**Sutureless Amniotic Membranes: When and How to Use Them**

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**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 conj 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 Roth
  - 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, descemetocele or perforations
  - Scleral melts
  - Limbal graft for partial or total limbal stem cell deficiency
  - Oculoplastic procedures including lid, fornix, and socket reconstruction
  - Glaucorea Surgery
  - Conjunctivochalasis and conjunctival reconstruction
  - Pterygium surgery
  - Bullous keratopathy
  - Band keratopathy

Acute Chemical Burns
- Two waves of intense inflammation
  - First Wave occurs 12-24 hours after chem injury with infiltration of peripheral cornea with PMN and mononuclear leukocytes.
  - Resulting from:
    - Blood elements from injured vessels in conj and uvea
    - Necrotic tissue of bulbar and tarsal conj
    - Chemotactically attracted byproducts of epi and stromal tissue
  - Second, more aggressive wave of inflammatory cell infiltration begins at 7 days and peaks when corneal repair and degradation are maximal (bet 14-21 day)

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

AM Mech of Action
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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
- 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 - > 1/2 involvement
- Loss of most limbal stem cells
- Stromal haze limits visualization of iris and lens
**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

**Pathophysiology**
- Faulty BM with poor adhesion complexes
  - Poor epithelization

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**Persistent Corneal Epithelial Defects / Neurotrophic Defects**

- 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

**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.

**Persistent Corneal Epithelial Defects / Neurotrophic Defects**

- 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.
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

Vernal Keratoconjunctivitis (VKC)

- Chronic, bilateral inflammation
- Common in hot, dry environments
- Seen more often in males between 4-20 years old
- Higher incidence with history of atopy

Clinical findings
- Sterile corneal ulcers
- Giant papillae
- Severe itching, photophobia
- Discharge

Vernal Keratoconjunctivitis

Several supportive studies
- Management, clinical outcomes, and complications of shield ulcers in vernal keratoconjunctivitis. Reddy et al
- Amniotic membrane transplantation in the management of shield ulcers of vernal keratoconjunctivitis. Sridhar et al

Vernal Keratoconjunctivitis

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

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Shield Ulcer
- Typically superior
- Can be sight threatening
- Opaque edges, deposition of mucus and cells centrally

Treatment
- Steroids
- Topical cyclosporin
- Amniotic membrane

Pathophysiology
- T helper type 2 cells and their cytokines, corneal fibroblasts along with various growth factors
- Poor re-epithelialization of shield ulcer

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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

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

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

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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

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

Salzmann’s Nodular Degeneration

- Clinical Findings
  - Multiple, superficial nodules in mid-peripheral cornea
  - Pathogenesis unknown
  - Usually asymptomatic

- Treatments
  - Lamellar or penetrating keratoplasty
  - Surgical removal
  - PTK
Nodular Degeneration
- Superficial keratectomy
  - Manual and mechanical
  - Combined with AMT
    - Helps reduce inflammation and restore ocular surface
    - Controls inflammation

Pathophysiology
- Chronic inflammation and/or irritation
- Associated with Dry Eye, EBMD, RCE, Rosacea, MGD,

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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

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

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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

Procurement
- Membranes are procured and processed according to standards established 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
An absolute guarantee of tissue safety is not possible. Allograft has the potential to transmit infections disease to the recipient and the pt 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.

**Available Sutureless Membranes**
- ProKera
- Ambio-Disk
- Bio-Optx

**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, III, 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 collagen
  - Preserves heavy chain hyaluronic acid

**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
  - 15,000 milestone in March 2013
  - 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

**Procurement**
- Cryopreserved
- Store in freezer
  - 1 year bet -49 deg C to 0 deg C (-56.2 F to 32 F)
  - 2 years bet -85 C to -50 C (-121 F to -58 F)- shelf life is 2 yr 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 then 30 days
  - Our cost PKS $949 / tissue + $69 overnight shipping = $1018 (PK $800, PKP $1049)
Tape-sorraphy

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

Complete the donor and recipient information form and return immediately

Recommendations

- 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
- What's new
  - ProKera-Slim
    - New comfort ring
    - Maximizes amniotic membrane contact time with cornea, limbus, and limbal stem cells
    - Outer diameter: 21.6mm
    - Inner diameter: 17.9mm
    - 0.7 thickness
  - ProKera-Plus
    - 200 micron thick
    - Thicker layers result in longer biologic action on ocular surface

AmbioDisk
AmbioDisk
- Dehydrated tissue
  - 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

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**BioD Optix**
- 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

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**BioD Optix**
- **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)

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

Billing Amniotic Membranes

- 1/01/2011 - 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 for wound healing; self-retaining (suture or glue is not needed to achieve ocular surface retention).

- 65779 Placement of amniotic membrane on the ocular surface for wound healing; single layer, sutured

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

- 10 day global period on membrane placement
Recurrent Corneal Erosion

- 87 yo WF with H/O RCE for 3+ years
- OCHx: BRVO, Cat Sx, Fuchs
- Otx: 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.

KeratoConjunctivitis Sicca

Had been suffering through minor occurrences almost every other week and major every 2-3 months. Has been 6+ months since prokera and no recurrence.
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

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 ilasik 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

One day follow up

Two week follow up
Recurrent Corneal Erosion

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

Recurrent Corneal Erosion

- Debridement
- ProKera

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)

Neurotrophic Keratitis
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.

**Conclusion**
- 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.

**Thank You.**

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