Major complications
- Increased costs
- Reduced QOL
- Increased chance of death
Why Amniotic Membrane?

• Barrier properties
  
• Modulates inflammation

• Reduces scar tissue formation

• Immunologically privileged

• Contains essential growth factors

• Enhances wound healing


# FDA Regulatory Classifications for Tissue & Cell Based Products

<table>
<thead>
<tr>
<th>Classification</th>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>361 HCT/Ps (Human Cell Tissue/Products)</td>
<td>Human Tissue (Allograft)</td>
<td>Minimally manipulated, intended for homologous use. No clearance or premarket approval required. Requires FDA Good Tissue Practices (GTP).</td>
</tr>
<tr>
<td>510(k) Clearance (351 HCT/Ps)</td>
<td>Medical Device (Example: decellularized human dermis, xenografts, collagen dressings, bone void filler).</td>
<td>Requires FDA Substantial Equivalence, shorter submission and less required verses PMA. Based on predicate device. Requires FDA Current Good Manufacturing Practice (cGMP).</td>
</tr>
<tr>
<td>Premarket Approval (PMA)</td>
<td>Medical Device (Example: human living skin substitutes, bone substitute).</td>
<td>Requires extensive FDA premarket approval process, including comprehensive clinical trials. Requires FDA Current Good Manufacturing Practice (cGMP).</td>
</tr>
<tr>
<td>Biologic License Application (BLA) (351 HCT/Ps)</td>
<td>Biological product (Example: Cell products, such as those containing hematopoietic progenitor cells, vaccines, and blood components).</td>
<td>Requires extensive FDA premarket approval process, including comprehensive preclinical and clinical trials. Requires compliance to FDA Current Good Manufacturing Practice (cGMP).</td>
</tr>
<tr>
<td>New Drug Application (NDA)</td>
<td>(Example: living stem cells non-autologous, second degree relative, or autologous stem cells that are expanded in the laboratory).</td>
<td>Requires extensive FDA premarket approval process, including comprehensive clinical trials. Requires FDA Current Good Manufacturing Practice (cGMP).</td>
</tr>
</tbody>
</table>
Placental Based Allografts

After Live Births

- Amniotic Fluid
- Amnion Membrane
- Chorion Membrane
- Umbilical Cord
- Placenta with Chorionic Plate
Tissue Processing

- Cleansing/Cell Removal
- Gentle Vs. Harsh
- Preservation Options
- Aseptic Vs. Sterilization
- Storage?
- Shelf-life?
## Step Process to Ensure Safety

<table>
<thead>
<tr>
<th>Major Process Steps</th>
<th>Criteria that Ensures Tissue Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor Screening</td>
<td>Acceptable donor medical history approved by Medical Director</td>
</tr>
<tr>
<td>Infectious Disease Testing</td>
<td>Negative results for all FDA approved tests that includes HIV, Hepatitis B and C, Syphilis, and Human T-cell lymphotropic virus (HTLV)</td>
</tr>
<tr>
<td>Processing</td>
<td>A validated process with proven Bacterial/Spore reduction capabilities that range from 1.4 – 5.6 Logs</td>
</tr>
<tr>
<td>Terminal Sterilization of all grafts</td>
<td>Require a validated process per ISO 11137 standards providing at least a $10^{-6}$ SAL with irradiation dose monitoring linked to the release of every tissue distributed</td>
</tr>
<tr>
<td>Double sterile barrier product packaging</td>
<td>A validated process per FDA recognized ASTM D4169 standards that ensures sterility is maintained post distribution</td>
</tr>
</tbody>
</table>
# Amniotic Membrane Grafts

## Not All Amniotic Membrane Products Are the Same

### Single Layer Grafts - Amnion

- **Decellularized (cells removed)**
  - Biovance® (Celgene/Alliqua)

- **Cellular (non-living cells)**
  - AmnioExcel® (BioD/DermaSciences)\(^1\)
  - Neox® 100 (Amniox)

- **Cellular (living cells)**
  - Grafix® Prime (Osiris Therapeutics)\(^2\)

#### Single Randomized Clinical Trial in DFUs

**REFERENCES:**


### Bilayer Grafts - Amnion/Chorion (dHACM)

- **Cellular (non-living cells)**
  - AmnioFix® & EpiFix® (MiMedx) \(^3\)-\(^8\)

#### Has completed randomized clinical trials in both DFUs and VLUs

**REFERENCES:**


Apligraf and Dermagraft are registered trademarks of Organogenesis, Inc. AMNIOEXCEL is registered trademark of BioD, LLC, an Integra Life Sciences company. BIOVANCE is a registered trademark of Alliqua Biomedical, Inc. Grafix is registered trademark of Osiris Therapeutics, Inc. NEOX is registered trademark of Amniox Medical, Inc. EpiFix and AmnioFix are registered trademarks of MiMedx Group, Inc.
Dehydrated Human Amnion Chorion Membrane (dHACM)

**Preserves:**

Extracellular Matrix
- Structurally intact tissue
- Collagens I, III, IV, V, VII
- Elastin, Laminin, fibronectin, proteoglycans, and glycosaminoglycans
- Non-viable cells

Evolution of Thinking

1 Function
1 Growth Factor

VS.

Multi-Functions
200+ Bioactive Proteins
226 Natural Bioactive Proteins Preserved in PURION® Processed dHACM\textsuperscript{1-4}

<table>
<thead>
<tr>
<th>Angiostatin</th>
<th>Galecin-7</th>
<th>TIMP-2</th>
<th>IL-1 F10</th>
<th>IFGBP-2</th>
<th>FLRG</th>
<th>IFGBP-6</th>
<th>Pentraxin 3</th>
<th>Resistin</th>
<th>CA9</th>
<th>OSM</th>
<th>TRAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiogenin</td>
<td>Thrombospin-5</td>
<td>WISP-1</td>
<td>S100A8</td>
<td>RGM-B</td>
<td>Marapsin</td>
<td>PGRP-S</td>
<td>Thrombospin-2</td>
<td>aGF</td>
<td>FABP2</td>
<td>OPG</td>
<td>Trappin-2</td>
</tr>
</tbody>
</table>

Relative Growth Factor Amounts Between PURION® Processed Amnion and Chorion

Growth Factor Release Profile for dHACM

**Method**

Combine Micronized dHACM and Saline Solution → Incubate at 4°C for 24 Hours → Centrifuge → Analyze Soluble Growth Factors bound in the Matrix

**Results**

- **PDGF-AA**
  - Released: 13%
  - Bound: 87%

- **PDGF-BB**
  - Released: 4%
  - Bound: 96%

- **bFGF**
  - Released: 44%
  - Bound: 56%

- **TGF-b1**
  - Released: 24%
  - Bound: 76%

- **EGF**
  - Released: 63%
  - Bound: 37%

Determined by ELISA Assay (N=5)

Effects of Extracts of dHACM on Adult Human Dermal Fibroblasts Proliferation

Method

Combine

Micronized dHACM
Saline Solution

Incubate

24 Hours @ 4°C

Centrifuge

dHACM Soluble Extract

Result: Fibroblast Proliferation

Human Dermal Fibroblast

Incubate

Cell Culture Dish

Incubate

Cell number (per well)

Result: Fibroblast Proliferation

Cell number (per well)

dHACM Extracts Stimulate Growth Factor Production by Human Dermal Fibroblasts

dHACM Extracts Stimulate Growth Factor Production by Human Dermal Fibroblasts

dHACM Recruits of Circulating Hematopoietic Stem Cells

dHACM Recruits of Circulating Hematopoietic Stem Cells

Intra-implant CD34 + progenitor cell engraftment was increased in the dHACM group compared with control.

Promotion of Angiogenesis within the dHACM Graft in an Ischemic *in vivo* Model

Biological Activity of PURION® Processed dHACM<sup>1-8</sup>

## Comparative Review of Advanced Biologically Active Skin Substitutes

### Diabetic Foot Ulcer - Level I Clinical Evidence

<table>
<thead>
<tr>
<th>Product</th>
<th>N=</th>
<th>Complete Healing at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td>EpiFix(^1) (dHACM)</td>
<td>100</td>
<td>85%(^*)</td>
</tr>
<tr>
<td>Grafix(^2)</td>
<td>97</td>
<td>15%(^\dagger)</td>
</tr>
<tr>
<td>Apligraf(^3)</td>
<td>208</td>
<td>20%</td>
</tr>
<tr>
<td>Dermagraft(^4)</td>
<td>245</td>
<td>11%(^\dagger)</td>
</tr>
<tr>
<td>AmnioExcel(^5)</td>
<td>21</td>
<td>-</td>
</tr>
</tbody>
</table>

* \(^*\) \( \leq 0.05\)
\(^\dagger\) not reported - estimated
\(^\dagger\) INTENT TO TREAT STUDY

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Multi-Center Comparative Effectiveness Study of Healing DFUs Using EpiFix®, Apligraf®, and Standard Care

DFU Trial Showed Superiority of EpiFix® over both Apligraf® and Standard Care for Complete Healing at 4, 6 and 12 Weeks


Multi-Center Comparative Effectiveness Study of Healing DFUs Using EpiFix®, Apligraf®, and Standard Care


VENOUS LEG ULCER STUDIES
Multi-Center, Randomized, Controlled, Venous Leg Ulcer Trial

\( n = 84 \)

\( \geq 40\% \) Wound Area Closure of Venous Leg Ulcers in 4 Weeks

PAIN Observation measured: EpiFix® showed a reduction in pain in 79.5% of the patients that received EpiFix® compared to 52.4% patients receiving only MLCT.

## Comparative Review of Advanced Biologically Active Skin Substitutes

### Venous Leg Ulcers - Level I Clinical Evidence

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<tr>
<td></td>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td><strong>EpiFix</strong>&lt;sup&gt;1&lt;/sup&gt; ongoing</td>
<td>36</td>
<td>17%</td>
</tr>
<tr>
<td><strong>Control</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>37</td>
<td>11%</td>
</tr>
<tr>
<td><strong>Apligraf</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>140</td>
<td>9%</td>
</tr>
<tr>
<td><strong>Control</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>110</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Dermagraft</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
<td>274</td>
<td>-</td>
</tr>
<tr>
<td><strong>Control</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
<td>263</td>
<td>-</td>
</tr>
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*Note: Apligraf, Dermagraft, and EpiFix studies are independent of one another*

* ≤ 0.05

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COMPLEX WOUND CASE EXAMPLES
Complex Wounds: Exposed Bone/Tendon

dHACM has been used for both primary closure and as an important adjunct in the treatment of complex wounds.
VLU Wound Healing

Initial Wound, Pretreatment and first application of dHACM
Mid-Feb 2015
Wound 25x19 cm
(475 cm²)

Pre-debridement and Second Application of dHACM
4/7/2015
Wound < 100 cm²

Week of 4/13/2015
Healed
Hypergranulation

Hypergranular wound to 2\textsuperscript{nd} PIPJ; Closed with 5 applications of dHACM sheet in 5 weeks
Pediatric Partial-Thickness Burn

- Toddler presented with a partial-thickness, typical scald burn on the face and head
- dHACM 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
- Returned one week later for follow-up; 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

Day 1  Week 1  Week 4
Post dHACM application  Regaining pigment, no signs of scarring
4 year old contact burn

Presentation

dHACM in place

dHACM Application

2 weeks after dHACM Application
Radiation Dermatitis

Dermagraft® and Oasis® treatments failed, then dHACM was applied.

One dHACM graft applied to the surface of the wound.

Week 1 Post application of dHACM
Keloid Scar Revision

Pre-Op

1 Year
Post-scar revision using dHACM on 1/3 portion of original scar

EpiFix Treated Area
Injection of dHACM Micronized and saline

Injection of dHACM Micronized and saline

2 weeks post injection  
6 weeks post injection  
12 weeks post injection
THANK YOU