Clinical Outcomes of Amnion Allografts with Living Cells in DFU Management

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Disclosures

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Learning Objectives

• Diabetic foot ulcer (DFU) statistics and when to use advanced wound care modalities containing living cells

• Structure and properties of human amniotic membrane and effects of different tissue preservation methods on tissue components

• Benefits of preserving all components of the amniotic membrane and currently available amniotic membrane products with living cells

• Key living amniotic membrane clinical studies and outcomes for chronic DFU management
Diabetic Foot Ulcers (DFUs)

- In 2015 30.3 million Americans, or 9.4% of the population, had diabetes¹

- DFU is one of the most common complications of diabetes²
  - Annual incidence 1% to 4% with lifetime risk of 15-25%³-⁶
  - ~15% of diabetic foot ulcers result in ~85,000/year lower extremity amputation⁵,⁷
  - ~85% of lower limb amputations in patients with diabetes are proceeded by ulceration⁸-⁹

- Annual cost of DFU management alone estimated to be between $9-13 Billion¹⁰

1. American Diabetes Association, Statistics about diabetes, 2018
When and Why to Use Wound Care Modalities Containing Living Cells?

- **When:** in high-risk patients
  - Advanced age is associated with impaired wound healing
  - Co-morbidities negatively affect wound healing
    - Diabetes
    - Obesity
    - Chronic renal failure
    - Smoking
    - Blood circulation insufficiency

- **Why:** patients have low cellular activities
  - Low number and functionality of stem cells
  - Cell senescence and apoptosis
  - Insufficient levels of growth factors & extracellular matrix produced by cells
  - Decreased cell migration, proliferation and maturation
Amniotic Membrane Structure & Properties

Amniotic membrane (amnion):
- Serve as a protective barrier for the developing fetus
- Contains
  - A single layer of epithelial cells attached to the basement membrane
  - Compact & fibroblast (with fibroblast and mesenchymal stem cells) layers
  - The spongy layer separate amnion from chorion, the second placental membrane
- Natural properties include:
  - Anti-inflammatory
  - Antimicrobial
  - Anti-scarring
  - Anti-adhesion
  - Angiogenic
- Amnion has a long history of clinical use for burns and chronic wounds

The goal of preservation is to retain all components of fresh tissue in their native state to be able to store for long time sufficient:

- To complete donor & tissue testing
- To make tissues a “point of care” product

Effect of Preservation Methods on Amniotic Membrane Components

PROCESSING OF FRESH TISSUE

- Cryopreservation & Refrigeration
- Freezing & Cryopreservation
- Drying & Radiation
- Decellularization & Radiation

Cryopreserved Amnion with Living Cells (vCPM) Retains Antimicrobial Properties of Fresh Tissue

- Fresh placental membranes (amnion and chorion) have an antimicrobial effect against a diverse panel of bacteria
- vCPM inhibits growth of ESKAPE bacteria associated with chronic wounds
- Devitalized CPM shows reduced antimicrobial activity

<table>
<thead>
<tr>
<th>ESKAPE Bacteria</th>
<th>Gram stain</th>
<th>Growth reduction compared with control (Log)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>Positive</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Positive</td>
<td>2.6 ± 1.5</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>Negative</td>
<td>5.1 ± 1.7</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Negative</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Negative</td>
<td>6.6 ± 1.8</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>Negative</td>
<td>3.6 ± 1.4</td>
</tr>
</tbody>
</table>

# Currently Available Amnion Products with Living Cells

<table>
<thead>
<tr>
<th>Product Feature</th>
<th>HSAM</th>
<th>vHAMA</th>
<th>vCPM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Hypothermically stored amniotic membrane</td>
<td>Viable human amnion membrane allograft</td>
<td>Cryopreserved placental membrane with living cells</td>
</tr>
<tr>
<td><strong>Storage &amp; Shelf Life</strong></td>
<td>3 weeks (42 days from the manufacturing date) at 1-10(^\circ) C</td>
<td>2 years at -70(^\circ) C or below</td>
<td>3 years at -75-85(^\circ) C</td>
</tr>
<tr>
<td><strong>Cell Viability testing for lot release</strong></td>
<td>No</td>
<td>Yes, no acceptance criterion for cell viability disclosed</td>
<td>Yes, ( \geq )70%</td>
</tr>
<tr>
<td><strong>Graft sizes</strong></td>
<td>2 (1.5x1.5 &amp; 2.5x2.5 cm)</td>
<td>6 (14 &amp; 18 mm disks, 2x2, 2x4, 3x4 &amp; 5x5 cm)</td>
<td>5 (16 mm disks, 1.5x2, 2x3, 3x4 &amp; 5x5 cm)</td>
</tr>
<tr>
<td><strong>HCPCS codes</strong></td>
<td>Q4159</td>
<td>Q4151</td>
<td>Q4133</td>
</tr>
<tr>
<td><strong>Regulatory class</strong></td>
<td>Tissue allograft, HCT/P 361</td>
<td>Tissue allograft, HCT/P 361</td>
<td>Tissue allograft, HCT/P 361</td>
</tr>
<tr>
<td><strong>Published DFU data</strong></td>
<td>No publications identified</td>
<td>1: Regulski M. WOUNDS, 2018, 30(3):E36-E40 (2 cases)</td>
<td>10+ publications including multicenter randomized trials</td>
</tr>
<tr>
<td><strong>DFU trials at clinicaltrials.gov</strong></td>
<td>3 registered: NCT02461641, active, not recruiting; NCT02880592, recruiting; NCT03205436, recruiting</td>
<td>None identified</td>
<td>3 completed</td>
</tr>
</tbody>
</table>

Key vCPM DFU Clinical Studies

• Level I prospective multicenter randomized controlled clinical trial

• Confirmation of level I trial results in real world setting

• Comparative prospective multicenter randomized clinical trial vs a bioengineered skin substitute
Cryopreserved Amnion Level I Multicenter RCT for Chronic DFUs: Study Design

- Level I multicenter RCT Based on 2006 FDA Guidelines*
- Independent Academic Research Organization (ARO) Oversight and monitoring clinical sites
- Wound closure was confirmed by a third party blinded image verification
- Cross-over arm
- The only DFU RCT that was stopped for overwhelming efficacy by an independent safety committee based on results of the pre-defined interim analysis at 50% enrollment


* Guidance for Industry, Chronic, Cutaneous Ulcer and Burn Wounds; Developing Products for Treatment
Cryopreserved Amnion Level I Multicenter RCT for Chronic DFUs: Key Study Outcomes

Fewer Adverse Events, More & Faster Wound Closure and Lower Cost with vCPM Use for Chronic DFUs

- The cost of care for vCPM-treated patients (n=50) was approximately $14,000 lower when compared to control patients (n=47), based on associated adverse events (AEs) and serious adverse events (SAEs).

- The lower costs for vCPM-treated patients were driven by faster wound closure, fewer AEs, SAEs, and hospitalizations.

Open-Label Extension Phase of the Chronic DFUs Multicenter RCT Confirms Benefits of vCPM for Wound Closure and Reduction of Adverse Events

Clinical outcomes for 26 patient cohort treated with SWC in the blinded phase and with vCPM in the extension phase of the trial

<table>
<thead>
<tr>
<th>Outcome</th>
<th>SWC (n=26), blinded phase</th>
<th>vCPM (n=26), open label phase</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound closure, n (%)</td>
<td>0 (0%)</td>
<td>17 (65.4%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Time to closure in days (median)</td>
<td>N/A**</td>
<td>34</td>
<td>N/A</td>
</tr>
<tr>
<td>Study visits (median)</td>
<td>12***</td>
<td>3</td>
<td>N/A</td>
</tr>
<tr>
<td>Subjects with at least one AE, n (%)</td>
<td>18 (69.2%)</td>
<td>11 (42.3%)</td>
<td>0.0465</td>
</tr>
<tr>
<td>AEs, n</td>
<td>52</td>
<td>24</td>
<td>0.019</td>
</tr>
<tr>
<td>Index wound infections, n</td>
<td>12</td>
<td>5</td>
<td>0.116</td>
</tr>
</tbody>
</table>

* - Fisher’s exact one-sided test and Wilcoxon test; ** - Wounds were not closed during the blinded phase; *** - Each patient in the blinded phase had 12 study visits
AE - adverse event; vCPM – viable cryopreserved placental membrane; N/A – not applicable; SWC – standard wound care
vCPM Wound Closure Rate in Real World Mirrors RCT Closure Rates

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Raspovic et al. Real World Study¹</th>
<th>Lavery et al. RCT²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Type</td>
<td>Retrospective, multicenter, nonrandomized</td>
<td>Prospective, multicenter RCT</td>
</tr>
<tr>
<td>Centers</td>
<td>58</td>
<td>20</td>
</tr>
<tr>
<td>Wounds</td>
<td>350</td>
<td>97 (50 Grafix, 47 Control)</td>
</tr>
<tr>
<td>Wounds Excluded</td>
<td>≤ 0.25 cm²</td>
<td>&lt; 1 cm², &gt; 15 cm²</td>
</tr>
<tr>
<td>Complex Wounds</td>
<td>Allowed</td>
<td>Excluded</td>
</tr>
<tr>
<td>Wound Closure at End of Treatment</td>
<td>59.4%</td>
<td>62.0%</td>
</tr>
<tr>
<td>Time to Closure (median)</td>
<td>42.0 days</td>
<td>42.0 days</td>
</tr>
<tr>
<td>Grafts to Close (median)</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

Comparative Multicenter Randomized Clinical Trial for Chronic DFUs: vCPM vs hFDS

Study Design

- Prospective, randomized, single-blind, multi-center, non-inferiority trial\(^1\,\,^2\) comparing clinical outcomes between vCPM (cryopreserved placental membrane with living cells) and hFDS (human fibroblast dermal substitute) for the treatment of chronic DFUs
  - Weekly applications up to 8 weeks with either vCPM or hFDS plus SOC
  - Intent-to-Treat (ITT): 75 patients (38 in vCPM arm, 37 in hFDS arm)
  - Per Protocol (PP): 62 patients (31 in vCPM arm, 31 in hFDS arm)

Study Endpoints

- Primary: Proportion of patients with complete wound closure
- Wounds ≤ 5 cm\(^2\) were specifically evaluated since this represents >75% of DFUs in real-world practice\(^3\)

Patient Demographics & Baseline Wound Characteristics (ITT)

<table>
<thead>
<tr>
<th>Age (mean, years)</th>
<th>vCPM (n = 38)</th>
<th>hFDS (n = 37)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age&gt;65 (%)</td>
<td>23.7</td>
<td>29.7</td>
<td>0.554</td>
</tr>
<tr>
<td>Male (%)</td>
<td>73.7</td>
<td>86.5</td>
<td>0.166</td>
</tr>
<tr>
<td>BMI (mean, Kg/m(^2))</td>
<td>33.7</td>
<td>31.1</td>
<td>0.171</td>
</tr>
<tr>
<td>Heart Disease (%)</td>
<td>92.1</td>
<td>94.6</td>
<td>1.000</td>
</tr>
<tr>
<td>Prior Amputation (%)</td>
<td>55.3</td>
<td>54.1</td>
<td>0.916</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>8.7</td>
<td>8.5</td>
<td>0.601</td>
</tr>
<tr>
<td>Wound Size (cm(^2))</td>
<td>7.2</td>
<td>5.7</td>
<td>0.732</td>
</tr>
<tr>
<td>Wound Duration (days)</td>
<td>199.3</td>
<td>146.3</td>
<td>0.022</td>
</tr>
<tr>
<td>Patients with Prior Advanced Therapy (%)</td>
<td>34.2</td>
<td>16.2</td>
<td>0.073</td>
</tr>
</tbody>
</table>

Comparative Multicenter Randomized Clinical Trial for Chronic DFUs: vCPM vs hFDS

Key Study Outcomes

- vCPM was not inferior to hFDS for the proportion of patients achieving complete wound closure: 48.4% (15/31) for vCPM vs 38.7% (12/31) for hFDS (90% CI: -10.6%, 28.9%)

- Fewer adverse events for vCPM (17 AEs, 4 SAEs) vs hFDS (24 AEs, 7 SAEs)
  - No adverse events were attributed to vCPM

- For typical DFUs (≤5 cm²), patients treated with vCPM had a significantly higher rate of complete wound closure (81.3% vs 37.5%) and the per patient product cost was significantly lower ($3,846 vs $7,969)
Summary

• Three amniotic membrane products (HSAM, vHAMA and vCPM) containing living cells are currently available, however, only one product has clinical evidence in DFU treatment.

• vCPM, a cryopreserved amniotic membrane that retains all components of fresh tissue including viable cells is an appropriate advanced wound care modality for chronic DFU management.

• Results of clinical trials show high rate of wound closure and reduction of wound-related infection, which is a costly complication often leading to hospitalization and amputation.