Sutureless Amniotic Membranes: When and How to Use Them

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Disclosure Statement:

Allergan Pharmaceuticals Speaker’s Bureau
Bio-Tissue
IOP Ophthalmics / Katena
BioDLogics, LLC
Seed Biotech / Blythe Medical

What is the Amniotic membrane?
What is the Amniotic membrane

- Thin but tough transparent pair of membranes, which hold a developing embryo (and later fetus) until shortly before birth.

- The primary function of the amniotic membrane is to protect the fetus from injury.
  - 1. Anti-inflammatory
  - 2. Anti-scarring
  - 3. Anti-angiogenic

Fetal Membranes

Amnion is avascular and a translucent membrane composed of an inner layer of epithelial cells which are planted on a basement membrane. Amnion is made of Collagen I, III, IV, V and VII, laminin and fibronectin of which IV, VII, laminin and fibronectin are also found in conjunctiva and cornea.
Historical Perspective

- Considered to be “lucky” and brought good fortune if born with intact caul
- As the healing properties became substantiated by scientific research, this folklore became established as clinical reality
- First used in Dermatology in 1910
  - First used in skin transplantation
  - Biological bandage to dress burns
  - Non-healing skin ulcers
  - Aid to physiological wound healing

Historical Perspective

- Ophthalmological use first occurred:
  - 1940 De Rotth
    - conjunctival defects
  - 1946 Sorsby & Symons
    - chemical burns
- Usage then disappeared from the literature for almost 50 years???
- Horacio Serrano of Caracas, Venezuela, visited Dr Muldachev in Ufa of the former Soviet Union and witnessed the use of a “special tissue” used in ocular sx with impressive results

Historical Perspective

- In May 1992 Dr. Juan F. Batlle presented case at Bascom Palmer, then as a poster at AAO Nov 1993
Historical Perspective

1995 and beyond Dr. Scheffer Tseng and numerous colleagues expanded the clinical applications

Patch vs. Graft

- Biological Bandage — PATCH
  - When used to cover an area of ocular surface and eventually is removed or falls off
  - Placed epithelial side down

- Substrate Basement Membrane — GRAFT
  - When used with expectation that it will become epithelialized and incorporated into the host tissue
  - Placed epithelial side up

Mechanisms of Action

- Promotes Epithelialization
- Suppresses Inflammation
- Inhibits Scarring
- Inhibits Angiogenesis
- Neurotrophic Factors
- Anti-Microbial Agent

All without the harmful side effects found in topical and oral medications
Indications

- Acute Chemical/Thermal Burns
- Recurrent Corneal Erosions
- Neurotrophic Defects / Persistent Corneal Epithelial Defects
- Filamentary Keratitis
- Vernal Keratoconjunctivitis
- Recalcitrant Dry Eye
- Microbial Keratitis
- Nodular Degeneration
- PRK

Indications

- Acute Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis
- Post-infectious Recalcitrant Corneal Inflammation (e.g. herpetic, vernal, and bacterial)
- In conjunction with:
  - Superficial Keratectomy
  - High-Risk Corneal Transplantation
  - Corneal ulcers, descemetocoele or perforations
  - Scleral melts
  - Limbal graft for partial or total limbal stem cell deficiency
  - Oculoplastic procedures including lid, fornix, and socket reconstruction
  - Glaucoma Surgery
  - Conjunctivochalasis and conjunctival reconstruction
  - Pterygium surgery
  - Bullous keratopathy
  - Band keratopathy

Acute Chemical Burns

- Extensive limbal ischemia
- Grade I - No limbal involvement
- Grade II - < 1/3 limbal involvement
- Grade III - 1/3 to 1/2 limbal involvement
- Grade IV - > ½ involvement
- Loss of most limbal stem cells
- Stromal haze limits visualization of iris and lens
Acute Chemical Burns

Two waves of intense inflammation

First Wave occurs 12-24 hours after chemical injury with infiltration of peripheral cornea with PMN and mononuclear leukocytes.

Resulting from:
- Blood elements from injured vessels in conjunctiva and uvea
- Necrotic tissue of bulbar and tarsal conj
- Chemotactically attracted byproducts of epithelium and stromal tissue

Second, more aggressive wave of inflammatory cell infiltration begins at 7 days and peaks when corneal repair and degradation are maximal (between 14-21 day)

Acute Chemical Burns

Pathophysiology
- Limbal ischemia w delayed or non-existent re-epithelialization
- 2 Waves of intense inflammation
- Stromal melt

AM Mech of Action
- Promotes Epithelialization
- Suppresses Inflammation
- Inhibits Scarring
- Inhibits Angiogenesis
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- Anti-Microbial Agent

Recurrent Corneal Erosions

(Courtesy of Ramamurthi et al)
Recurrent Corneal Erosions

Epithelial cells rest on the basement membrane - 128nm
- Lamina Lucida – made of glycoprotein laminin
  - secreted by overlying epi
- Lamina Densa – Made of Type IV collagen
  - secreted by overlying epi
- Lamina Reticularis – Made of fibronectin
  - secreted by underlying stroma

Normal adherence to BM maintained by “adhesion complexes”:
- Hemidesmosomes (arrowhead)
- Lamina lucida and densa
- Anchoring fibrils (arrows)
- Laminin
- Fibronectin
- Type IV and VII Collagen

Recurrent Corneal Erosions

Matrix metalloproteinase (MMP)
- Name for group of enzymes that break down the structure of the extracellular matrix (collagenase)
- Gelatinase
  - Composed of MMP-9 and MMP-2
  - Degrades collagen type IV and VII and Laminin
  - all major components of BM

Elevated levels of MMP-9 and MMP-2 have been observed in tears of patients with RCE
Increased MMP-9 and MMP-2 expression have been implicated in the pathogenesis of RCE’s
- upregulation may lead to BM degradation and poor epithelial basement membrane adhesion.
- Higher than required levels of MMP may dissolve old and newly forming BM

Recurrent Corneal Erosions

Pathophysiology
- Faulty BM with poor adhesion complexes
- Poor epithelialization
- Increased MMP

AM Mech of Action
- Promotes Epithelialization
- Suppresses Inflammation
- Inhibits Scarring
- Inhibits Angiogenesis
- Neurotrophic Factors
- Anti-Microbial Agent
An epithelial defect is defined as persistent when it has failed to heal within a 2 week period.

(PED) occur when there is a failure of the mechanisms promoting corneal epithelialization. results in disassembly of hemidesmosomes accompanied by degradation of Bowman’s layer and stroma.

PED commonly occur in patients with:
- Neurotrophic corneas
- LSCD such as chemical injury
- Immune-mediated ocular surface disorders including atopic keratoconjunctivitis
- Ocular mucus membrane pemphigoid
- Stevens–Johnson Syndrome
- Peripheral ulcerative sclerokeratitis.

Pathophysiology
- Impaired function of the trigeminal nerve
- Insufficient supply of neural factors.
- Deficit in sensory neurotransmitter Substance P

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Filamentary Keratitis

Chronic and recurrent disorder of the cornea characterized by the formation of epithelial and mucous filaments on the corneal surface.

Patients with filamentary keratitis generally experience foreign body sensation, chronic pain, tearing, mucoid discharge, photophobia, and blepharospasm.

Filamentary Keratitis

Inflammatory cells and fibroblasts under the basal epithelium that infiltrate Bowman's layer and damage the epithelial basement membrane.

First step in formation of the filaments

Pathophysiology
- Inflammatory cells damage the epithelial basement membrane
- Focal epithelial basement membrane detachments form and become elevated by the shearing force of blink

AM Mech of Action
- Promotes Epithelialization
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- Neurotrophic Factors
- Anti-Microbial Agent
Recalcitrant Dry Eye

- Clinical findings
  - Tear film instability
  - Ocular inflammation
  - Pro-inflammatory cytokines are upregulated
  - Elevated levels of MMP noted

- Sutureless amniotic membranes contain anti-inflammatory mediators, growth factors and cytokines
  - Help restore a healthy and non-inflamed ocular surface
  - Maintain a stable tear film

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Recalcitrant Dry Eye

Pathophysiology
- Elevated Pro-inflammatory cytokines
- Elevated levels of MMP

AM Mech of Action
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- Anti-Microbial Agent

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Microbial Keratitis

- Excavation and necrosis of corneal tissue from epithelium through stroma
- Common in CL wearers
- Often central, often > 1 mm wide

- Typical findings
  - Pain
  - Redness
  - Photophobia
  - Discharge
Microbial Keratitis

- Amniotic membrane for microbial keratitis
  - Promote healing, reduce haze/scarring

Supportive studies

- Effect of amniotic membrane transplantation on the healing of bacterial keratitis.
  - 3 treatment groups
    - Cefazolin and AMT
    - Non-preserved saline and AMT
    - Cefazolin without AMT
  - Best outcomes were with cefazolin and AMT group
    - Less haze
    - Less neovascularization

Microbial Keratitis

Pathophysiology

- Corneal scarring secondary to stromal involvement

AM Mech of Action

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- Anti-Microbial Agent

PRK Haze

- Steroids used to modulate healing
- Risk factors noted in past
  - UV exposure
  - Increased laser energy
  - Deeper ablations
    - Large optical zones
    - High myopia
    - Previous corneal surgery
PRK Haze

Treatment options
- Manual debridement, steroids
- MMC
- Superficial PTK with MMC
  - May induce more haze
- Amniotic membrane
  - Can be used in conjunction with PTK to reduce haze
  - Can be used during early healing to prevent haze
  - Used as dressing
    - May induce rapid epithelial healing and minimize inflammation
    - May inhibit the irregular synthesis of stromal collagen that is associated with corneal haze

Pathophysiology
- Transforming growth factor beta 1 (TGFβ1) - induced corneal fibrosis

AM Mech of Action
- Promotes Epithelialization
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Procurement
- Membranes are procured and processed according to standards estab by Am Assoc of Tissue Banks (AATB) and FDA
- All recovered under full informed consent
  - From Caesarean vs. vaginal
- A thorough medical and social history of donor is obtained. Screened for:
  - HIV-1
  - HIV-2
  - HIV type 1 Nucleic Acid Test
  - HTLV-1
  - HTLV -2
  - Syphilis RPR
  - CMV
  - Hep B Core antibody
  - Hep B surface antigen
  - Hep C Antibody
  - Hep C Virus Nucleic Acid test
Procurement

- An absolute guarantee of tissue safety is not possible. Allograft has the potential to transmit infections disease to the recipient and the patient should be made aware
- Keep track of tissue used and lot numbers
- All data on file in regard to testing for the tissue
- Do Not use:
  - Areas with active or latent infection
  - Disorder that would create unacceptable risk of post op complications
  - Not to be used in eyes with GLC drainage devices or blebs

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Procurement

- Cryopreserved by CryoTek
  - Preserves the active extracellular matrix (ECM) components of the amniotic membrane
  - Heavy chain hyaluronic acid
  - PTX-3 [HC-HA activator]
  - Collagens (types I, II, IV, V, and VI)
  - Fibronectin
  - Laminin
  - Proteoglycans
  - Growth Factors

- Dehydrated by Purion
  - Dehydration step preserves the delicate collagen matrix
  - Delivers essential growth factors and cytokines
  - Promotes cell proliferation
  - Promotes cell migration

- Dehydrated by DryFlex
  - Optimizes the handling characteristics
  - Retains the growth factors, cytokines, and collagens
  - Preserves extracellular matrix
  - Type I, II, III, IV, VII Collagen
  - Laminin & Fibronectin

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Available Sutureless Membranes

- ProKera®
- Ambio-Disk
- Sky™ OculoMatrix

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Optix International, LLC
9375 Gordon Bernard Cove
Bartlett, TN 38133
Cryopreserved Amniotic Membranes

Prokera

<table>
<thead>
<tr>
<th>Product Specifications</th>
<th>PROKERA</th>
<th>PROKERA</th>
<th>PROKERA PLUS</th>
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<td>Outer Ring Diameter</td>
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<td>Device Height</td>
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<td>Tissue Thickness</td>
<td>Single Layer</td>
<td>Single Layer</td>
<td>Multiple Layers</td>
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<td>Ring Description</td>
<td>Ring &amp; Elastomeric Band System (polycarbonate)</td>
<td>Dual Ring System (polycarbonate)</td>
<td>Dual Ring System (polycarbonate)</td>
</tr>
</tbody>
</table>
Prokera

- Approved by FDA Dec 2003 as a Class II medical device comprised of cryopreserved amniotic membrane graft fastened to thermoplastic ring-set.
- Launched in April 2005.
- 17,000 milestone in September 2014.
- Dual action promotes healing of ocular surface and controls inflammation.
- Stored in medium made of Dulbecco's Modified Eagle Medium / Glycerol containing Ciprofloxacin and Amphotericin B.
- Do not use on patients with a history of drug Rxn to Cipro or amphotericin B.

Prokera

- Cryopreserved.
- Store in refrigerator x 3 months 1°C to 10°C (33.8°F to 50°F).
- Store in freezer:
  - 1 year between -49°C to 0°C (-56.2°F to 32°F).
  - 2 years between -85°C to -50°C (-121°F to -58°F).
- Shelf life is 2 years from date of manufacturer.
- Allow to thaw to room temperature unopened for 5-10 min.
- Open inner pouch and remove using blunt forceps.
- Rinse with saline to reduce stinging sensation.
- Do not leave in eye longer than 30 days.

Prokera

- No content available.

Prokera

- No content available.
Tape-orrhaphy

A tape over the lid crease- Narrows the eye opening, Keeps ProKera centered, and Minimizes discomfort

ProKera

- Specific to rep in your area but if interested in trying, can request a demo tissue to use (cannot bill)
- Volume discounts
  - Order 3 = 5% reduction, Order 5 = 10% reduction

Dehydrated Amniotic Membranes
AmbioDisk (IOP Inc. / Katena)
BioDOptix (BioDLogics)
Aril (Seed Biotech)
Oculomatrix (Skye Biologies)
Alpha Patch (Optix LLC)

Dehydrated Membranes
- All stored at room temperature
- Shelf life typically 2-5 years
- Do not need to be rehydrated
- All require the use of BCL

Ambio Disk
- Ambio 2 (35μ)
- 9 or 15 mm
- Ambio 5 (100μ)
- Comes with a Kontur Precision Spherical CL
  - 8.9 bc
  - 16mm*, 18mm or 20mm

BioDOptix
- Two Disc Sizes
- 12mm or 15mm
- BCL of choice
- Careful with sizing
- 40-60μm thick membrane
AmbioDisk

- Dehydrated tissue
- FDA approval Sept 2006, launched in Oct 2007
- 40,000 tissues placed ocularly

- Ambio 2 ~35um thick 15mm dia
- Ambio 5 ~100+um thick 15mm dia
  - Thicker = longer duration of contact

- Store at controlled room temp 0-38 deg C, 32-100 deg F (can be refrigerated but does not need to be)
- Expires approximately 5 years after receipt
- Processed with Streptomycin Sulfate and Gentamicin Sulfate
  - Caution in patients with allergies to these

- Comes with a Kontur Precision Spherical CL
  - 8.9 bc
  - 16mm, 18mm or 20mm OAD.
- Our Cost $595 (for both 35um and 100um) – includes shipping
Basement membrane side (epithelium) noted by correct right-to-left nomenclature orientation of “IOP”

Apply to cornea with IOP down, i.e. basement membrane (epithelium) of tissue directly in contact with cornea.
**Ambio Insertion**

- Courtesy Eyetube.net Dr. John Hovanesian, MD

![Ambio Insertion Image]

**Dehydrated Membranes**

- **Aril**
  - 8 mm disc
  - 15 mm disc
  - 2 cm x 3 cm ellipse
  - BCL of choice

- **OculoMatrix**
  - 1 cm² or 2 cm²
  - 2 thicknesses
  - 45µ
  - 200µ

![Aril Image]

![OculoMatrix Image]

**Dehydrated Membranes**

- **AlphaPatch**
  - 1.5cm x 2cm
  - 2cm x 3cm
  - 2cm x 6cm
  - 4cm x 4cm
  - 4cm x 8cm

- Thought to maintain growth and healing factors
- Not disrupted as may be the case in other dehydrated membranes
- Used currently in wound care
- Extending into ophthalmic setting

![AlphaPatch Image]
BioD Optix

Product Features
- Dehydrated, extracellular membrane allograft derived from human amniotic tissue
- Product Features
- Dehydrated using patent-pending DryFlex® processing technology
- Adheres well to sclera and conjunctiva when placed on the ocular surface
- Generally placement does not require suture or glue
  - BSCL of choice
- Allograft typically incorporates into tissue in 4-7 days
- Circular or rectangular sizes for optimal fit
- Can be stored at room temperature
  - Shelf life of 2 years
- No advance ordering or preparation is required

Two Disc Sizes
- 12mm
- 15mm

Cover with bandage contact lens of choice.
- If ordering 15mm disc make sure have CL coverage

- 40-60um thick membrane

- All amnion (no chorion), so no basement membrane.
  - Stromal side adheres to cornea better and packaged with that side down
- Dehydration process preserves heavy chain hyaluronic acid

Cost
- 12mm $545
- 15mm $595
- Buy 3 get one free ($400)
Complete the donor and recipient information form and return immediately.

BioDOptix

Recipient form
- Age
- Gender
- Date
BioDOptix
- Tissue recovered from live, healthy donors
  - Pre-screened during pregnancy
  - Caesarean section only
  - Aseptic recovery
  - Operating entities registered with the FDA and accredited by the American Association of Tissue Banks
- Donor testing
  - Pre-natal medical records and test results
  - Comprehensive medical history and behavior risk assessment from the donor prior to donation
  - Discussions with the physician and/or the donor mother are conducted
    - Identify circumstances that may lead to the exclusion

Suggestions
- Create a routine for using these
- Consent Form
- Home going instructions help
  - Antibiotic
  - Corticosteroid
  - Cycloplegic
  - Oral narcotic
- Debridement prior
- Follow up call

Billing
Billing Amniotic Membranes

- 1/01/2014 - Two new CPT codes exist for the use of amniotic membrane along with a series of additional instructions and a revision to the existing ocular surface reconstruction code.

- **65778** — Placement of amniotic membrane on the ocular surface; without sutures

- **65779** — Placement of amniotic membrane on the ocular surface; single layer, sutured

- Do not report 65778, 65779 in conjunction with 65430 (corneal culture), 65435 (debridement), 65780 (ocular surface reconstruction)

- 10 day global period on membrane placement
Case Studies

Recurrent Corneal Erosion

- 45 year old white male– Marathon runner
- October 2012: First visit seen on emergent basis
  - Scratched OD by his Dog
  - 2 linear abrasions detected
  - Healed as expected, Educated on possibility of RCE
- February 2013: RCE but reports minor events on and off for last couple of months
  - EW BSCL
- April 2013: RCE and on and off for weeks
  - EW BSCL and DCN
- Oct 2013: RCE
  - EW BSCL, DCN, Azasite, Muro
Dec 2013
- Corneal Debridement
- Amniotic Membrane – Prokera Slim
- Corticosteroid for 8 weeks
- EW BSCL for 12 weeks

Been symptom free and no recurrences since December 2013
87 yo WF with H/O RCE for 3+ years

OcHx: BRVO, Cat Sx, Fuchs

OcTx: punctal plugs, Restasis, ointments, gels, tears, Steroid drops, BSCL

MedHx: Kidney removed (one kidney), HTN, osteoarthritis, osteoporosis

RTO C/O pain and discomfort with morning awakening. OS Terrible pain 7 out of 10 and photophobia

Noted to have 2mm epi defect on inf nasal cornea OS. 2+ injection and tr cell in AC.

Had been suffering through minor occurrences almost every other week and major every 2-3 months. Has been >18 months since Prokera and no recurrence.
Keratoconjunctivitis Sicca

- Sandra, 75 years old
- Medical Hx:
  - HTN, Osteoporosis
- Ocular History
  - Successful cataract surgery 2012 OU
  - Longstanding dry eye syndrome
- Medications
  - Lotrel
  - Fosamax
  - Restasis
  - FreshKote as needed

Keratoconjunctivitis Sicca

- Ophthalmic Exam
  - Decreased TBUT
  - Dense and diffuse SPK
  - Patient very photophobic
Keratoconjunctivitis Sicca

Options?

Chemical Injury
32 WF, reported to office C/O blurry vision OD since 3pm that day. She reports one hour earlier she had a flat tire and used fix-a-flat to repair her flat car tire.

- Intense pain and photophobia OD
- H/O lasik 4 months earlier
- OD 20/400 last post op visit 20/20, OS 20/20

- pH taken in office was 8.5. MSDS reports fix-a-flat between 8.5-9.5. Immed irrigated in office and after 20 min pH was back to 7.0
- Debrided loose area, applied Ambiodry2:
- Started Ocuflox QID, Pred Forte q2h and Ultram PO
KeratoConjunctivitis Sicca

Case Study -TG

- 61 year old female
- Initially seen in practice 4 years ago
- Ocular History
  - (+) h/o Radial Keratotomy
  - (+) longstanding dry eye / filamentary keratitis
  - treated successfully in past with Restasis, tears

- Patient feels worsening of condition
- Add FreshKote

Case Study -TG

- But.....

- Add Autologous Serum 4-6x/day
- Controlled for a while
- Patient feels worsening of condition
48 yo female suffered with herpes zoster one and half years earlier

Had been tx elsewhere for non healing area on cornea w Pred forte and viroptic. Vision was 20/20 uncorrected prior. After uncorrected 20/100 corrected 20/40-. Complained of pain

Haze on cornea with staining and whorl like healing pattern. As would imagine significant SPK

D/C Viroptic and Pred and started on tears, gels, and Restasis (2 fold, for dry eye and t cell inhibition for potential stromal involvement of HSK)
Recurrent Corneal Erosion
- WR, 50 year old male
- Initial visit August 2011
- Presented with c/o foreign body/irritation OD
- Medical Hx: HTN, hyperlipidemia
- Ocular Hx: Unremarkable

Case Study
- Clinical Exam (September 2012)
  - BCVA 20/20 OD, OS
  - Slit lamp exam
    - Blepharitis/Meibomitis
    - DFE
    - Unremarkable
- Clinical Exam (July 2013)
  - Presents with c/o symptoms of RCE OD
  - Cornea clear OD/OS
  - Treatment: Start Muro 128 ointment QHS OD

Case Study
- Clinical Exam (August 2013)
  - Patient more symptomatic
  - Change treatment course
Case Study

Clinical Exam (August 2013)
- Patient more symptomatic
- Change treatment course
  - Debrided cornea OD
  - BCL x 2 months
  - Add Azasite BID
- Less symptomatic until January 2014

Case Study

Clinical Exam (March 2014)
- New Plan

- Debrided cornea
- ProKera Slim AM inserted
Case Study

Clinical Exam (April 2014)

- Doxycycline 20 mg BID x 2 months
- Lotemax gel TID OD x 1 month

KeratoConjunctivitis Sicca
Use of sutureless amniotic membranes has shown to provide valuable tool to control inflammation and promote epithelialization.

Indications for use are increasing and recommending considering its usage earlier in the treatment paradigm.

When to use a Sutureless AM?
- Promote Epithelialization
- Suppress Inflammation
- Inhibit Scarring

How to use a Sutureless AM?
- Practice makes perfect
- Don’t wait for last resort treatment
Questions?

Thank you

Please feel free to contact us:
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Case Study - PK
- PK, 70 year old Caucasian female
- Significant DES, Sjogren's
- Initial visit December 2010
  - Artificial Tears
  - Salagen
- Eventually added
  - FreshKote
  - Restasis
  - Lotemax
Clinical Findings - PK

- Conjunctiva
  - Lissamine Green Stain
- Cornea
  - Decreased TBUT
  - Lissamine Green, Diffuse SPK

Some improvement clinically initially, but patient still symptomatic and dry eye findings still present
Findings eventually started to worsen

Treatment

- AmbioDry
- Rejuvenate corneal surface
- Informed consent
- Review expectations
Current Treatment

- Restored corneal integrity
- Using Restasis and Rapeseed oil
- Dry eyes still present
- Condition controlled and patient comfortable

KeratoConjunctivitis Sicca

- 64yo Caucasian female
- Referred in for sjogrens syndrome dry eye, previously tried everything under the sun
- Rated dryness irritation 9/10
- Prokera to rejuvenate and concurrent tx with AS OU
- Each ring remained in place for approx 10-15 days
- Best she has felt and seen in years.
Recurrent Corneal Erosion

50 year old Asian male
FBS every morning
Previous treatments:
   - BCL
   - DCN
   - AzaSite

Next Step
Recurrent Corneal Erosion

- Debridement
- ProKera