The Amniotic Membrane

- The amniotic membrane is the innermost lining of the placenta (amnion)
- Biologically active components that facilitate repair and regeneration

Amnion arises from the cell line of the fetus
Biologically Active Tissue Creates a Fetal Environment to Achieve Corneal Healing

“Regeneration” rather than Repair

“Scarless” Fetal Wound Healing

DUAL ACTION

Transition from fetal regenerative healing phenotype → scarring/adhesion phenotype correlates directly with the amount of inflammatory reaction at the wound site.

Slide 6

AM ‘In Utero’ vs. AM on the Ocular Surface

AM Around the Fetus

- In utero, AM acts as a physical barrier against the external environment.
- In utero, AM acts as anti-scarring agent.
- In utero, AM acts as anti-inflammatory agent.
- In utero, AM acts as anti-angiogenic agent.
- In utero, AM supports epithelial adhesion and differentiation.

AM on the Ocular Surface (OS)

- On OS, AM acts as a physical barrier against the external environment.
- On OS, AM acts as an anti-scarring agent.
- On OS, AM acts as an anti-inflammatory agent.
- On OS, AM acts as an anti-angiogenic agent.
- On OS, AM supports epithelial adhesion and differentiation.
Amniotic membrane procurement

- Consensual donation after planned cesarean section delivery
- Donors are screened and tested for infectious diseases and lifestyle risk factors
- Placentas supplied by contracted tissue banks

Amniotic Membrane Technology Highlights

Contains natural growth factors and optimal scaffolding properties within a complex extracellular matrix that are:

- Anti-inflammatory
- Anti-scarring
- Anti-angiogenic

Therapeutic actions:

- Promotes Stem Cell Expansion
- Suppresses pain
- Promotes cellular migration
- Expedites recovery

Amniotic membrane utilization in medicine

- First reported in 1910 at Johns Hopkins by the suggestion of a 3rd year medical student.
- Preservation processes developed in the 1940s.
BioTissue

**AminoGraft®** and **PROKERA®** are cryopreserved amniotic membrane grafts processed using the CRYOTEK™ Method to ensure that the tissue is able to deliver anti-inflammatory, anti-angiogenic, anti-scarring, and wound healing actions.

---

Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

- SJS (Stevens-Johnson syndrome) and TEN (Toxic Epidermal Necrolysis) are potentially fatal adverse reactions to medications such as antibiotics, anti-convulsants, anti-depressants and anti-inflammatory drugs (prescription and non-prescription such as ibuprofen).
- SJS patients have severe blistering of the skin and mucous membranes including the mouth, eyes and genitals.
- When the involvement of the body is greater than 30% the classification is changed to TEN which literally means "toxic skin death".
Ten-day postop Stevens-Johnson patient treated by Dr. Gregory with amniotic membrane. (Fig. 1). As the membranes degraded they appeared mucopurulent but were not infected. Four months later the patient was completely recovered, with no dry eye or other sequelae. (Fig. 2). Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

Bio-Tissue Amniograft (handling wet tissue paper)
IOP Ophthalmics: Ambiodry –
Ambiodry2 – Ambio 5 – AmbioDisk

Lyophilized: dried by freezing in a high vacuum
Ambiodry2=40 microns vs
Ambio5=110 microns—retained chorionic elements

Lyophilized:

Cryopreservation vs AirDrying

• Ensures key active components of the Extracellular Matrix (ECM) are retained
  – The integrity of the tissue structure
  – The key active (ECM) components
• Bio-Tissue Cryopreserved Amniotic Membrane is the ONLY Amniotic membrane product granted wound healing indication by the FDA.

June 2005 FDA letter to OKTO Ophthalmics

• FDA has recognized that amniotic membrane that has not been dehydrated or decellularized may be used for wound repair and wound healing. However, the dehydration and decellularization of AmbioDry alters the characteristics of the original amniotic tissue in a way that could have a meaningful bearing on how the HCT/P performs when used for wound repair or wound healing. More specifically, based on review of the published literature and other data available to the agency, FDA has concluded that removal of cytokine containing cells from this tissue would interfere with human amniotic membrane’s ability to actually mediate wound repair and wound healing. Therefore, when intended for these uses, AmbioDry is more than minimally manipulated and does not meet the criteria in 21 CFR 1271.10(a)(1) for regulation solely under section 361 of the PHSA and 21 CFR Parts 1270 and 1271. The wound repair and wound healing claims described in your website require a pre-market application or notification before the product may be legally marketed.
PROKERA®

• Class II medical device comprising of amniotic membrane in a symblepharon polycarbonate ring to create a unique treatment option for delivering dual action to:
  • Reduce Inflammation
  • Promote Healing
• A safe and effective method to control inflammation and promote scarless healing of the corneal surface
• Provides pain relief and reduces haze, resulting in improved visual acuity


PROKERA® Products: ProKera, ProKera Slim, ProKera Plus
• 21.6 outer diameter for ProKera, Slim, and Plus
• 17.9 inner diameter for Slim, 15.5 for ProKera and Prokera Plus
• Device width 0.7 for Slim, 1.1 for the other two
• Ring and elastomeric band system for Slim
• Dual polycarbonate ring system for the other two
• Prokera Plus with doubled membrane

The Wound Healing Process

Not all wound healing pathways are the same.
Slide 22

Regenerative Healing
ECM components regulate and promote regenerative processes

HC-HA/PTX3 Complex
Promotes Regenerative Healing

- HC-HA (High molecular weight hyaluronic acid covalently linked to Heavy Chain 1 of inter alpha trypsin inhibitor)
- PTX3 (Pentraxin 3) (HC-HA activator)
- Collagens I, III, IV, V and VI
- Fibronectin
- Laminin
- Growth factors

Slide 23

Regenerative Healing

- HC-HA binds to surface neutrophils and accelerates apoptosis of neutrophils
- HC-HA promotes formation of of M2 type macrophages, which facilitates removal of neutrophils
- HC-HA activates TH1 and TH17 lymphocytes which reduces activation of other inflammatory lymphocytes

Slide 24

Other factors present in Amniotic Membrane

- Promoter of epithelialization
  - Epidermal growth factor (EGF), keratinocyte growth factor (KGF), transforming growth factor-α (TGF-α), basic fibroblast growth factor (bFGP), transforming growth factor-β (TGF-β), keratinocyte growth factor receptor (kGFR), hepatocyte growth factor receptor (hGFR), trefoil factor family 3 peptide (TFF3)
- Suppresses angiogenesis
collagen receptor inhibitors, matrix metallopeptidase-2 (MMP-2), MMP-9
- Suppression of inflammation
  - IL-10, IL-1β, IL-6, TNF-α, IL-8, IL-1α, IL-1β, IL-8, TNF-α, IL-1α, IL-1β, IL-8
- Suppression of scarring
  - TGF-β1, TGF-β2, TGF-β3, TGF-β receptor II
- Antimicrobial
  - Human defensins 1–4, defensin, secretory leukocyte protease inhibitor (SLPI), cathepsin G
- Downregulates: Matrix metalloproteinase 2 (MMP-2), MMP-9
Ocular Surface Inflammation

- Uncontrolled inflammation leads to:
  + Chronic pain and discomfort/irritation
  + Delayed healing, more tissue damage
  + Vision-threatening complication, e.g., scar/haze
- Effective control of inflammation is an important strategy to promote healing

Different Outcomes of Tissue Injury

**Passive Pathway**
- Uncontrolled inflammation
- More tissue damage
- Deficient healing
- Ulceration
- Scar formation
- Vision loss

**Active Pathway**
- Proper healing
- Exact replacement
- Regeneration

Current Anti-Inflammatory Approaches:
**Passive, Some Delay Healing**

<table>
<thead>
<tr>
<th>Approach</th>
<th>Targets</th>
<th>Wound Healing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Len/Goggles</td>
<td>Protection</td>
<td>Passive</td>
</tr>
<tr>
<td>Steroids</td>
<td>PMNs and MØ</td>
<td>Delay</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Prostaglandins</td>
<td>Delay (some)</td>
</tr>
<tr>
<td>Topical antibiotics</td>
<td>Microbes</td>
<td>Delay (some)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Microbes, MMPs</td>
<td>No effect</td>
</tr>
<tr>
<td>Omega-3 fatty acids</td>
<td>Prostaglandins</td>
<td>No effect</td>
</tr>
<tr>
<td>Lid scrub</td>
<td>Pure cleansing</td>
<td>No effect</td>
</tr>
</tbody>
</table>
PROKERA® Indications

Diseases with Pre-existing Epithelial Defects
- Neurotrophic persistent corneal epithelial defect
- Post-infectious recalcitrant corneal ulcers (e.g. herpetic, vernal, and bacterial)
- Non-healing epithelial defect after PRK/PTK
- Acute chemical/thermal burns
- Acute Stevens-Johnson Syndrome / Toxic Epidermal Necrolysis
- Following PKP

Diseases without Epithelial Defects
- Dry eye syndrome / Superficial (punctate) keratitis
- Filamentary keratitis
- Radiation keratitis
  - limbal stem cell injury
- Exposure (Graves) keratopathy

To prevent further damage and promote regeneration (no debridement/PTK)
Slide 31

Diseases with Unhealthy Epithelium or Basement Membrane

- Recurrent corneal erosion, EBMD
- Salzmann’s nodular degeneration
- Bullous keratopathy
- Haze after PTK
- Partial limbal stem cell deficiency
- Corneal dystrophy

Slide 32

PROKERA® Insertion

- Set patient expectations!
  Inform the patient they may experience some initial stinging and foreign body sensation
- Apply topical anesthesia
- Rinse the PROKERA® with a sterile solution (saline, BSS etc.)
- Hold the upper eyelid
- Ask the patient to look down
- Insert the PROKERA® into the superior fornix
- Slide the PROKERA® under the lower eyelid

Slide 33

Post Treatment Protocol

- Continue medications
- Apply Temporary Tarsorrhaphy (PRN)
  - Tape tarsorrhaphy
  - Tegaderm strips
Slide 34

Tegaderm/Tape-sorrhaphy

Narrow eye opening, Keep Prokera centered & minimize discomfort

Slide 35

How do I know when to remove PROKERA®?

• Determining when to remove PROKERA® is patient and case dependent
• Follow your usual protocol for follow-up
• Fluorescein stain while PROKERA remains on the eye to determine the progress of healing
• Expect to see the amniotic membrane in PROKERA® decay
• PROKERA® will decay faster when significant inflammation is present
• If there has been over exposure to air, the membrane will likely decay prematurely (less than 3 days)

Slide 36

What about taking medications or IOP readings with PROKERA®?

• Patients can take all their topical medications while having PROKERA® in place, i.e. glaucoma, NSAIDS, antibiotics, steroids etc.
• IOP readings can be taken with PROKERA® on the eye via TonoPen.
**PROKERA® Removal**

- Topical Anesthetic
- Pull the lower eyelid
- Lift the lower edge of PROKERA® using a Q-tip or forceps
- Ask the patient to look down
- Slide the PROKERA® out with gentle pressure on the upper eyelid

**Neurotrophic Keratitis**

**Overview**
- Decreased corneal sensation, epithelial breakdown & poor healing
- Associated with dry eye, exposure keratitis, LSCD, prior HSV or HZV infection
- May lead to corneal infection and melting/perforation
- Conventional treatments fail to promote prompt healing and tend to leave a corneal scar
- Cryopreserved Amniotic Membrane contains Nerve Growth Factor which facilitates epithelial healing and helps recover corneal sensitivity

**Treatment Strategy**
- Restore corneal integrity by reducing inflammation, promoting healing, and preventing haze (PROKERA®)
- Treat underlying cause (e.g., Antiviral)
- Treat associated dry eye (Punctal occlusion and consider autologous serum)
- Prevent further damage (Withdraw unnecessary topical drugs)

**PROKERA® Case Studies**
Case Study 1
A 67 year-old patient had a history of HSV keratitis and dry eye. She presented with mild ocular discomfort and progressive diminution of vision (20/400) for several weeks. Examination revealed a central corneal epithelial defect surrounded by a rim of loose epithelium, stromal edema and anterior chamber inflammatory reaction (Fig. A, B).

**Treatment:**
PROKERA® was placed along with punctal plug, tapingorrhaphy, and oral Acyclovir.

**Results:**
Complete healing occurred within one week, resulting in clear cornea, 20/20 vision, and improved tear meniscus (Fig. C, D).

Neurotrophic Ulcer, commonly associated with:
- Dry Eye
- Punctal Occlusion
- Tapingorrhaphy
- Tarsorrhaphy
- Keeps the eye moist
- Prevents rapid membrane dissolution
- Accelerate healing
- More than one PROKERA®

Recurrent Corneal Erosion
**Overview:**
Disturbance at the level of the corneal epithelial BM → Defective adhesions and recurrent breakdowns of the epithelium

Increased levels of matrix metalloproteinases (MMPs) that dissolve the BM and its anchoring components including integrins, laminin, and type VII collagen

Conventional treatments still have a high recurrence rate and carry the risk of developing haze

Cryopreserved AM contains MMPa inhibitors and active matrix components including collagen type VII and laminin; essential for regenerative healing and prevention of recurrence

**Treatment Strategy**
- Relief acute symptoms e.g. pain (Debridement/PROKERA®)
- Restore corneal integrity, Regain epithelial attachments & Prevent recurrence (PROKERA®)
Case Study 2

52 year-old female presented with ocular pain and blurred vision (20/200) for 2 weeks. She had a history of similar attacks & diagnosed as RCE. Epithelial debridement, lubricants, and DRC, failed to relieve pain and halt recurrence.

Treatment

Epithelial debridement to remove loose epithelium (Fig. A, B) followed by placement of PROKERA® (Fig. C).

Results

On the 2nd day, the patient had no pain. Complete healing occurred within 3 days, resulting in clear corneas, 20/20 vision (Fig. D). A smooth surface remained stable with no recurrence for more than 2 years follow-up.

Debridement + PROKERA® can be considered as an option after lubricants have failed.

Salzmann’s Nodular Degeneration

Overview

Dense irregular collagen tissue between epithelium & Bowman’s layer or beyond
PTK may restore a smooth-ocular surface, however deep laser ablation induce haze and refractive error
Superficial keratectomy/PTK followed by placement of PROKERA® is effective in restoring corneal surface integrity without haze

Treatment Strategy

Removal of corneal lesion (Superficial Keratectomy +/- PTK)
Restoring corneal surface integrity by promoting healing and preventing haze (PROKERA®)
Case Study 3
A 36-year-old male presented with progressive distortion of vision in both eyes for 20 years. Examination showed Salzmann’s Nodular degeneration encroaching on the visual axis resulting in 20/70.

Treatment
Superficial keratectomy was performed, followed by topical mitomycin C (MMC) for 25 sec and placement of PROKERA®.

Results
Complete epithelialization was achieved without haze in 6 days and PROKERA® was removed (Fig. C). The cornea was crystal clear 3 weeks later and vision improved to 20/20 (Fig. D). No recurrence was noted during a 2 years follow-up.

PROKERA® allows regenerative healing to restore corneal surface integrity.

Filamentary Keratitis
Overview
- Filamentary keratitis is a chronic corneal condition characterized by multiple filaments that attach to areas of compromised corneal epithelium. The filaments can be quite extensive and may embed calcareous granules, bacteria, and dust particles, stimulating the pain-sensitive corneal nerves and leading to multiple epithelial defects. Patients often experience foreign body sensation, discomfort, photophobia, pain, and blurry vision. Filamentary keratitis most often accompanies dry eye syndrome and patients may also have underlying systemic conditions, particularly conjunctival pseudomembrane. Ocular preserved amniotic membrane contains anti-inflammatory mediators and complex arrays of growth factors and cytokines, which help regenerate a healthy corneal epithelium and may reduce recurrence.

Treatment Strategy
- Treat ocular surface inflammation (non-preserved steroids)
- Treat associated dry eye (artificial tears/punctal occlusion)
- Restore corneal integrity (PROKERA®)

Case Study #4
A 68-year-old female presented with acute ocular pain, photophobia, and blurred vision (20/70) for 3 days. She had a history of similar repeated attacks as well as dry eye (treated with artificial tears and punctal plugs).

- Diagnosis of filamentary keratitis was confirmed based on the clinical findings of positively stained mucus strands attached to the cornea (Fig. A, C).
- Numerous treatment regimens were implemented, including non-preserved artificial tears, lubricating ointment, and topical steroids for 2 months without success.
- Without development, PROKERA® was placed for 3 days. Her symptoms were completely relieved, filaments disappeared, and the cornea became clear (Fig. B, D).
Case Study #5

• 58 year old contact lens wearer with culture positive S. aureus ulcer, previously treated with (unknown) antibiotics x 2 weeks.
• Placed on fortified vancomycin and tobramycin hourly. 4 days later, culture was negative and antibiotic therapy suspended x 24 hours.

ProKera was inserted in the left eye under topical anesthesia and topical medication was switched to Polymyxin eye drops 4 times/day. The pain rapidly subsided on day 1 after instillation
• Conjunctival inflammation was reduced and corneal epithelialization started centripetally on day 3 postinsertion, when preservative-free 0.1% dexamethasone eye drops were added 4 times a day. On day 5, the patient had no pain, ocular surface inflammation was notable reduced, and the epithelial healing progressed centripetally (Fig. 2C and D).

On day 7, the amniotic membrane dissolved and ProKera was replaced with a therapeutic bandage contact lens.
• However, the epithelial defect enlarged on day 9; therefore, a new ProKera was inserted.
• At day 14 after the second insertion, the corneal surface was healed completely, leaving a 2-mm paracentral faint cornea haziness, and her best corrected visual acuity improved to 20/70 (Fig. 2E and F).
AMT itself does not impede penetration of topical antibiotics, and that AM soaked with antibiotics actually prolong the bactericidal effect. Insertion of ProKera as early as 72 hours after intensive fortified antibiotic treatment in patients after the pathogen was identified could deliver AM's desirable actions with success. Similarly, although insertion of ProKera appeared to allow concomitant topical administration of preservative-free steroid in all 3 cases, future studies are still needed to determine whether it is necessary to use steroids at all when ProKera is inserted for managing infectious keratitis.

Slide 53

Indications Overview
- Band keratopathy
- Post-PK/PTK haze
- Chemical burns
- Corneal epithelial defects - RE, EBMD
- Corneal ulcers, acute and non-healing
- Superficial keratectomy/debridement for Salzmann's, bulous keratopathy post DSEK
- Keratitis - infectious, exposure, filamentary, LCSD, neurotrophic-FED (DM, aging, post HSV, post hx)
- Pterygium sx, conjunctival tumor reaction
- Stevens-Johnson Syndrome