Ambiodisk vs Cryopreserved Duke Eye Center Poster

The attached poster was presented at this year's ASCRS and will also be presented at WIO by Dr. Lucy Hui, MD. It was independently funded by Duke Eye Center.

The outcomes of the poster are clear: AmbioDisk is just as clinically effective as ProKera but with greater convenience to the facility!

- Both types of amniotic membrane show similar clinical outcomes
- AmbioDisk has the advantage of room temperature storage
- AmbioDisk has the advantage of a 5-year shelf life
- AmbioDisk does not require any pre-thawing or rinsing before use

AOA e-Poster

testimonial from Dr. Balani, OD from LCA Vision, Inc regarding our AmbioDisk product:

- *"We recently placed a Clearify processed Amniotic Membrane allograft in-office on a patient's cornea for the treatment of neurotrophic keratitis. She had a tremendous improvement in both signs and symptoms within a few days. The technology is simply incredible!"*

Dr. Balani presented the attached e-poster at the AOA Optometry meeting, which describes this exact case.

A few key takeaways:

- Slide 1: The use of a dehydrated amniotic membrane (specifically our product) is as an effective treatment for LASIK induced NK – good case study
    - The title "dehydrated amnio graft" is another market term for amniotic membrane graft – not a specific product or brand name
- Slide 3: Highlights key points for amniotic membrane usage including the benefit of "HCHA" heavy chain hyaluronic acid
    - Corza's multi-layer ambio grafts contain 80% more HA than previous versions
- Slide 4: Comparison between dehydrated vs. cryopreserved – specifically calling out our Clearify process
    - *"Clearify™ is a technique that retains greater volume of the intermediate layer (including HCHA) which results in a dehydrated complete human placental membrane (dCHPM)."*
    - *"Well tolerated, ease of application, lower cost to doctor, shelf stable at room temperature for 5 years, expanded availability"*

We are working with Dr. Balani and other physicians on further opportunities to study the clinical outcomes of our dehydrated technology vs. cryopreserved.

Freeze-Dried Versus Cryopreserved Amniotic Membranes in Corneal Ulcers

Recently published, retrospective paper comparing dehydrated amniotic membrane to cryopreserved amniotic membrane to treat corneal ulcers.

This paper was published in the March 2022 edition of Cornea. You can read through the abstract here and attached:
https://journals.lww.com/corneajrnl/Abstract/2022/03000/Freeze_Dried_Versus_Cryopreserved_Amniotic.3.aspx

Summary:

- Baseline characteristics and clinical features of both groups were comparable
- There was no statistically significant difference in corneal healing rate between the two groups
- Conclusion: *"This is the first study that provides positive insight into the effectiveness of FD-AM compared with C-AM when used as overlay transplantation for treating corneal ulcers."*

Outcomes of dried versus cryopreserved amniotic membranes on corneal epithelial defects

Lucy Hui, MD; James Tian, MD; Hazem M. Mousa, MD; Alexander J Snyder, MD; C. Ellis Wisely, MD, MBA

Duke Eye Center

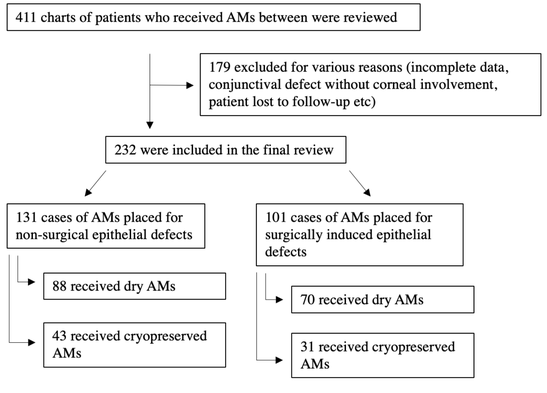
Background

The amniotic membrane (AM) is a collagenous membrane that is derived from the innermost layer of the placenta closest to the fetus.[1] It has been shown to promote epithelial wound healing and can serve as a scaffold for cell growth.[1-5] AMs can be processed into cryopreserved and dried forms.[1,5-6] The dried form is thought to contain lower amounts of the growth factors that contribute to its wound healing properties.[5-6] However, there is insufficient data on whether this translates to differences in clinical outcomes between cryopreserved and dried AMs. The aim of this study is to compare the outcomes of corneal epithelial defects after treatment with cryopreserved versus dried AMs.

Purpose and Methods

A retrospective chart review of patients who received AMs for corneal epithelial defects at Duke was performed. The type of AM, presenting best corrected visual acuity, indications for AM, and anterior segment exam were collected. Based on clinical documentation, patients were separated into epithelial defect fully healed, partially healed, no change, or worse groups. Subgroup analysis of surgically versus non-surgically induced epithelial defects was done. Statistical analysis was performed using R studio.

Figure 1: Study design



Results

Table 1: Overall results

Characteristic	Dry AM (n=158)	Cryopreserved AM (n=74)	P-value
Presenting logMAR, mean (SD)	1.30 (0.85)	1.24 (0.92)	0.70
Follow-up interval days, mean (SD)	10.75 (5.71)	12.49 (11.33)	0.21
Epithelium outcomes, No. (%)			
Healed	59 (37.3)	33 (44.6)	0.36
No change	24 (15.2)	12 (16.2)	0.99
Partially healed	60 (38.0)	22 (29.7)	0.28
Worse	15 (9.5)	7 (9.5)	1.0

Notes: P-values reflect comparison between dry and cryopreserved using 2-sided student's t tests and χ^2 comparisons where appropriate

Table 2: Surgically induced epithelium defect group results

Characteristic	Dry AM (n=70)	Cryopreserved AM (n=31)	P-value
Presenting logMAR, mean (SD)	1.11 (0.83)	1.22 (0.87)	0.57
Follow-up interval days, mean (SD)	10.22 (5.28)	10.29 (6.15)	0.96
Epithelium outcomes, No. (%)			
Healed	34 (48.6)	14 (45.2)	0.92
No change	9 (12.9)	7 (22.6)	0.35
Partially healed	20 (28.6)	8 (25.8)	0.96
Worse	7 (10.0)	2 (6.5)	0.84

Notes: P-values reflect comparison between dry and cryopreserved using 2-sided student's t tests and χ^2 comparisons where appropriate

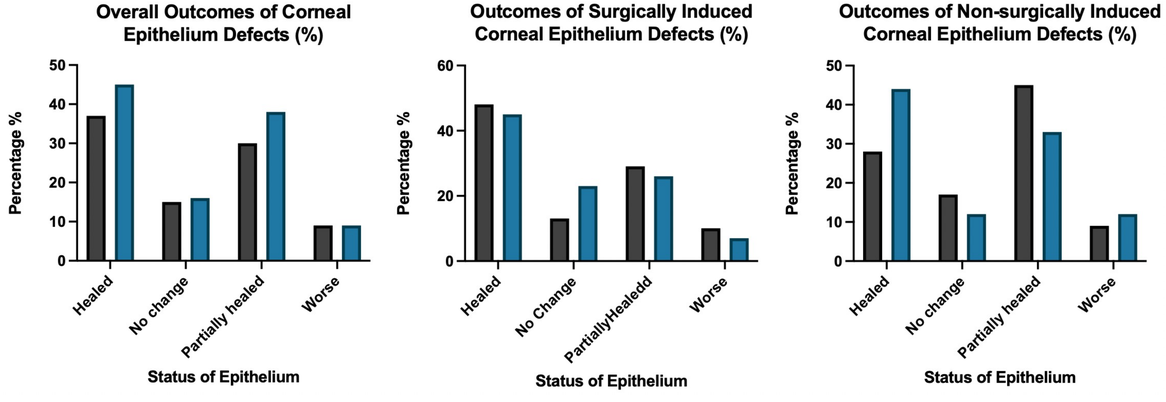
As shown in *Figure 1*, 411 charts of AM placements were reviewed and 232 involved corneal epithelial defects. Of these, 131 did not involve surgery and 101 were surgically induced. *Table 1* focuses on overall results. Patients treated with dried and cryopreserved AMs had similar presenting BCVA (1.30 vs 1.24 logMAR, p = 0.70). The dried and cryopreserved patients also had similar follow-up times after AM placement (10.75 vs 12.49 days, p=0.21). Epithelium outcomes for healed (37.3 vs 44.6 percent, p=0.36), partially healed (38.0 vs 29.7 percent, p=0.28), no change, (15.2 vs 16.2 percent, p=0.99) and worse (9.5 vs 9.5 percent, p=1) groups were not statistically significant between dried and cryopreserved AMs. Similarly, no significant results was found in the two subgroups (*Tables 2 and 3*).

Table 3: Non-surgically induced epithelium defect group results

Characteristic	Dry AM (n=88)	Cryopreserved AM (n=43)	P- value
Presenting logMAR, mean (SD)	1.44 (0.84)	1.26 (0.96)	0.31
Follow-up interval days, mean (SD)	11.16 (6.03)	14.07 (13.78)	0.19
Epithelium outcomes, No. (%)			
Healed	25 (28.4)	19 (44.2)	0.11
No change	15(17.0)	5 (11.6)	0.58
Partially healed	40 (45.4)	14 (32.6)	0.22
Worse	8 (9.1)	5 (11.6)	0.88

Notes: P-values reflect comparison between dry and cryopreserved using 2-sided student's t tests and χ^2 comparisons where appropriate

Figure 2: Corneal epithelial outcomes overall and for each subgroup



Conclusion

In this study, we have shown that dried and cryopreserved AMs resulted in similar outcomes when used to treat corneal epithelial defects. Cryopreserved AMs are stored frozen and must be thawed to room temperature before use while dried AMs have stable shelf lives of many years.[1,6] Our result is clinically important because these dried AMs can be much more easily utilized and accessed especially in resource limited circumstances.

Limitations and future directions

In this study, visual acuity was used as a proxy for overall eye health, and this was found to be not statistically significant between the two AM groups. However, this is only one parameter and more data collection of other variables representing eye health (such as number of prior surgeries, other ocular comorbidities, etc) could be done to make a better overall proxy for eye health and determine whether there are differences in AM use and outcomes depending on eye health.

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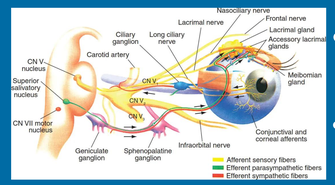
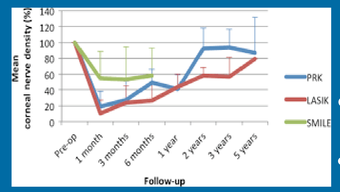
Use of a Dehydrated Amnio Graft for the Treatment of LASIK-induced Neurotrophic Keratitis

Natasha K. Balani, OD; Peter Hovander, OD

LCA Vision, Inc.

Background





- **Neurotrophic keratitis (NK)** is a degenerative process whereby *impaired sensitivity of the corneal nerves* results in *damage and poor healing* of the epithelium.
- While *healthy corneal nerves stimulate* tear production and help *maintain homeostasis* of the ocular surface, *damaged nerves disrupt the feedback loop* between the corneal nerves, lacrimal gland and epithelium, cascading a series of events including *reduced blinking, altered tear film dynamics and poor epithelial healing*.
- **LASIK** has been linked to NK as it *alters the morphology* of the sub-basal corneal nerve plexus.
- In this case, we report on the use of a **dehydrated amniotic membrane** as an effective treatment for LASIK induced NK.

Case History

A 46 year-old female presented in July 2020 with photophobia, redness and decreased acuity, especially at near. She reported fluctuating vision and difficulty working on a computer.

Ocular history was significant for **hyperopic LASIK (monovision-OS near)** 18 months prior and a working diagnosis of dry eye disease

Exam Findings

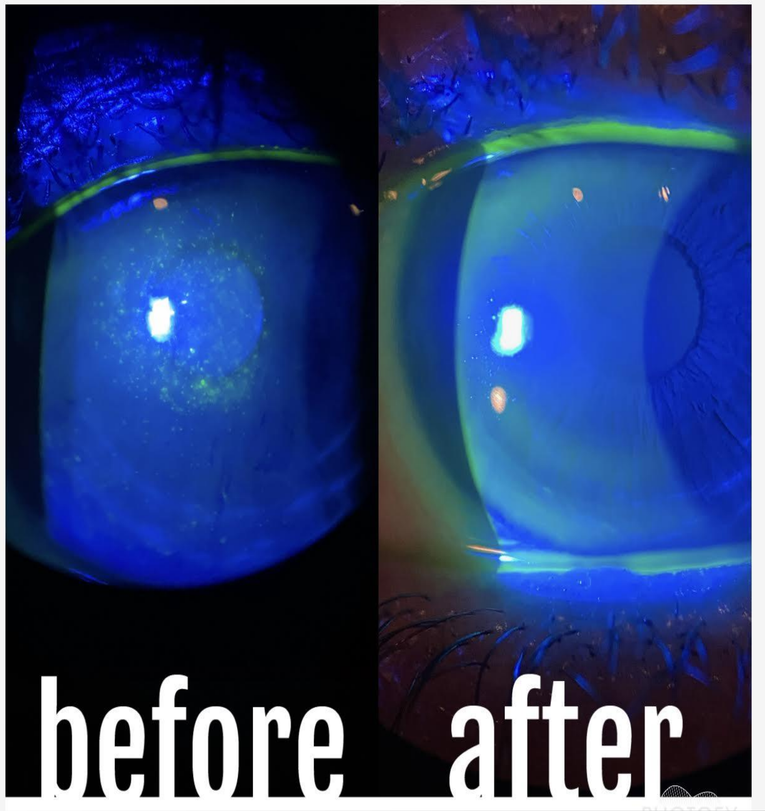
Exam was notable for:

- meibomian gland impissation, cloudy corneal epithelium, punctate epithelial defects (1+ OD, 3+ OS), reduced TBUT
- VAs: 20/25, 20/70
  & **20/50 at near**
- OD: +0.50-0.25x088 20/20
- OS +1.50-0.50x070 20/20-

- Corneal esthesiometry: sensation in the left eye dramatically reduced centrally & in all 4 quadrants when compared to baseline in the right eye – *NK confirmed*

Interventions

- Artificial tears, punctal plugs, Omega 3's, ointment qhs
- Loteprednol etabonate 0.25%, cyclosporine 0.1%., and lifitegrast 5%
- Readers advised for near.
- A *dehydrated graft* was placed in the left eye.

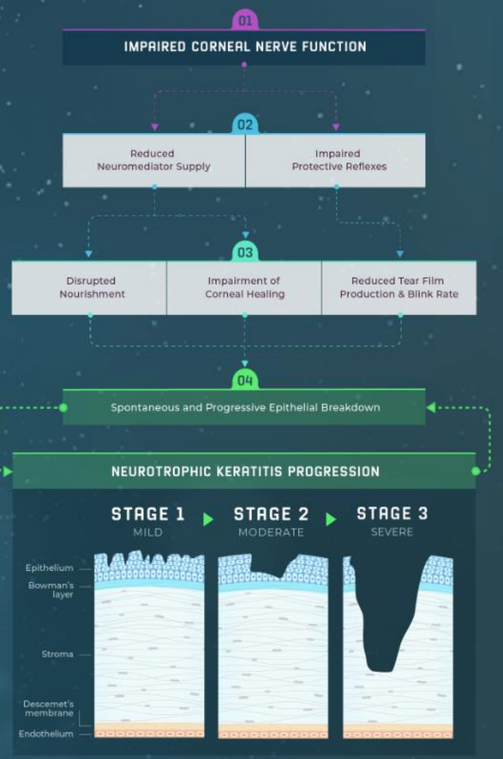


AOA AOSA OPTOMETRY'S MEETING®

Neurotrophic Keratitis

- **Causes of NK**:
  - Herpetic keratitis
  - Trauma
  - ***Corneal surgery such as LASIK, PRK or SMILE***
  - Diabetes
  - Neurosurgical procedures
  - Chronic (over)use of topical medications
  - Corneal dystrophies
  - Chemical burns
  - Other childhood, systemic or genetic diseases

Stages of NK



01 **Impairment of corneal nerves** prompts a series of events that cause progressive damage.[3]

02 A **reduction in neuromediator supply** leads to impairment of neurotrophic factors and a decreased corneal healing rate.[3]

Corneal nerve impairment also causes **impaired protective reflexes** (reduced tear film production and blink rate).[3]

03 The combination of **disrupted nourishment** and **reduced corneal healing, tear film production, and blink rate** contribute to the spontaneous breakdown of the corneal epithelium.[3]

04 **Spontaneous epithelial breakdown leads to NK**, ranging from an irregular, dry, and cloudy corneal epithelium or punctate keratitis (Stage 1), to persistent epithelial defects (Stage 2), and corneal ulcers with stromal melting and perforation (Stage 3). Damage to the corneal epithelium leads to a lack of nourishment that is essential to the survival, differentiation, and maturation of corneal nerve fibers.[1,3]

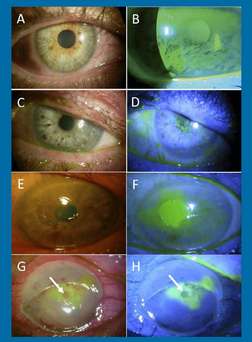
Downstream Effects and Importance of Early Intervention

THE CONSEQUENCES OF UNDIAGNOSED NK:
If left undiagnosed, neurotrophic keratitis (NK) can progress from mild to moderate to severe, and may ultimately lead to corneal perforation, stromal melting, and profound vision loss.[1,]

Aim 1
Diagnose early:
Cotton whisp or
Cochet Bonnet

Aim 2
Reduce
Inflammation
and scarring

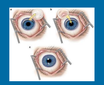
Aim 3
Promote
Re-epithelialization



Remember:
NK is graded by the severity of corneal damage as proposed by Mackie:
Stage 1 (mild) is characterized by epithelial keratitis – *STAIN WITHOUT PAIN*
Stage 2 (moderate) exhibits recurrent or persistent epithelial defect
Stage 3 (severe) exhibits stromal ulceration, can progress to stromal melt or perforation

Treatment options target inflammation and promote re-epethelialization:
They may include artificial or autologous serum tears, scleral contact lenses, tarsorrhaphy, amniotic membranes, cenegermin-bkbj 0.002%, or keratoplasty

oxervate™ 0.002% (20 mcg/mL)
(cenegermin-bkbj) ophthalmic solution

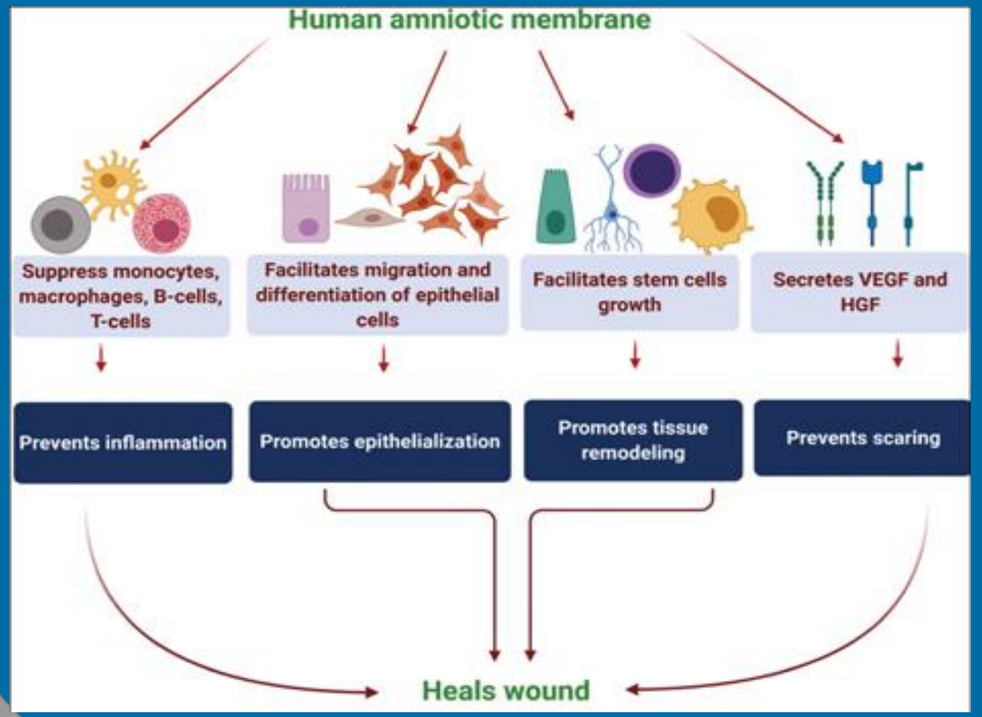


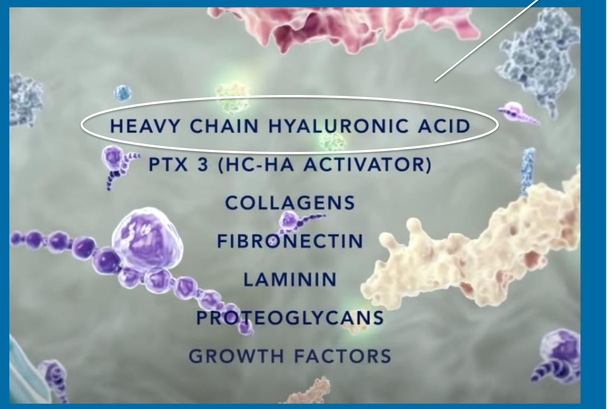
Amniotic Membranes

What are amniotic membranes? Biologic barrier grafts derived from human placental tissue

Clinical Indications: Corneal erosions, Neurotrophic ulcerations, Acute chemical/thermal burns, Non-healing epithelial defects, Conditions associated with excessive dry eye, and Post-infectious keratitis (herpetic, vernal, bacterial).

How do they work?





AM KEY POINTS

- Non-immunogenic
- Reduce inflammation
- Dry (Dehydrated) vs Cryopreserved
- Extracellular matrix, proteins, collagen I-VI, proteoglycans (GAGs), growth factors (PDGF, TGF-B, FGF, IGF) and cytokines
- **HCHA** binds to the surface of neutrophils and accelerates their apoptosis, promotes the formation of phagocytic M2 type macrophages, reduces activity of TH1 and TH17 lymphocytes
- Helps provide the optimal environment to repair, reconstruct and replace wound tissue
- Anti-fibrotic, anti-angiogenic, antimicrobial

Application of the Dehydrated Membrane:

- 1 gtt 0.5% proparacaine
- Lid Speculum
- Dry the cornea
- Open & place AM graft using forceps
- Use a Weck cell to smooth the graft
- Cover with clean BCL
- Remove speculum
- 1 gtt antibiotic

It's All About the *Process*: Dehydrated vs Cryopreserved Amniotic Membranes

	Dehydrated	Cryopreserved
Processing	**Clearify™** is a technique that retains greater volume of the intermediate layer (including HCHA) which results in adehydrated complete human placental membrane (dCHPM). Traditional dehydrated AMs are preserved to room temperature through the application of heat or forced cold air	**CryoTek®** preserved via a proprietary technique which preserves HCHA/PTX3, an immune-signaling complex known to have anti-inflammatory, anti-scarring, and anti-angiogenic effects Membrane is attached to a PMMA ring
Pros	Well tolerated, ease of application, lower cost to doctor, shelf stable at room temperature for 5 years, expanded availability	Retains structural integrity of ECM
Cons	Some preparations are void of the ECM which hold many healing properties	Poorer patient tolerance, Shelf-life limited (2yrs vs %yrs): requires -112°F → 39.2°F storage, can be cost prohibitive
Efficacy	Varied based on study and processing technique	Varied based on study, some studies cite 7-9% greater likelihood of needing secondary intervention due to partial re-epithelialization

Description of Results

Ultimately, a dehydrated membrane was selected and placed in this patient's left eye. Four days later, the membrane was removed and cleared all epithelial defects, restored tear film volume and achieved a **3D resolution** of induced refractive error which fully restored near function.

Cost & Insurance

Procedure code: 65778 "Placement of amniotic membrane on the ocular surface; without sutures." Reimbursement = $1470.83

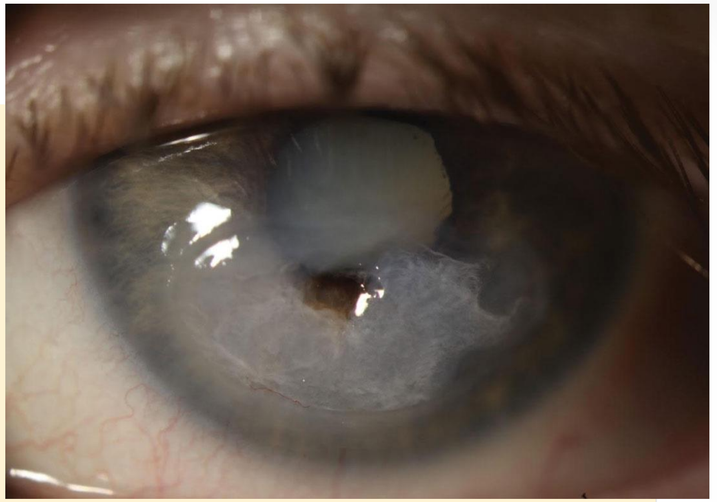
Future Yield

Market for amniotic membrane tissue: 300 million in 2018, forecasted to 660 million in 2026. One of the fastest growing biologics available!

Clinical Pearls

NK is rare disease, with a prevalence estimated at 5 out of every 10,000.

Left untreated it can lead to *devastating* consequences.



Diagnostic Pearls:

- Hallmark symptoms: Dryness, photophobia, blurry vision especially at near, reduced blinking
- *Think NK when signs of PEK/PEE/SPK or PEDs don't respond to standard therapeutic options*
- Identification of NK is performed through corneal nerve sensitivity using a cotton wisp or Cochet Bonnet

Application

Amniotic Membranes can profoundly rehabilitate the ocular surface in a matter of days when conventional treatments fall short.

Just one of the many tools in your toolbox!

Opportunities for Further Research and Development:

Studies comparing efficacy between dehydrated complete (HCHA) and cryopreserved (HCHA-PTX3) amniotic membranes are limited

Development of and more widespread adoption of tools that can predict the development of NK during refractive surgery screening

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Freeze-Dried Versus Cryopreserved Amniotic Membranes in Corneal Ulcers Treated by Overlay Transplantation: A Case–Control Study

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Abstract

Purpose:

The purpose of this study was to assess cryopreserved amniotic membrane (C-AM) versus chorion-free freeze-dried amniotic membrane (FD-AM) overlay transplantation for corneal ulcers in a French tertiary ophthalmology hospital.

Methods:

Between March and July 2020, when C-AMs were not available because of the COVID-19 pandemic, 28 corneal ulcers underwent FD-AM overlay transplantation and were retrospectively compared with 22 corneal ulcers treated with C-AM during the same period in 2018. All patients had at least 3 months of follow-up, and those who underwent combined surgeries were excluded. Ulcers were assessed at baseline and then at 72 hours, 1 month, and 3 months. Population demographics, follow-up time, ulcer etiologies, epithelial defect size, ulcer depth, and complications were also recorded.

Results:

Baseline characteristics and clinical features of both groups were comparable. There was no statistically significant difference in the number of overlay AM transplantations ($P = 0.52$) or early detachments ($P = 0.57$). At 3 months, the corneal healing rate was almost the same in both groups (89% and 91% for FD-AM and C-AM, respectively; $P = 0.87$). Complications were equally uncommon (11% and 9%, respectively; $P = 0.92$). In logistic regression, the type of the membrane did not influence corneal healing at 1 month ($P = 0.42$) or 3 months ($P = 0.99$), regardless of the depth of the ulcer. However, whatever the type of AM used, the deeper the ulcer was, the less likely it was to heal at 3 months ($P = 0.02$).

Conclusions:

This is the first study that provides positive insight into the effectiveness of FD-AM compared with C-AM when used as overlay transplantation for treating corneal ulcers.