Sutureless Amniotic Membranes: When and How to Use Them
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Disclosure Statement:
• Allergan Pharmaceuticals
• BioTissue
• IOP Ophthalmics

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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

What is the Amniotic membrane

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 VII, laminin and fibronectin are also found in conj and cornea
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

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.

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

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

**Acute Chemical Burns**
- Extensive limbal ischemia
  - 1/3 to 1/2 of limbus
- Loss of most limbal stem cells
- Stromal haze limits visualization of iris and lens

**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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**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
  - Glaucoma Surgery
  - Conjunctivochalasis and conjunctival reconstruction
  - Pterygium surgery
  - Bullous keratopathy
  - Band keratopathy

**Acute Chemical Burns**
- 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)**

**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

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

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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 keratoconjunctivitis.

Pathophysiology
- Impaired function of the trigeminal nerve
- Insufficient supply of neural factors
  - Deficit of 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.

**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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**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

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

**Treatment**
- Steroids
- Topical cyclosporin
- Amniotic membrane

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

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Recalcitrant Dry Eye

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

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**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

**Pathophysiology**
- Corneal scarring secondary to stromal involvement

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**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
**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, RCS, 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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**PRK Haze**

- 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
Membranes are procured and processed according to standards established by the American Association of Tissue Banks (AATB) and FDA. All recovered under full informed consent. A thorough medical and social history of the 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 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

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

Available Sutureless Membrane’s

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
Prokera

- 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 than 30 days
- Our cost PKS $949 / tissue + $69 overnight shipping
  = $1018 (PK $800, PKP $1049)
Complete the donor and recipient information form and return immediately.

Prokera

Tape-sorrhaphy

A tape over the lid crease: Narrows the eye opening, keeps Prokera centered, and minimizes discomfort.

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 memb contact time with cornea, limbus, and limbal stem cells
  - ProKera-Plus
    - 200 micron thick
    - Thicker layers result in longer biologic action on ocular surface

AmbioDisk

Dehydrated tissue

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

- Ambio dry 2 40um thick 15mm dia
- Ambio5 100um 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 approx 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 DIA.

- Our Cost $595 (for both 40 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.

Suggestions:
- Create a routine for using these
- Consent Form
- Home going instructions help
  - Antibiotic
  - Corticosteroid
  - Cycloplegic
  - Oral narcotic
- Debridement prior
- Follow up call
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 (corneal culture), 65435 (debridement), 65780 (ocular surface reconstruction)

10 day global period on membrane placement
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.

KeratoConjunctivitis Sicca

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
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

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
One day follow up

Two week follow up

Recruent Corneal Erosion

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

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.

When to use a Sutureless AM?
- Promotes Epithelialization
- Suppresses Inflammation
- Inhibits Scarring

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

Thank You.

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