



Amniotic Membrane Transplantation for Ocular Surface Reconstruction

Scheffer C. G. Tseng, M.D., Ph.D.

Professor and Charlotte Breyer Rodgers Chair in Ophthalmology
Ocular Surface & Tear Center, Bascom Palmer Eye Institute
Departments of Ophthalmology and Cell Biology & Anatomy
University of Miami School of Medicine

McKnight Vision Research Building
1638 NW 10th Ave.
Miami, FL 33136, USA

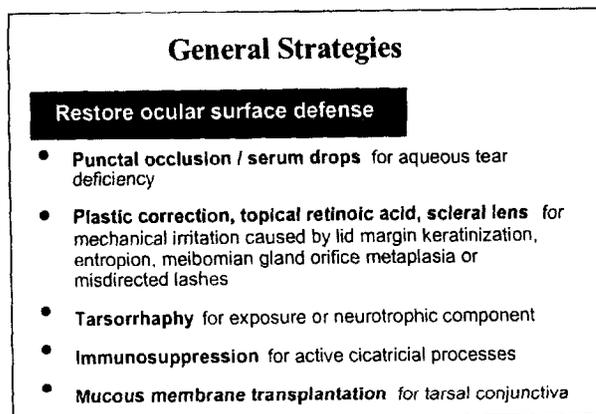
305-326-6046 (voice), 305-326-6306 (fax), e-mail: stseng@bpei.med.miami.edu

Strategies of Ocular Surface Reconstruction

The first strategy is to restore ocular surface defense so that there will be minimal, if not totally excluded, contraindications for surgical grafting (to be described below). Some key measures are summarized in Fig. 1.

If corrected, surgical reconstruction employs two major strategies. The next strategy is to restore the stem cell population by transplanting corneal epithelial stem cells in the procedure termed "limbal transplantation" or by transplanting conjunctival epithelial stem cells in the procedure termed "conjunctival transplantation"¹. The third strategy is to restore the damaged stroma by amniotic membrane transplantation based on the action mechanism (to be shown in Fig. 4).

Fig. 1



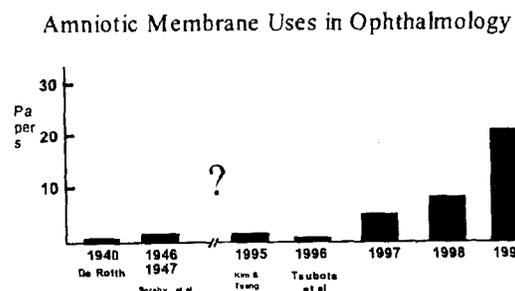
Amniotic Membrane Transplantation

Amniotic membrane, or amnion, is the innermost layer of the placenta and consists of a thick basement membrane and an avascular stromal matrix. Amniotic membrane transplantation has been described for reconstruction in different medical subspecialties in early literature [for review see ²]. In English literature, a *live* fetal membrane including both amnion and chorion was first used by De Roth in 1940 as a graft for conjunctival surface

reconstruction ³. It may be due to his method of preparation and inclusion of chorion, the reported result was not impressive (i.e., the success rate of 1 out of 6 cases in symblepharon lysis), and might explain why others did not follow suit. Based on the observation made by Brown ⁴, who successfully promoted healing and prevented spread of necrosis using a rabbit peritoneum to cover the burned ocular surface, Sorsby et al ^{5,6} in 1946 and 1947 reported a similar success of using amniotic membrane as a patch for treating acute ocular burns.

For reasons still not clear, the use of amniotic membrane disappeared from the literature until 1995 when Kim and Tseng ⁷ reintroduced it for various ophthalmic uses. As will be described in detail below, encouraging results have since been reported from different investigators presumably attributable to improved methods of processing and preservation. There have been increasing interests in using this new procedure as evidenced by the surge of literature reports on this subject (Fig. 2).

Fig. 2



When appropriately processed and preserved (Fig. 3), amniotic membrane can be used in a number of indications, either as a graft to replace the damaged ocular surface stroma matrix or as a patch to prevent unwanted inflammatory insults from gaining access to the damaged ocular surface.

Fig. 3

Preparation of Amniotic Membrane

- Rinse and clean human placenta from elective C/S delivery after screening against HIV-1, HIV-2, HTLV-1, HTLV-2, HBV, HCV, Syphilis at delivery and 6 month

- Separate amnion from chorion blunt dissection



- Flatten amnion onto nitrocellulose paper with epithelium/basement membrane surface up

- Store in DMEM / glycerin (1:1) at - 80 ° C until use

- Available at Bio-Tissue (South Miami)



conjunctival epithelial basement membrane ⁸. The side of the basement membrane is an ideal substrate for supporting the growth of epithelial progenitor cells of the corneal and conjunctival epithelial cells ^{9,10} by prolonging their life span and maintaining their slow-cycling and thus clonogenicity ¹¹. This action supports why amniotic membrane transplantation can be used to expand remaining limbal stem cells and corneal transient amplifying cells during the treatment of partial limbal deficiency ¹² and to facilitate epithelialization for persistent corneal epithelial defects with stromal ulceration ¹³⁻¹⁶.

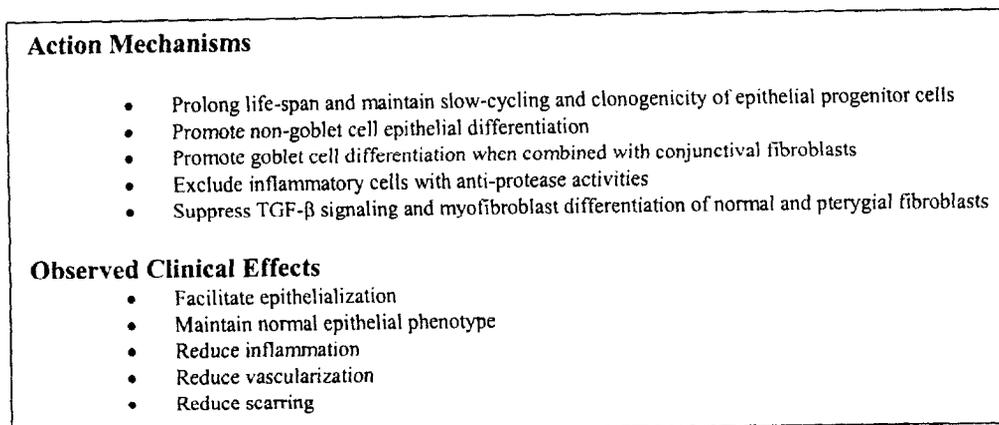
In tissue cultures, amniotic membrane supports limbal epithelial cells grown from explant cultures and resultant amniotic membrane with ex vivo expanded limbal epithelial progenitor cells can be transplanted back to reconstruct the damaged corneal surface in a short-term rabbit study ¹⁷ and in human patients ¹⁸⁻²⁰.

The amniotic membrane can also be used to promote non-goblet cell differentiation of the conjunctival epithelium ¹⁰.

The reported clinical effects and action mechanisms can be summarized in Fig. 4.

The potential action mechanisms might include the following.

Fig. 4



The basement membrane component of the amniotic membrane resembles that of the

This data supports why conjunctival goblet cell density is promoted following amniotic membrane transplantation in vivo ²¹. The stromal side of the membrane contains a unique matrix

component that suppresses TGF- β signaling, proliferation and myofibroblast differentiation of normal human corneal and limbal fibroblasts²², and pterygial body fibroblasts²³. This action explains why amniotic membrane transplantation helps reducing scars during conjunctival surface reconstruction²⁴, preventing recurrent scarring after pterygium removal^{25,26}, and reducing corneal haze following PTK and PRK²⁷⁻²⁹. Several growth factors have been identified in the amniotic membrane³⁰. The stromal matrix of the membrane can also exclude inflammatory cells by rendering them into rapid apoptosis^{28,29}, and contains various forms of protease inhibitors^{31,32}. This action explains why stromal inflammation is reduced after amniotic membrane transplantation^{13,24} and corneal neovascularization is mitigated³³, actions important for preparing the stroma for supporting limbal stem cells to be transplanted either at the same time or later^{12,26,34,35,35}. This action also explains why keratocyte apoptosis can be reduced and hence the stromal haze is prevented in PRK or PTK by amniotic membrane^{28,29}.

Application #1: AMT as a Graft for Treating LSCD

Following diagnosis of limbal deficiency, the damaged corneal surface can be restored with limbal stem cell transplantation with or without the use of amniotic membrane transplantation and^{12,34,35}. The former is intended to restore the limbal stem cell population, and the latter is to restore the damaged limbal stromal environment. Our recent clinical experience showed that this combined approach is effective to treat various extents of limbal deficiency according to the following parameters extents of limbal deficiency, presence of absence of the central corneal transient amplifying cells (TAC), and depth of central corneal involvement¹² (Fig. 5).

When limbal deficiency is diffuse or focal, but as long as the central corneal surface still retains intact and functional TAC, medical treatments should be considered first. These remaining TAC can still provide improved vision with the use of a bandage contact lens, which protect the integrity by eliminating surface breakdowns and by ensuring a smooth optical surface. For this reason, it is advised that the eye be avoided from the use of toxic medications including preservatives and from unnecessary trauma to the remaining TAC by debridement. These medical treatments may last for a period of time before surgical treatments have to be considered.

Although a case report has appeared showing that limbal deficiency caused by radiation may be reversible³⁶, it should be noted that limbal deficiency in general is a progressive disease and hence it should be followed up carefully. Recently we noted that central corneal TAC can also be expanded, if not reversible to SC, by the use of amniotic membrane. Future studies are needed to determine if early intervention with amniotic membrane transplantation is warranted in patients with total limbal deficiency but still retaining central corneal TAC.

When limbal deficiency is partial (involving part of the limbal circumference) and the central corneal surface is no longer covered by intact corneal TAC, amniotic membrane transplantation can be considered. After removing the perilimbal pannus and abnormal epithelium on the corneal surface, a procedure first advocated by Dua et al^{37,38}, the perilimbal bulbar conjunctiva is recessed 5 to 7 mm from the limbus, and the denuded corneal and perilimbal scleral surfaces are covered by a sheet of amniotic membrane. This membrane is anchored to the conjunctival edge by interrupted 10-0 Vicryl or nylon sutures with scleral fixation and to the limbus by a running 10-0 nylon purse-string. After AMT, The membrane covering the corneal surface dissolved but the portion covering the sclera became integrated into the host tissue and epithelialized. The resultant corneal surface and the perilimbal tissue become stable, avascular and non-inflamed. This action is important and permits us to use in

Fig. 5

Strategies for Treating Limbal Deficiency			
Central TAC	Extent of LD	Depth of Corneal Scar	
① Presence	Total/Partial	No	➡ Medical +/- AMT
② Absence	Partial	Superficial	➡ Debridement of Conj + AMT
③ Absence	Total	Superficial	➡ AMT + Allograft LT (first) Autograft LT (last)
④ Absence	Total	Deep	➡ AMT + Allograft LT + PKP/DLKP

conjunction with limbal autograft transplantation to expand limbal stem cell populations on the donor site after removal of the limbal tissue and for the recipient site. Because impression cytology applied to the corneal surface still revealed conjunctivalization²¹, the reconstructed corneal surface might still retain areas with limbal deficiency, where the corneal epithelial phenotype is not recovered.

Because the central corneal surface is now covered with healthy smooth corneal TAC, patient's vision has improved. The advantage of AMT alone in treating this type of partial limbal deficiency is the fact that these patients do not need to take systemic immunosuppressives, and the healthy fellow eye will not take the risk of removing its limbus for autograft limbal transplantation.

When limbal deficiency is unilateral and diffuse but severe enough so that the visual outcome is not certain or when limbal deficiency is bilateral, AMT and allogeneic limbal transplantation in the form of keratolimbal allograft (KLAL) can be considered. If the recipient eye is inflamed with epithelial erosion or granuloma, it is advised that AMT be performed first to suppress the inflammation. In this case, a piece of amniotic membrane is used to cover 360-degree of perilimbal sclera and the entire corneal surface after peritomy and recession of the perilimbal conjunctiva to 5 mm from the limbus. If however, the recipient eye is not inflamed, AMT can be performed with KLAL simultaneously.

The surgical procedure starts with the use of a 1:1000 dilution of epinephrine for vasoconstriction. Incision as a peritomy is made through the upper limbus. The subconjunctival fibrovascular scar tissue is removed, and the entire corneal pannus is removed through the plane usually easily identified via blunt dissection. This results in the denuded corneal and perilimbal sclera up to 5 to 7 mm from the limbus.

The limbal graft is then prepared in the following manner. The donor cornea is removed from the storage medium and the central corneal button is removed by an 8-mm trephine with the epithelial surface facing up. The excessive scleral tissue is first removed by a pair of scissors. The remaining corneoscleral ring is then protected by a layer with Healon. The graft is then turned with the corneal endothelium facing up and the posterior 2/3 of the stroma is removed by scissors. The corneal margin and the scleral margin are both tapered off by scissors trimming

of additional stroma. The finished corneolimbal lamellar graft is then protected on the epithelial side with additional Healon.

Amniotic membrane is then removed from the storage medium and peeled off from the nitrocellulose filter paper, and laid to cover the denuded ocular surface. The membrane is sutured to the conjunctival edge at the bulbar sclera close to the fornix with interruptive 10-0 Vicryl sutures with episcleral fixation. The donor corneolimbal graft is then placed over the cornea and sutured to the sclera and the membrane with interruptive 10-0 Vicryl sutures with episcleral fixation and to the cornea if necessary with interruptive 10-0 nylon sutures or combined with a continuous running suture, if necessary.

Due to the allograft tissue transplantation to vascularized limbal region, potential allograft rejection can occur. To avoid this complication, systemic immunosuppression has to be used.

One major advance made by amniotic membrane transplantation is that partial limbal deficiency can now be reconstructed by this technique without the use of limbal transplantation¹². This result, first observed in rabbit experiments at the time when no explanation was given⁷, indicates that patients with partial limbal deficiency can now be treated without the use of allogeneic limbal stem cell transplantation and hence avoid the long term use of oral cyclosporin. The second advance is the low incidence of limbal allograft rejection when systemic cyclosporin is concomitantly used when amniotic membrane transplantation is performed as the first stage procedure to restore the limbal stromal environment. This effect is presumably attributed to the restoration of a non-inflamed limbal stroma. The remaining difficulty remains in those patients who suffer from severe and deep limbal deficiency leading to concomitant transplantation of corneal grafts, of which the rejection rate is high¹².

Application #2: AMT as a Graft For Treating Other Corneal Indications

Amniotic membrane can also be applied to treat other corneal surface diseases as a graft. These indications are summarized in Fig. 6.

Fig. 6

Surgical Indications of AMT as a Graft

For Corneal Diseases

- Persistent Corneal Epithelial Defect with or without Ulceration
- Partial Limbal Stem Cell Deficiency
- Total Limbal Stem Cell Deficiency (with Limbal Transplantation)
Chemical burns, Stevens Johnson Syndrome
- Painful Bullous Keratopathy with Erosion
- Band Keratopathy

When used as a graft, amniotic membrane can promote healing of persistent corneal ulcers from different causes including neurotrophic keratopathy¹³⁻¹⁶. This approach is superior to conjunctival flaps or tarsorrhaphy as it preserves a cosmetically more acceptable appearance. A recent multi-center trial shows that amniotic membrane transplantation can be used to treat symptomatic bullous keratopathy caused by aphakia, pseudophakia or failed corneal grafts to ameliorate pain and prevent recurrent erosion and microbial superinfection³⁹. Our preliminary study also points out that AMT as a graft can also help treating band keratopathy with or without bullous keratopathy [Anderson et al, manuscript submitted, 2000]. The surgical techniques will be presented in video for discussion.

Application #3: AMT as a Graft for Conjunctival Surface Reconstruction

Based on the action mechanism outlined in Fig. 17, amniotic membrane transplantation can be used to reconstruct conjunctival surface as an alternative to conjunctival graft following removal of large conjunctival lesions such as pterygium^{25,26}, conjunctival intraepithelial neoplasia and tumors²⁴, scars and symblepharon^{24,40}, and conjunctivochalasis^{24,41}.

Other reported indications include improvement of trabeculectomy⁴², and repair of scleral perforation in Marfan's syndrome in conjunction with a sclera patch graft⁴³.

These results indicate that the reconstructed area can be very large so long as the underlying bed is not ischemic and the bordered conjunctiva has a normal epithelium and subconjunctival

stroma. These indications are summarized in Fig. 7. Details of surgical procedures will be presented in video for discussion.

Fig. 7

Surgical Indications of AMT as a Graft

For Conjunctival Diseases

- Pterygium
- Bulbar Conjunctival Reconstruction after Removal of Large Lesions or Scars
- Symblepharon Lysis
- Conjunctivochalasis
- With or without preserved sclera or pericardium for
 - Bleb Leakage or Revision
 - Scleral Melt
 - Lid Reconstruction
 - Orbit Reconstruction

Application #4: AMT as a Temporary Patch for Ocular Surface Reconstruction

Taking the advantage that amniotic membrane may exert anti-inflammatory, anti-angiogenic and anti-scarring effects, which collectively are important to improve the damaged or diseased surfaces, AMT can also be used as a temporary patch as proposed by Kim et al⁴⁴. Specifically, it has been shown very effective to suppress inflammation, relieve pain, and promote epithelialization in the acute stage of chemical or thermal burns both in animals³² and in human patients⁴⁵. Based on the same principle, this technique can also be used for treating patients suffering from Stevens Johnson syndrome at the acute stage⁴⁶. Therefore, it is valuable to suppress uncontrolled inflammation in recalcitrant HZO or HSV keratitis and vernal keratoconjunctivitis (manuscript submitted, 2000 and personal communication).

Based on the same principle, AMT as a patch on a temporary basis has also been used experimentally to reduce corneal haze following PRK or PTK²⁷⁻²⁹. For this reason, it is also useful to protect transplanted PKP when used simultaneously at the conclusion of the surgery to augment the success in severely damaged corneas. These indications are summarized in Fig. 8. It is envisioned that one may expand this list of indications in the future.

Fig. 8

Surgical Indications of AMT as a Patch

- Acute Stage of Chemical or Thermal burns, Stevens Johnson Syndrome
- Preventing Scar after PRK or PTK
- Refractory or Recalcitrant Inflammatory or Ulcerative Keratitis: HSV, HZO, and Vernal

Application #5: AM as a Carrier for Supporting and Expanding Limbal Epithelial Stem Cells *Ex Vivo* for Treating LSCD

The fact that the amniotic membrane can help preserve and expand limbal epithelial stem cells indicates that it can also be used as a carrier

to expand them *in vitro* culture. This new approach is applicable to those patients with limited limbal reserve or who are concerned about having a large part of the healthy limbus removed from the fellow eye or from a living-related donor. In this case, a small limbal biopsy will be performed and the sample will be placed on the amniotic membrane and appropriately cultured. Within 3 to 4 weeks, such an *ex vivo* expanded culture together with the amniotic membrane can then be transplanted to restore the normal corneal surface on limbal deficient corneas. The feasibility of this new approach based on an autologous source has been demonstrated in in a short-term rabbit study¹⁷ and in long-term human patients¹⁸⁻²⁰. This new procedure will be presented in video and its potential uses for allogeneic transplantation will also be demonstrated.

REFERENCES

1. Holland EJ, Schwartz GS. The evolution of epithelial transplantation for severe ocular surface disease and a proposed classification system. *Cornea*. 1996;15:549-556.
2. Trelford JD, Trelford-Sauder M. The amnion in surgery, past and present. *Am J Obstet Gynecol*. 1979;134:833-845.
3. de Roth A. Plastic repair of conjunctival defects with fetal membrane. *Arch Ophthalmol*. 1940;23:522-525.
4. Brown AL. Lime burns of the eye: Use of rabbit peritoneum to prevent severe delayed effects. *Arch Ophthalmol*. 1941;26:754-769.
5. Sorsby A, Symons HM. Amniotic membrane grafts in caustic burns of the eye. *Br J Ophthalmol*. 1946;30:337-345.
6. Sorsby A, Haythorne J, Reed H. Further experience with amniotic membrane grafts in caustic burns of the eye. *Br J Ophthalmol*. 1947;31:409-418.
7. Kim JC, Tseng SCG. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea*. 1995;14:473-484.
8. Fukuda K, Chikama T, Nakamura M, Nishida T. Differential distribution of subchains of the basement membrane components type IV collagen and laminin among the amniotic membrane, cornea, and conjunctiva. *Cornea*. 1999;18:73-79.
9. Cho B-J, Djalilian AR, Obritsch WF, Mattteson DM, Chan CC, Holland EJ. Conjunctival epithelial cells cultured on human amniotic membrane fail to transdifferentiate into corneal epithelial-type cells. *Cornea*. 1999;18:216-224.
10. Meller D, Tseng SCG. Conjunctival epithelial cell differentiation on amniotic membrane. *Invest Ophthalmol Vis Sci*. 1999;40:878-886.
11. Meller D, Pires RTF, Tseng SCG. *Ex vivo* preservation and expansion of human limbal epithelial progenitor cells by amniotic membrane. *Invest Ophthalmol Vis Sci*. 40, S329. 1999.
12. Tseng SCG, Prabhasawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. *Arch Ophthalmol*. 1998;116:431-441.

13. Lee S-H, Tseng SCG. Amniotic membrane transplantation for persistent epithelial defects with ulceration. *Am J Ophthalmol*. 1997;123:303-312.
14. Taylor RJ, Wang MX. Rate of re-epithelialization following amniotic membrane transplantation. *Invest Ophthalmol Vis Sci*. 39, S1038. 1998.
15. Kruse FE, Rohrschneider K, Völcker HE. Multilayer amniotic membrane transplantation for reconstruction of deep corneal ulcers. *Ophthalmology*. 1999;106:1504-1511.
16. Chen H-J, Pires RTF, Tseng SCG. Amniotic membrane transplantation for severe neurotrophic corneal ulcers. *Br J Ophthalmol*. 2000;84:826-833.
17. Koizumi N, Inatomi T, Quantock AJ, Fullwood NJ, Dota A, Kinoshita S. Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. *Cornea*. 2000;19:65-71.
18. Schwab IR. Cultured corneal epithelia for ocular surface disease. *Trans Am Ophthalmol Soc*. 1999;97:891-986.
19. Tsai RJF, Li L-M, Chen J-K. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Eng J Med*. 2000;343:86-93.
20. Schwab IR, Reyes M, Isseroff RR. Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. *Cornea*. 2000;19:421-426.
21. Prabhasawat P, Tseng SCG. Impression cytology study of epithelial phenotype of ocular surface reconstructed by preserved human amniotic membrane. *Arch Ophthalmol*. 1997;115:1360-1367.
22. Tseng SCG, Li D-Q, Ma X. Suppression of Transforming Growth Factor isoforms, TGF- β receptor II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. *J Cell Physiol*. 1999;179:325-335.
23. Lee S-B, Li D-Q, Tan DTH, Meller D, Tseng SCG. Suppression of TGF- β signaling in both normal conjunctival fibroblasts and pterygial body fibroblasts by amniotic membrane. *Curr Eye Res*. 2000;20:325-334.
24. Tseng SCG, Prabhasawat P, Lee S-H. Amniotic membrane transplantation for conjunctival surface reconstruction. *Am J Ophthalmol*. 1997;124:765-774.
25. Prabhasawat P, Barton K, Burkett G, Tseng SCG. Comparison of conjunctival autografts, amniotic membrane grafts and primary closure for pterygium excision. *Ophthalmology*. 1997;104:974-985.
26. Shimazaki J, Shinozaki N, Tsubota K. Transplantation of amniotic membrane and limbal autograft for patients with recurrent pterygium associated with symblepharon. *Br J Ophthalmol*. 1998;82:235-240.
27. Choi YS, Kim JY, Wee WR, Lee JH. Effect of the application of human amniotic membrane on rabbit corneal wound healing after excimer laser photorefractive keratectomy. *Cornea*. 1998;17:389-395.
28. Wang MX, Gray T, Parks WC, Prabhasawat P, Culbertson WW, Forster RK, Hanna K, Tseng SCG. Corneal haze and apoptosis is reduced by amniotic membrane matrix in excimer laser photoablation in rabbits. *J Cat Refract Surg*. 2000;in press.
29. Park WC, Tseng SCG. Keratocyte death linked with acute inflammation modulated by suturing, blood and amniotic membrane in trans-epithelial PRK in rabbits. *Invest Ophthalmol Vis Sci*. 2000;in press.
30. Koizumi N, Inatomi T, Sotozono C, Fullwood NJ, Quantock AJ, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res*. 2000;20:173-177.
31. Na BK, Hwang JH, Kim JC, Shin EJ, Kim JS, Jeong JM, Song CY. Analysis of human amniotic membrane components as proteinase inhibitors for development of therapeutic agent of recalcitrant keratitis. *Trophoblast Res*. 1999;13:459-466.

32. Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits protease activity on wound healing following acute corneal alkali burns. *Exp Eye Res.* 1998;70:329-337.
33. Kim JC, Tseng SCG. The effects on inhibition of corneal neovascularization after human amniotic membrane transplantation in severely damaged rabbit corneas. *Korean J Ophthalmol.* 1995;9:32-46.
34. Tsubota K, Satake Y, Ohyama M, Toda I, Takano Y, Ono M, Shinozaki N, Shimazaki J. Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. *Am J Ophthalmol.* 1996;122:38-52.
35. Shimazaki J, Yang H-Y, Tsubota K. Amniotic membrane transplantation for ocular surface reconstruction in patients with chemical and thermal burns. *Ophthalmology.* 1997;104:2068-2076.
36. Fujishima H, Shimazaki J, Tsubota K. Temporary corneal stem cell dysfunction after radiation therapy. *Br J Ophthalmol.* 1996;80:911-914.
37. Dua HS. The conjunctiva in corneal epithelial wound healing. *Br J Ophthalmol.* 1998;82:1411.
38. Dua HS, Forrester JV. The corneoscleral limbus in human corneal epithelial wound healing. *Am J Ophthalmol.* 1990;110:646-656.
39. Pires RTF, Tseng SCG, Prabhasawat P, Puangsricharern V, Maskin SL, Kim JC, Tan DTH. Amniotic membrane transplantation for symptomatic bullous keratopathy. *Arch Ophthalmol.* 1999;117:1291-1297.
40. Franch A, Rama P, Lambiase A, Ponzin D, Caprioglio G. Human amniotic membrane transplantation. *Invest.Ophthalmol.Vis.Sci.* 39, S90. 1998.
41. Meller D, Maskin SL, Pires RTF, Tseng SCG. Amniotic membrane transplantation for symptomatic conjunctivochalasis refractory to medical treatments. *Cornea.* 2000;In press.
42. Fujishima H, Shimazaki J, Shinozaki N, Tsubota K. Trabeculectomy with the use of amniotic membrane for uncontrolled glaucoma. *Ophthalmic Surg Lasers.* 1998;29:428-431.
43. Rodriguez-Ares MT, Tourino R, Capeans C, Sanchez-Salorio M. Repair of scleral perforation with preserved scleral amniotic membrane in Marfan's syndrome. *Ophthalmic Surg Lasers.* 1999;30:485-487.
44. Kim JC. Use of temporary amniotic membrane graft for corneal diseases. Inaugural Scientific Meeting of Asia Pacific Society of Cornea and Refractive Surgery, 49-49. 1998.
45. Meller D, Pires RTF, Mack RJS, Figueiredo F, Heiligenhaus A, Park WC, Prabhasawat P, John T, McLeod SD, Steuhl KP, Tseng SCG. Amniotic membrane transplantation for acute chemical or thermal burns. *Ophthalmology.* 2000;107:980-990.
46. John T. Transplant successful in Stevens-Johnson syndrome: Human amniotic membrane technique treats acute damage, preserves child's eyesight. *Ophthalmology Times.* 1999;15:10-13.



A-STATE LEVA-40722 04- 04** SECY-ED

4th Ocular Surface and Tear Conference

A Unique Meeting
for Focused and In-depth Discussion

**Amniotic Membrane Transplantation
for Ocular Surface Reconstruction**
Exploring Scarless Wound Healing

Friday, May 14, 1999

This program is sponsored by



**Ocular Surface
& Tear Center**

BASCOM PALMER EYE INSTITUTE

University of Miami School of Medicine

And supported in part by an unrestricted educational grant from

Bio-Tissue, Inc.

Program of 4th Ocular Surface & Tear Conference

Amniotic Membrane Transplantation for Ocular Surface Reconstruction *Exploring Scarless Wound Healing*

7:30am-8:00am Registration and Continental Breakfast (Retter Auditorium)

8:00-8:05am *Scheffer Tseng* Welcome & Historical Overview of Amniotic Membrane Transplantation

8:05-8:30am *Thomas Krummel* Scarless Tissue Repair "Lessons from the Fetus"

I. Corneal Surface Reconstruction for Persistent Corneal Epithelial Defect

Moderator: Friedrich Kruse

8:30-8:40am Friedrich Kruse Multi-layer use for neurotrophic corneal ulcers in Germany

8:40-8:50am Jun Shimazaki For persistent corneal ulcer in Japan

8:50-9:00am Renato Pires For painful bullous keratopathy without visual potential

9:00-9:10am Stephen Stechschulte For persistent corneal ulcer and limbal deficiency

II. Corneal Surface Reconstruction for Limbal Stem Cell Deficiency

Moderator: Jun Shimazaki

9:10-9:20am Craig McCabe Limitation to promote epithelial healing

9:20-9:30am Scheffer Tseng For nearly total limbal deficiency

9:30-9:40am Edward Holland Reconstruction with keratolimbal allografts

9:40-9:50am Ray Tsai Reconstruction with autologous limbal stem cells expanded ex vivo in humans

9:50-10:00am Daniel Meller For maintaining slow-cycling property of epithelial progenitor cells

10:00am-10:10am Coffee Break

III. Conjunctival Surface Reconstruction

Moderator: Charles Bouchard

10:10-10:20am Charles Bouchard Variability and indications for various procedures of ocular surface reconstruction

10:20-10:30am Jose Gomes For cicatricial diseases

10:30-10:40am Marian Macsai Combined with accessory salivary gland transplantation for Stevens Johnson syndrome

10:40-10:50am Daniel Meller For conjunctivochalasis

10:50-11:00am Steve Maskin Experience in an urban cornea referral practice in USA

11:00-11:10am Robert Mack Experience in a suburban cornea referral practice in USA

11:10-11:20am John Kanellopoulos Combined experience in New York and Greece

11:20-11:30am Harminder Dua Experience in UK

11:30-11:40am Bernard Duchesne Experience in Belgium

11:40-11:50am Joseph Frucht-Pery Experience in Israel

11:50-12:00pm Pinnita Prabhasawat 118 eye experience in Thailand

12:00noon-1:10PM

Lunch at University of Miami Faculty Club (Retter Patio)

IV. As a Temporary Patch and Issues Related to Preservation and Storage

Moderator: Jae Chan Kim

1:10-1:20pm	Jae Chan Kim	An overview of its uses as a temporary patch for PED with ulcers
1:20-1:30pm	Thomas John	For acute Stevens Johnson syndrome and acute chemical burn
1:30-1:40pm	Gary Foulks	For acute Stevens Johnson syndrome
1:40-1:50pm	Donald Tan	For refractory vernal conjunctivitis and large tumor excision
1:50-2:00pm	Woo Chan Park	For PRK in humans
2:00-2:10pm	Arnd Heilingenhaus	For necrotizing HSV keratitis in mice
2:10-2:20pm	Elsa Mai	For experimental bacterial keratitis as an adjunctive therapy
2:20-2:30pm	Luis Mejia	Use of non-preserved amniotic membrane
2:30-2:40pm	Clark Springs	Storage and outcome in pterygium

V. Action Mechanisms

Moderator: Scheffer C. G. Tseng

2:40-2:50pm	Per Fagerholm	Presence and distribution of hyaluronan
2:50-3:00pm	Teruo Nishida	Basement membrane components in amniotic membrane
3:00-3:10pm	Noriko Koizumi	Reconstruction with autologous limbal stem cells expanded ex vivo in rabbits
3:10-3:20pm	Abraham Solomon	Suppression of IL-1 β production by epithelial cells seeded on the basement membrane side
3:20-3:30pm	Friedrich Kruse	Suppression of IL-8, Gro- α , ENA expression by epithelial cells seeded on the basement membrane side
3:40-3:50pm	De-Quan Li	Suppression of TGF- β signaling by fibroblasts seeded on the stromal side
3:50-4:00pm	Tae Hoon Choi	Demonstration of anti-scarring effects by intracorneal implantation
4:00-4:10pm	Akira Kobayashi	Anti-angiogenic effect by the stromal side
4:10-4:20pm	Feng Zhang	Presence of anti-neovascularization proteins

4:20pm-4:30pm

Coffee Break

V. Other Applications

Moderator: Jonathan Dutton

4:30-4:40pm	Jonathan Dutton	For fornix reconstruction
4:40-4:50pm	Juan Murube	For punctum patch
4:50-5:00pm	Eva Dafgard Kopp	For oculoplastic surgeries
5:00-5:10pm	Ivan Hwang	For dermal abrasion by CO ₂ laser
5:10-5:20pm	Donald Budenz	For leaking bleb in a prospective randomized trial
5:20-5:30pm	Keith Barton	For glaucoma surgery in rabbits
5:30-5:40pm	Francisco Fantes	Problem solving in glaucoma surgeries
5:40-5:50pm	Thomas John	For exposed Ahmed tube
5:50-6:00pm	Philip Rosenfeld	For subretinal implantation

6:00pm-6:00pm

Conclusion

An Overview of the Development of Amniotic Membrane Transplantation for Ocular Surface Reconstruction

Presenter: Scheffer C. G. Tseng, M.D., Ph.D.

Affiliation: Ocular Surface and Tear Center, Bascom Palmer Eye Institute,
University of Miami School of Medicine, Miami, Florida

Amniotic membrane, the innermost layer of fetal (or placental) membrane, consists of a thick basement membrane and an avascular stroma. Its function is to protect the fetus from unwanted maternal insults during development. It is likely that such a protective function is inherently built in the unique property of the membrane, explaining the observed effects following amniotic membrane transplantation for ocular surface reconstruction. These effects include rapid epithelialization, return of normal epithelial phenotype, and reduced inflammation, vascularization, and scarring. Past history and recent development of this new surgical procedure will briefly be reviewed and the key issues will be laid down for the rest of this conference. It is hoped that through many thorough presentations and intense discussions among presenters and participants, we may begin to understand its action mechanisms and to use amniotic membrane for other new indications.

The Key Issue is: What is the secret for amniotic membrane to achieve these therapeutic effects?

[Note]:

Scarless Tissue Repair “Lessons from the Fetus”

Presenter: Thomas M. Krummel, M.D.

Associates: H. Paul Ehrlich, M.D.

Affiliation: Emile Holman Professor and Chair, Department of Surgery
Stanford University School of Medicine

Scarless repair has been demonstrated in numerous models of fetal integumentary wound healing. The response of the midgestational fetal dermis to wounding is characterized by a rapid restoration of tissue architecture without an acute inflammatory reaction and with a limited, highly ordered deposition of collagen fibers to fill the defect. As term approaches, with abundant disorganized collagen fibers filling the defect resulting in the formation of a scar.

The Key Issue is: Understanding the cellular and biochemical mechanisms of fetal tissue repair in order to apply it to the many problems associated with repair in the adult.

[Note]:

Multilayer Amniotic Membrane Transplantation for Neurotrophic Ulcers

Presenter: Friedrich E. Kruse, M.D.

Associates: Klaus Rohrschneider, M.D., H. E. Völcker, M.D.

Affiliation: Department of Ophthalmology, University of Heidelberg Medical School, Heidelberg, Germany

Purpose. To evaluate the intermediate-term effect of multilayer amniotic membrane transplantation (AMT) for reconstruction of deep neurotrophic corneal ulcers.

Methods. 12 patients with persistent epithelial defects and deep corneal ulcers were followed for 18 months. Patients suffering from metaherpetic keratitis (n = 7), cerebral hemorrhage/trauma (n=2), post keratoplasty (n=2), buphthalmus with multiple intraocular surgeries (n=1). Multilayer AMT with 2 or more layers of membrane was performed with bandage lens for 4 weeks. **Results.** In the early phase all patients showed a remarkable reduction of ocular inflammation. Upon removal of the contact lens (at 4 weeks) the epithelium was closed in all patients. Epithelium and stroma remained stable in 9/12 after one year with 2 patients requiring a second AMT later on. All membranes dissolved after 1 year. **Conclusion.** Multilayer AMT reduces ocular inflammation and allows rapid epithelial healing. In about 50% of the patients long term stability was achieved even when membrane had dissolved.

The Key Issue is: Multilayer use of amniotic membrane for deep corneal ulcers.

[Note]:

Amniotic Membrane Transplantation for the Treatment of Corneal Ulceration

Presenter: Jun Shimazaki, M.D.

Associates: Kazuomi Hanada, M.D., Shigeto Shimmura, M.D.
Hiroshi Fujishima, M.D., Kazuo Tsubota, M.D.

Affiliation: Department of Ophthalmology, Tokyo Dental College

Purpose. To investigate the usefulness of amniotic membrane (AM) transplantation for the treatment of corneal ulceration. **Methods.** Ten eyes of corneal ulceration unresponsive to medical treatments were treated with AM transplantation. Multiple layers of preserved human AM were placed on the ulceration site, which was further covered by another AM. **Results.** The ulceration was successfully treated in 7 eyes. The AMs in the stroma were covered either by corneal or conjunctival epithelium. AMs were rejected in 2 eyes with rheumatoid arthritis-related ulceration, which were then successfully treated by penetrating keratoplasty. **Conclusion.** AM transplantation is a safe, and useful treatment for deep corneal stromal ulceration.

The Key Issue is: A new application of amniotic membrane transplantation for corneal surface reconstruction.

[Note]:

Amniotic Membrane Transplantation for Symptomatic Bullous Keratopathy with Poor Visual Potential

Presenter: Renato T. F. Pires, M.D.¹

Associates: Pinnita Prabhasawat, M.D.², Vilavun Puangsrichareern, M.D.³, Steven L. Maskin, M.D.⁴, Jae Chan Kim, M.D.⁵, Donald T. H. Tan, M.D.⁶
Scheffer C. G. Tseng, M.D., Ph.D.¹

Affiliation: ¹Ocular Surface and Tear Center, Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, Florida, ²Department of Ophthalmology, Siriraj Hospital, Mahidol University, Bangkok, Thailand, ³Department of Ophthalmology, Chulalongkorn University Hospital, Bangkok, Thailand, ⁴Cornea and External Disease of the Eye, Tampa, Florida, USA, ⁵Department of Ophthalmology, Yong-San Hospital, Chung-Ang University College of Medicine, Seoul, Korea, ⁶Singapore National Eye Center, Singapore

We have recently reported that amniotic membrane transplantation can be used as an alternative method for treating persistent epithelial defects and sterile ulceration (American Journal of Ophthalmology 123:303-312, 1997). Now, in this presentation, a study with 50 patients (50 eyes) will be presented to illustrate how amniotic membrane transplantation alone can be considered as an alternative to conjunctival flaps in alleviating pain, promoting epithelial healing, and preserving cosmetic appearance in patients with symptomatic bullous keratopathy and poor visual potential. The underlying causes of bullous keratopathy included aphakia (9 eyes), pseudophakia (19 eyes), failed grafts (9 eyes) and others (13 eyes). The basic surgical technique include the removal of the loose epithelium creating a large corneal epithelial defect, and fastening of amniotic membrane tightly onto the corneal surface to the epithelial edge with running, interrupted, or combination of both sutures.

The Key Issue is: Amniotic membrane transplantation as an alternative method for corneal surface reconstruction in symptomatic bullous keratopathy displaying poor visual potential.

[Note]:

Amniotic Membrane Transplantation for Persistent Epithelial Defects and as an Adjuvant for Surface Reconstruction

Presenter: Stephen Stechschulte, M.D.¹

Associates: Kenneth R. Kenyon, M.D.^{2,3}, Claes Dohlman, M.D.²
Martin Gruterich, M.D.¹

Affiliation ¹Cornea Augenklinik, Munchen
²Cornea Consultants Service, Massachusetts Eye and Ear Infirmary, Boston
³LMU, Boston

Preserved human amniotic membrane has recently become recognized for its usefulness and ability to provide connective tissue and extracellular matrix support for regenerating ocular surface epithelium. Thus it is particularly applicable in situations such as persistent epithelial defects (e.g., neurotrophic keratitis) as well as widespread limbal stem cell deficiency (e.g. chemical burns). **Methods.** 20 eyes of 19 patients were treated with human preserved amniotic membrane transplantation to rehabilitate the ocular surface. Amniotic membranes were used in two ways; to cover a localized defect or to cover the entire cornea and limbus and was sutured to the surrounding conjunctiva. In 15 patients the goal of surgery was to close an epithelial defect. In 7 patients amniotic membranes were used in conjunction with limbal stem cell allograft transplantation in an attempt to rehabilitate the corneal epithelium. Patients were followed for 2-12 months. The area of epithelial defect, degree of neovascularization, and corneal clarity were evaluated. **Results.** Most patients (11/15) showed resolution of epithelial defects, and stabilization of existing neovascularization. Four of seven patients who underwent limbal stem cell transplantation showed increased corneal clarity and improvement of visual acuity. One Stevens-Johnson patient required a second membrane transplantation. One post-penetrating keratoplasty patient developed unrelated bacterial keratitis but re-epithelialized following repeat amnion transplantation. **Conclusion.** Amniotic membrane transplantation can be used in high risk patients to promote resolution of persistent epithelial defects and as an effective adjuvant for ocular surface reconstruction.

The Key Issue is: Amniotic membrane transplantation can be used successfully as an adjuvant for surface reconstruction in a group of challenging patients.

[Note]:

Experience with Eleven Consecutive Cases of Human Amniotic Membrane Transplantation

Presenter: Craig McCabe, M.D.

Associates: Brett Steinwand, M.D., Rebecca Taylor, M.D. Ming Wang, M.D., Ph.D.

Affiliation: Vanderbilt Department of Ophthalmology, Nashville, TN

Purpose. To evaluate the outcomes of human amniotic membrane transplantation (AMT) in a series of 11 consecutive patients. **Methods.** Preserved human amniotic membrane was grafted onto the ocular surface of 11 eyes of 11 patients. Indications included autoimmune corneal melting, pseudophakic bullous keratopathy, persistent corneal epithelial defect, and repeated graft failures for the following conditions: aniridia, keratoconus, trauma, aphakic bullous keratopathy, congenital rubella keratopathy, ocular cicatricial pemphigoid, and ICE syndrome. Nine patients subsequently underwent penetrating keratoplasty (PK), 8 of these had simultaneous limbal allografts (LA) with systemic immune suppression. Visual acuity was assessed. A subset of 8 patients had re-epithelialization rates assessed. **Results.** For the 8 patients for whom the epithelial healing rates were assessed, the average extent of epithelial closure was $21\% \pm 26\%$ of the original epithelial defect on post-operative day 4, and $65\% \pm 35\%$ on day 11. One eye with progressive autoimmune corneal thinning stabilized following AMT alone. For the 9 patients who had AMT + PK, the average time between AMT and PK was 3 months (range 1.25 to 5.5 months). The pre-AMT vision ranged from light perception to 20/200. The best post-AMT + PK/LA vision ranged from 20/50 to light perception. Visual acuities at the time of the last exam ranged from 20/400 to no light perception. The average length of follow up after AMT + PK/LA was 12 months (range 5 to 15 months). Five patients had progression of the original disease process or increased ocular surface inflammation after surgery, resulting in decreased post-operative visual acuity compared to pre-operative. Three patients had no

significant change in post-operative visual acuity compared to pre-operative. Six of the 9 PK's failed. One eye was enucleated due to infection and perforation. **Conclusion.** AMT appears to be efficacious in assisting epithelial healing on the diseased ocular surface. The visual outcome after AMT + PK/LA is not satisfactory, necessitating further discrimination of surgical indications, better control of post-operative inflammation and disease progression, as well as appropriate comparison among non-treated controls, conventional treatment (PK alone), and AMT + PK / LA.

The Key Issue is: What are the limiting factors?

[Note]:

***Amniotic Membrane Transplantation for
Nearly Total Limbal Deficiency***

Presenter: Scheffer C. G. Tseng, M.D., Ph.D.

Affiliation: Ocular Surface and Tear Center, Bascom Palmer Eye Institute,
University of Miami School of Medicine

We have recently reported that amniotic membrane transplantation alone is sufficient to treat partial limbal deficiency by expanding the remaining limbal stem cells to the deficient region (Arch Ophthalmol 116:431, 1998). This novel therapeutic effect is important because it avoids stem cell transplantation and potential side effects of systemic cyclosporin A. Several new cases will be presented to illustrate how amniotic membrane transplantation alone can be used for treating patients with nearly total limbal deficiency, i.e., less than one clock hour of the normal limbus or those with total limbal deficiency but with intact central corneal epithelial cells (transient amplifying cells). The basic surgical procedures include peritomy and recession of the perilimbal conjunctiva at the limbal deficient circumference, removal of the conjunctivalized pannus, and amniotic membrane transplantation to the perilimbal sclera as a graft and covering the corneal surface as a patch.

The Key Issue is: (1) Can corneal transient amplifying cells be reversed to stem cells by amniotic membrane transplantation? (2) Limbal deficiency is a progressive disease. (3) Avoid blood trapped under amniotic membrane during reconstruction to achieve a maximal effect.

[Note]:

*Ocular Surface Reconstruction for
Severe Limbal Stem Cell Deficiency*

Presenter: Edward J. Holland, M.D.

Associates: Ali R. Djalilian, M.D., Gary S. Schwartz, M.D.

Affiliation: Department of Ophthalmology, University of Minnesota

Purpose: To present the clinical outcomes of keratolimbal allograft (KLAL) in patients with severe ocular surface disease secondary to limbal stem cell deficiency. **Methods:** A retrospective review of all patients who underwent KLAL at the University of Minnesota with 6 or more months of follow-up. **Results:** Forty eyes of 33 patients underwent KLAL. The main etiologies of the stem cell deficiency included: aniridia, 16 eyes; chemical injuries, 12 eyes; Stevens-Johnson syndrome, 4 eyes; and multiple surgeries, 4 eyes. The mean follow-up was 32 months (range 6-126). At last follow-up, 25 of 40 eyes (63%) had a stable ocular surface. Aniridia patients had the best outcome with 12 years (75%) having stable surface. Twenty-two of the 40 eyes (55%) had an improvement in the visual acuity. No eyes lost any lines of best-corrected visual acuity. **Conclusion:** KLAL is useful technique in the management of severe ocular surface disease due to limbal stem cell deficiency.

The Key Issue is: Ocular surface reconstruction for severe limbal stem cell deficiency.

[Note]:

Human Amniotic Membrane Expands and Preserves Human Limbal Epithelial Progenitor Cells in Vitro

Presenter: Daniel Meller, M.D.

Associates: Renato T. F. Pires, M.D., Scheffer C. G. Tseng, M.D., Ph.D.

Affiliation: Ocular Surface and Tear Center, Bascom Palmer Eye Institute,
University of Miami School of Medicine, Miami, Florida

Amniotic membrane transplantation is effective in restoring the corneal surface by expanding the remaining limbal epithelial stem cells in patients with partial limbal deficiency. We thus wonder if such an action is also maintained *ex vivo* by amniotic membrane. Primary human limbal epithelial cells (HLEC) were established by explant cultures on Dispase-pretreated amniotic membrane fastened on a culture insert on plastic. Numerous BrdU label-retaining cells were seen on day 1 and 21, indicating that both rapid- and slow-cycling progenitor cells were maintained for at least 3 weeks. Such cultures were strongly positive for K14, positive for K3, ASGP1, ASGP2, and negative for K12, AMEM1, AMEM2 and AM3, indicating that they retained limbal basal epithelial phenotype. After transplanted onto a 3T3 feeder layer, the outgrowth of such cultures still maintained slow-cycling cells. After implanted into athymic mice, the resultant epithelium exhibited a stratified corneal epithelial phenotype, which was positive for K14, negative for K12, AM3, AMEM1, AMEM2, ASGP1, ASGP2 and only suprabasal and superficial cells were positive for K3. These data support that amniotic membrane preserves and expands limbal epithelial progenitor (stem) cells *in vitro*, and may be used to treat patients with limbal stem cell deficiency.

The Key Issues are: (1) What is the mechanism to maintain the slow-cycling nature of epithelial progenitor cells? (2) Is the supporting factor derived from the basement membrane or stroma of the amniotic membrane? (3) Can amniotic membrane substitute the effect of 3T3 fibroblast feeder layer in supporting epithelial stem cells?

[Note]:

The Variability of Amniotic Membrane Transplantation

Presenter: Charles Bouchard, M.D.

Associates: Thomas John, M.D.

Affiliation: Loyola University Medical Center, Maywood, Illinois

To present a series of cases demonstrating the outcomes of amniotic membrane transplantation (AMT) for ocular surface reconstruction. The use of AMT in the management of symblepharon (ocular cicatricial pemphigoid, chronic alkali injury, scarring following infectious eyelid cellulitis), filtering bleb leak and persistent epithelial defects will be presented.

The Key Issue is: The efficacy of AMT depends on the indication for the procedure, the technique used, and the inflammatory condition of the eye.

[Note]:

Clinical Aspects of Amniotic Membrane Use for Surface Reconstruction in Ocular Cicatricial Diseases

Presenter: Jose A.P. Gomes, M.D.

Associates: Janete D.O. Pena, M.D., Namir Santos, M.D., Alessandra Chaves, M.D.
Ciro Komagomet, M.D., Denise de Freitas, M.D.

Affiliation: Federal University of Sao Paulo (UMFESP), Sao Paulo, Brazil

Purpose. To report surgical outcome of human amniotic membrane (AM) use for surface reconstruction in ocular cicatricial diseases. **Methods.** AM was preserved at -80°C in glycerol and cornea culture media (1:1). Eleven eyes of 10 patients underwent AM transplantation associated (9 eyes) or not (2 eyes) with corneal limbal graft. Ocular surface reconstruction was performed after chemical burns (6 eyes), trauma (1 eye) and Stevens-Johnson syndrome (SJS) (4 eyes). **Results.** Mean follow-up time was 90 days. One case of early post-operative infection was excluded from the analysis. Successful ocular surface reconstruction was achieved in 8 eyes (80%). Surgical failure was observed in 2 cases of SJS who presented melting at the time of surgery (20%). **Conclusion.** This study suggests that AM transplantation is an effective alternative for surface reconstruction in stabilizing ocular cicatricial diseases.

The Key Issue is: To report surgical outcome of amniotic membrane transplantation for surface reconstruction in ocular surface cicatricial diseases.

[Note]:

Can Buccal Mucosal Grafts and Amniotic Membrane Transplantation with Limbal Stem Cell Transplantation be Used Successfully to Treat Stevens-Johnson Syndrome?

Presenter: Marian S. Macsai, M.D.

Affiliation: West Virginia University, Morgantown, West Virginia

A 94-year old patient with Stevens-Johnson syndrome was referred for treatment of bilateral ankyloblepharon keratoconjunctivitis sicca and decreased vision. Fornix reconstruction was performed with a combination of amniotic membrane transplantation on the bulbar surface and buccal mucosal grafts on the bulbar surface. In addition, living related conjunctival allografts were obtained from her daughter and transplanted to the limbus during surgical reconstruction. Postoperatively the patient's keratinization resolved with re-wetting of the ocular surface, presumably due to transplantation of accessory salivary glands with the buccal mucosa, and epithelialization of the bulbar surface. However, over the first postoperative year, the buccal mucosa has aggressively grown over the bulbar and corneal surface with inflamed fibrovascular tissue.

The Key Issue is: How can buccal mucosal accessory salivary gland transplants be successfully combined with amniotic membrane transplantation for ocular surface reconstruction?

[Note]:

AMNIOTIC MEMBRANE TRANSPLANTATION FOR CONJUNCTIVOCHALASIS

Presenter: Daniel Meller, M.D.

Associates: Steve L. Maskin, M.D.
Scheffer C. G. Tseng, M.D.

Affiliation: Ocular Surface and Tear Center, Bascom Palmer Eye Institute, University
of Miami School of Medicine, Miami, Florida

Conjunctivochalasis is defined as a redundant, loose, non-edematous inferior bulbar conjunctiva interposed between the globe and lower eyelid. In symptomatic patients, surgical removal with crescent excision can potentially lead to wound healing-induced complications such as visible scarring, cicatricial entropion of the lower lid, retraction of the lower fornix, restricted motility, and/or corneal problems. Based on our recent reports showing that amniotic membrane transplantation (AMT) can successfully restore the conjunctival surface in a variety of external diseases, we performed AMT in 40 consecutive cases (47 eyes) with conjunctivochalasis refractory to conventional treatments for reconstructing the large conjunctival defect created during surgical removal. For a follow-up period of 3.9 ± 2.8 months, 43 (91.4%) eyes recovered a smooth, quiet and stable conjunctival surface. No recurrence was observed. The remaining 4 eyes showed either an inflammation at the host-graft border (initially 4 but resolved in 2 eyes), the formation of a pseudopterygium (1 eye) or residual conjunctivochalasis (1 eye). In two cases a minor scar formation was noted in the lower fornix as complication. Improvement in tearing (23/26, 88%), redness (5/12), itching (9/11), burning (2/7), foreign body sensation (8/11), crust formation (0/2) and tiredness (9/16). Residual symptoms were related to conjunctival inflammation due to pingueculae or aqueous tear deficiency. AMT can be considered as an alternative for conjunctival surface reconstruction during removal of conjunctivochalasis refractory to conventional treatments.

Key issues: (1) Is amniotic membrane suitable for ocular surface reconstruction of large surgically induced excisions of the conjunctiva? (2) Does the beneficial effect of amniotic membrane in conjunctivochalasis also include its anti-inflammatory action? (3) What is the pathogenesis of conjunctivochalasis?

[Note]:

Amniotic Membrane Transplantation Experience in an Urban Referral Practice in U.S.A.

Presenter: Steven L. Maskin, M.D., F.A.C.S.

Affiliation: Cornea and External Diseases of the Eye, Tampa, Florida

Between January 1998 through February 1999, amniotic membrane was used for surface reconstruction in 113 cases. This retrospective briefly reviews the use of amniotic membrane for a variety of indications from an urban referral practice in U.S.A. Results show success in 93% of cases. Amniotic membrane holds great promise as a major advancement in therapy for ocular surface disease.

The Key Issue is: Indications for amniotic membrane transplantation in a referral practice

[Note]:

Amniotic Membrane Transplantation in a Suburban Cornea Referral Practice

Presenter: Robert J.S. Mack, M.D.

The indications and outcomes for amniotic membrane transplantation in a suburban cornea referral practice will be presented and discussed. 12 procedures were performed, 2 for acute alkali burn, 2 for acute thermal burn, 3 for complicated pterygium, (recurrent or primary with two heads), 2 for persistent epithelial defects in cornea transplants, and 3 for the late effects of severe necrosis following thermal burn. Two of these later three were performed in conjunction with stem cell transplantation.

The Key Issue is: Various applications of amniotic membrane transplantation in a suburban cornea referral practice.

[Note]:

Human Amniotic Membrane Transplantation for the Management of Severe External Disease of the Eye

Presenter: Anastasios John Kanellopoulos, M.D.

Affiliation: Manhattan Eye, Ear and Throat Hospital, New York & Orasis Hellenic Eye Center, Athens, Greece

Purpose. To evaluate the safety and effectiveness of human amniotic membrane transplantation (AMT) in patients with severe external disease of the eye. **Methods.** 8 consecutive eyes underwent amniotic membrane transplantation either as the primary procedure, or combined with corneal transplantation (PK), or conjunctival autograft transplantation (CAT). With a follow-up of six to twelve months, the following parameters were evaluated: visual acuity, improvement of ocular surface disease, patient comfort, intra-operative and post-operative complications. **Results.** There were 3 cases of combined PK and AMT; 3 cases of CAT and AMT and two primary AMT. All patients experienced dramatic ocular surface disease improvement within the first month post-operatively. There was one minor complication noted. **Conclusion.** Amniotic membrane transplantation may be a useful alternative in the management of severe external disease of the eye, and can be utilized in combination with other ocular procedures such as penetrating keratoplasty and conjunctival autograft transplantation.

The Key Issue is: Clinical experience of amniotic membrane transplantation in different ocular surface disorders.

[Note]:

Amniotic Membrane Transplantation in UK

Presenter: Harminder S Dua MD, PhD.

Affiliation: Queens Medical Centre, Nottingham, England

The following three aspects of amniotic membrane transplantation (in the UK) will be presented.

- (a) The UK transplant support service authority stipulates that the donors should be tested for HIV at the time of donation and six months later (to cover the window period), while the tissue is held in storage. This precludes use of “fresh” material.
- (b) At least four centres are actively using amniotic membranes in ocular surface reconstruction. We have developed a technique for total ocular surface cover with amniotic membrane in the management of acute, extensive chemical burns. Briefly this consists of tucking a large sheet of amniotic membrane into all four fornices of the affected eye and holding it in place with “fornix-deepening mattress sutures” tied externally over bolsters.
- (c) Data from an Electron microscopic study to demonstrate the in-vitro re-epithelialization of amniotic “basement membrane” will be presented. This data begs the question: Is amniotic membrane transplantation really basement-membrane transplantation?

The Key Issue is: Experiences in UK

[Note]:

Amniotic Membrane Transplantation for Ocular Surface Reconstruction in Belgium

Presenter: Bernard Duchesne, M.D.

Associates: C. Marechal-Courtois, M.D., A. Galand, M.D.

Affiliation: Department of Ophthalmology, CHU Sart-Tilman, University of Liege, Belgium

Purpose: To assess efficacy and safety of the use of human amniotic membrane (HAM) for ocular surface reconstruction. **Methods:** Using the procedure described by Tseng to preserve HAM, 21 patients have been treated by this technique. Surgery was performed by the same surgeon (BD) for 6 recurrent pterygium, 4 chemical burns (3 alkali burns, 1 acid burn), 2 band keratopathy and 1 ulcerative band keratitis, 2 perforations (HAM +Tissucoll, Immuno), 1 persistent epithelial defect (after lids surgery), 1 Staph. aureus ulcer, 1 limbal deficiency, and 1 symblepharon. After the first six consecutive cases, the suture technique was improved and HAM was sutured to the sclera and placed under the conjunctiva. In certain cases the whole corneal surface was covered. **Results:** The average time between the beginning of the pathology and surgery was 244 days (8 months). The average follow-up was 8 months. Re-epithelialization was observed after 20 days. A persistent inflammation occurred in 7 of the 21 cases. Recurrence rate was observed in 8/21. Pain decreased in 14/18. One had a sterile infection (no laboratory findings). No tissue rejection occurred. **Conclusion:** In this series, HAM seems to be effective to treat epithelial defect and ulcer, and for symblepharon lysis, but not for recurrent pterygium. A combination of HAM and Tissucoll is effective to cure a perforation smaller than 3 mm in diameter. Furthermore, pain was effectively controlled. The amniotic membrane can be considered as an additive approach to ocular surface disorder but is not the sole answer to all difficult problems.

Supported by FRO and CHU Research grants.

The Key Issue is: Our experience in Belgium

[Note]:

*Amniotic Membrane Transplantation for
Severe Ocular Surface Disorders*

Presenter: Joseph Frucht-Pery, M.D.

Associates: Abraham Solomon, M.D., David Landau, M.D.

Affiliation: Department of Ophthalmology, Hadassah University Hospital
Jerusalem, Israel

Purpose: To evaluate the safety and the efficacy of amniotic membrane transplantation (AMT) for severe ocular surface disorders. **Methods:** In 5 eyes of 5 patients, 3 male and 2 females, 24 to 72 y.o., we performed AMT for cicatricial pemphigoid (CP), alkali/thermal burns and atopic keratoconjunctivitis (AKC). **Results:** The AMT failed in alkali burn and in AKC. It significantly improved the visual acuity (6/15) in the patient with CP but fungal superinfection after 4 months caused rapid regression and keratinization of the cornea. In thermal burn the ocular surface significantly improved. **Conclusion:** AMT has a limited effect for treatment of severe ocular surface disorders.

The Key Issue is: What is the limiting factor?

[Note]:

Application of Preserved Human Amniotic Membrane for Ocular Surface Reconstruction

Presenter: Pinnita Prabhasawat, M.D

Associates : Panida Kosrirukvongs, M.D., Wipawee Booranapong, M.D.
Yongyutra Watcharadul, M.D.

Affiliation: Department of Ophthalmology, The Bangkok Biomaterial Center, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Purpose. To study the efficacy of amniotic membrane transplantation for ocular surface reconstruction. **Method.** Amniotic membrane transplantations have been performed in Siriraj Hospital from September 1997 to January 1999 in 118 eyes of 108 patients for ocular surface reconstruction. The indications for 57 eyes (51 patients) of the corneal surface group were bullous and band keratopathy (19 eyes), limbal stem cell deficiency due to Steven Johnson syndrome or chemical burn (21 eyes), persistent epithelial defect and dellen (9 eyes), corneal ulcer (1 eye) and acute chemical burn (1 eye). The indication for 61 eyes (57 patients) of the conjunctival group were grafts for pterygial excision (36 eyes), conjunctival tumors (17 eyes), symblepharon (6 eyes), and covering the scleral graft (2 eyes). **Result.** The success were achieved in 77.1% (91/118 eyes), the partial success was 16.1% (19/118 eyes), and the failure was 6.8% (8/118 eyes) for the mean follow-up of 5.5 months (1 to 15 months). The success and partial success rates were 82.4% (47/57) and 12.3% (12/57), respectively, in the corneal surface group and were 72.1% (44/61) and 19.7% (7/61) in the conjunctival group. No patient developed major immediate postoperative complications or graft rejection, and a majority of patients felt more comfortable and were pleased with the surgical results. **Conclusion.** Amniotic membrane transplantation can solve difficult problems in ophthalmology. It is not only a graft but also a membrane that can promote epithelial healing and decrease inflammation and scarring.

The key issue is: Amniotic membrane transplantation used for Ocular surface Reconstruction.

[Note]:

*Temporary Amniotic Membrane Graft for
Persistent Epithelial Defects with Ulceration*

Presenter: Jae Chan Kim, M.D.¹

Associates: W.C. Park, M.D.², K.S. Kim, M.D.³, H.M. Kim, M.D.⁴, S.H. Choi, M.D.⁵, and W.R. We, M.D.⁶.

liation: Department of Ophthalmology, Yongsan Hosp. Chung-Ang Univ., Seoul¹. Dong-A Univ. Medical Center, Pusan². Keimyong Univ. Dongsan Medical Center, Pusan³. Korea Univ. Hospital, Seoul⁴. Chungnam National Univ. Hospital, Taechun⁵. Seoul National Univ. Hospital, Seoul⁶. Korea.

Purpose: We determined whether temporary amniotic membrane graft (TAMG) can be used as a combined approach to treat vision-threatening persistent epithelial defect (PED) with ulceration. **Method:** Human amniotic membrane was prepared and preserved using our previously described method with minor modification. TAMG was performed in total 62 patients with PED and ulceration, of which the causes were chemical burn (n=6), thermal burn (n=2), post-infectious (n=21), neurotrophic condition (n=11), bullous keratopathy (n=13), post-photorefractive keratotomy (n=8), and etc. **Results:** Mean duration time of TAMG lasted for 7.1 days. Mean numbers of TAMG application were 1.5 times. Corneal epithelial defects healed within mean 15.1 days, which were significantly shorter than that before TAMG (P<0.01). Postoperatively, visual acuity and ocular surface were much improved in most cases. TAMG failed in two cases of preexisting corneal perforation and two cases of long-standing PED with marked stromal thinning. **Conclusions:** This new approach with TAMG showed promise in treating recalcitrant PED with ulceration, and should be considered before conjunctival flap, tarsorrhaphy and keratoplasty.

Supported by grant of Good Health RND project (HMP-97-M-5-0055), Ministry of Health and Welfare, R.O.K.

The Key Issue is: What is the mechanism?

[Note]:

Amniotic Membrane Transplantation for Acute Alkali Burn and Stevens Johnson Syndrome

Presenter: Thomas John, M.D.

Affiliation: Loyola University Medical Center, Maywood, Illinois

A 53-year-old male sustained an alkali injury to his left eye in January 1998. He had an opaque cornea with almost total epithelial defect and surrounding conjunctival defect. He developed a persistent epithelial defect. On February 10, 1998 he underwent a preserved human amniotic membrane (HAM) transplantation to cover the cornea and conjunctiva during the acute stage of alkali burn. Detachment of the HAM required a repeat HAM transplantation on February 17, 1998. Epithelial defects healed in two weeks. One year following the procedure, he continues to do well. His vision has improved from legal blindness preoperatively to 20/50⁺¹ at the present time. This is the first case of amniotic membrane transplantation in acute alkali burn of the cornea and conjunctiva.

A 6-year-old boy developed acute Stevens-Johnson syndrome after the use of trimethoprim and sulfamethoxazole (Septra) medication for chronic right otitis media. There was total involvement of both eyes and all four eyelids. On October 21, 1998, he underwent bilateral lysis of symblepharon and lysis of adhesions, and left total amniotic membrane transplantation (AMT) of the entire ocular surface from lashline-to-lashline with corneal exposure for vision. The following day, he underwent right lysis of symblepharon and adhesions, and right total AMT. Exposure of eyelid musculature with total loss of eyelid skin required amniotic membrane transplantation of all four eyelids on the external eyelid surfaces and strip AMT at the mucocutaneous junction of the eyelid margins. At four months, there is no symblepharon, good ocular surface wetting, and a vision of 20/20 OU. This is the first case of ocular and lid AMT in acute Stevens Johnson syndrome.

The Key Issue is: Use of amniotic membrane in the acute stage of alkali burn, and transplantation in acute stage of Stevens Johnson syndrome.

[Note]:

Amniotic Membrane Transplantation in Acute Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

Presenter: Gary N. Foulks, M.D., F.A.C.S.

Associates: Kenneth Chang, M.D., Dean Hu, M.D.

Affiliation: Eye and Ear Institute, Department of Ophthalmology, University of Pittsburgh School of Medicine

Amniotic membrane transplantation was beneficial in treatment of acute severe mucosal disease in an eight year old female with severe toxic epidermal necrolysis (TEN) that presented initially as acute Stevens-Johnson Syndrome (SJS) associated with mycoplasma pneumonia. A different technique of application of the amniotic membrane was used in each eye, but both methods prevented adhesion of the eyelid to the globe and allowed corneal re-epithelialization without significant scar formation. The benefits and limitations of each technique of application will be discussed.

The Key Issue is: Potential benefit of amniotic membrane transplantation in acute SJS and TEN. Advantages and limitations of two techniques of application.

[Note]:

Amniotic Membrane Transplantation in Vernal Shield Ulceration, and in Conjunctival Squamous Papilloma Excision

Presenter: Donald Tan, M.D. FRCS(G), FRCS(E), FRCOphth¹

Associates: Lim Li, M.D., ¹, Cordelia Chan, M.D., ¹
Scheffer C. G. Tseng, M.D., Ph.D.²

Affiliation: ¹Singapore National Eye Center and Singapore Eye Research Institute
²Bascom Palmer Eye Institute

The use of amniotic membrane transplantation (AMT) is described in 2 pediatric patients with diverse ocular surface disorders.

A 9-year-old boy with severe vernal keratoconjunctivitis with recurrent vernal shield corneal ulceration failed to respond to a 6 week treatment regime of high dose topical steroids. AMT surgery allowed full epithelial healing to occur within 10 days, with minimal scarring, and no recurrence of shield ulceration has occurred at 9 months.

An 8-year-old boy with extensive conjunctival squamous papillomas affecting the total inferior tarsal conjunctiva, inferior bulbar conjunctiva and nasal canthal conjunctiva underwent total excision of the lesions. Extensive conjunctival surface reconstruction was successfully achieved with the use of amniotic membrane transplantation and a conjunctival autograft.

The Key Issue is: Amniotic membrane transplantation can be useful in the management of refractory vernal shield ulcers, and in reconstructing the ocular surface after extensive excision of conjunctival squamous papillomas.

[Note]:

The Effects of Amniotic Membrane Patch in Human Post-PRK Wound

Presenter: Woo Chan Park, M.D.¹
Associates: Jae Chan Kim, M.D.²

Affiliation: ¹Dong-A University Hospital, Pusan, Korea
²Chung-Ang University Hospital, Seoul, Korea

Purpose. To investigate the difference of clinical outcome between amniotic membrane (AM) patch and conventional patch after photorefractive keratectomy (PRK). **Methods.** Immediate after PRK, a 9mm disk of amniotic membrane was used to cover the ablated cornea by drying and a contact lens. The AM and contact lens were removed 24 hours later. Authors compared the post-operative pain, corneal haze and regression at 1, 4, 8, 12 weeks. **Results.** Preoperative spherical equivalent was $-7.6 \pm 1.7D$ in AM group and $-4.1 \pm 1.0D$ in control. Epithelial defect was healed within 3 days in both groups. Post-operative pain and myopic regression in AM group were less than control ($P < 0.05$). Corneal haze at 8 and 12 weeks in AM group was less than control ($P < 0.05$). **Conclusion.** Amniotic membrane patch after PRK might be effective for pain relief, corneal haze and myopic regression.

The Key Issue is: To investigate the difference in clinical outcome between amniotic membrane patch and contact lens after PRK.

[Note]:

Improvement of Murine HSV-1 Stromal Keratitis with Amniotic Membrane Transplantation

Presenter: Arnd Heiligenhaus, M.D.

Associates: D. Bauer, M.D.¹, S. Mrzyk, M.D.¹, D. Meller, M.D.^{1,2}
K.P. Steuhl, M.D.¹, S.C.G. Tseng, M.D., Ph.D.²

Affiliation: ¹Department of Ophthalmology, University of Essen, Germany
²Ocular Surface & Tear Center, Bascom Palmer Eye Institute, and
Department of Cell Biology & Anatomy, University of Miami
School of Medicine, Miami, Florida²

HSV-1 stromal keratitis (HSK) is an immune-mediated disease. Neutrophils are implicated in the destructive corneal process. BALB/c mice were corneally infected with the KOS strain of HSV-1. In mice with severe ulcerating HSK, amniotic membrane transplantation was performed. At different time points after surgery, the mice were examined clinically for the signs of HSV-1 keratitis, and the eyes were studied histologically and immunohistochemically (CD11b mAb) and by the TUNEL assay. The HSV-1 keratitis progressed in all mice of the untreated control group. In contrast, within 2 days the corneal inflammation and ulceration improved in the amniotic membrane-covered eyes, the number of neutrophils and CD11b+ cells in the cornea significantly decreased, and neutrophils showed the typical histological characteristics of apoptosis and were TUNEL-positive. Stromal inflammation and ulceration in HSV-1 keratitis improves with amniotic membrane transplantation, possibly by the induction of neutrophil apoptosis.

The Key Issue is: To study the effect of amniotic membrane transplantation on the course of HSV-1 stromal keratitis, especially on neutrophils.

[Note]:

Adjunctive Amniotic Membrane Transplant in Therapy of Severe Experimental Bacterial Keratitis

Presenter: Elsa L. C. Mai¹

Associates: De Quan Li, M.D. Ph.D.², Terrence P. O'Brien, M.D.¹
Scheffer C.G. Tseng, M.D., Ph.D.²

Affiliation: ¹Wilmer Ocular Microbiology & Immunology Laboratories, Johns Hopkins
University School of Medicine

²Bascom Palmer Eye institute, University of Miami Medical School

Purpose: To investigate the potential use and the protective mechanism of preserved human amniotic membrane transplantation (AMT) for bacterial ulcerative keratitis. **Methods:** Pseudomonas aeruginosa keratitis leading to severe keratolysis and descemetocele formation was induced in corneas of 15 NZW rabbits. Ten of these animals were treated frequently with 3 mg/ml ciprofloxacin (Ciloxan®). Five of the ten received AMT in addition to the ciprofloxacin. Statistical outcome analysis was based on gross slit-lamp examination, post mortem dissection measurement and histological staining. Zymography based on PMN elastase was performed with amniotic membrane extracts in Triton X-100 lysis buffer. **Result:** Reduction of keratolysis with less tissue loss was observed in the AMT group as compared to eyes treated with ciprofloxacin alone and the control group ($P < 0.0333$). Zymography showed that the caseinolytic effect of 23-26 KDa PMN elastase was dose-dependently inhibited by an increasing AM extract. **Conclusion:** Amniotic membrane has a protective effect against corneal melting and stromal keratolysis in moderately severe bacterial keratitis. This effect is in part mediated by an inhibitory effect on the PMN elastase enzymatic activity.

The Key Issue is: Can amniotic membrane prevent cornea melting in bacterial infections? If so, How?

[Note]:

Use of Non-preserved Human Amniotic Membrane in the Reconstruction of the Ocular Surface

Presenter: Luis Mejia, M.D.¹

Associates: Mauricio Jaramillo, M.D.²

Affiliation: ¹Director, Cornea Service, Instituto de Ciencias de la Salud, CES, Medellin, Colombia

²Third year ophthalmology resident. Instituto de Ciencias de la Salud, CES. Medellin, Colombia

Purpose. To present the evolution and results of non-preserved human amniotic membrane transplantation in 7 patients (7 eyes) (alkali burn, limbal deficiency reconstruction, large conjunctival squamous carcinoma, persistent central corneal sterile ulcer, OCP). **Methods.** Non-preserved human amnion was employed to cover large limbal-conjunctival defects (6 eyes) and a persistent central corneal sterile ulcer (1), using limbal autograft transplantation when deemed necessary. Sutures were removed at 21 days. Mean follow up was 6 months. **Results.** 6 eyes had a successful reconstruction of the ocular surface, with a restored limbal anatomy and physiology. One patient (alkali burn) responded favorably initially, but one of the limbal grafts subsequently failed. We observed a longer re-epithelization time (18 days) when compared with published articles using preserved amnios. **Conclusion.** This is a safe and suitable technique for the reconstruction of severe ocular surface in third world countries where preserved human amniotic membrane is not available.

The Key Issue is: Use of non-preserved human amniotic membrane in third world countries

[Note]:

The Effect of Time between Amniotic Membrane Preservation and Subsequent Transplantation of Graft Viability

Presenter: Clark Springs, M.D.

Associates: Terry Kim, M.D., William Lipham, M.D.

Affiliation: Duke University Eye Center
Corneal and External Disease Service, Oculoplastics Service

Purpose. To determine if time between amniotic membrane preservation and subsequent transplantation has any correlation with graft viability. **Methods.** We retrospectively reviewed the relevant data for amniotic membrane transplants used in pterygium surgery and persistent epithelial defects. **Results.** All amniotic membrane transplants re-epithelialized without complications. One transplant that was used more than 1 year after the recommended expiration date did result in a recurrent pterygium. **Conclusion.** Time between amniotic membrane preservation and transplantation appears to have no effect on graft viability.

The Key Issue is: Does time between amniotic membrane preservation and subsequent transplantation have any correlation with graft viability?

[Note]:

Presence and Distribution of Hyaluronan in the Human Amniotic Membrane Before and After Storage

Presenter: Per Fagerholm, M.D.

Affiliation: Karolinska Institute, St. Eriks Eye Hospital Stockholm, Sweden

Purpose. To investigate if hyaluronan is present in the human amniotic membrane. **Methods.** Bits, 1x1 cm, of human amniotic membrane were fixed in 4% formaldehyde containing 1% cetylpyridinium chloride and embedded in paraffin. Likewise human amniotic membrane stored frozen in Dulbecco modified Eagles medium and glycerol at the ratio 1:1 (v/v) at -70° for 2 weeks was thawed and fixed and embedded in paraffin. 2 amniotic membranes were analyzed before and after storage. The sections were stained with Mayers haematoxylin and histochemically for hyaluronan. **Results.** Hyaluronan was present throughout the thickness of the amniotic membrane. There was a gradient in staining intensity being weakest at the epithelial side. Storage caused swelling of the amniotic membrane up to two times its original thickness. The staining distribution for hyaluronan was basically the same after storage although the staining intensity was generally weaker and more varying compared to fresh membrane. **Conclusion.** Hyaluronan is present throughout the amniotic membrane and seems little affected by storage.

The Key Issue is: What is the role of hyaluronan in amniotic membrane?

[Note]:

Differential Distribution of Subchains of the Basement Membrane Components Type IV Collagen among the Amniotic Membrane, Cornea, and Conjunctiva

Presenter: Teruo Nishida¹

Associates: Ken Fukuda, M.D.¹, Tai-ichiro Chikama, M.D.¹, Masatsugu Nakamura, M.D.¹,
Yoshikazu Sado, M.D.², Yoshifumi Ninomiya, M.D.³

Affiliation: ¹Yamaguchi University School of Medicine
²Shigei Medical Research Institute,
³Okayama University Medical School

Amniotic membrane (AM) transplantation has been applied for the reconstruction of ocular surface. But the role of transplanted AM has not been fully understood. We compared the distributions of subchains of type IV collagen, laminin and other extracellular matrix proteins in AM to those in the cornea and conjunctiva by immunofluorescent microscopy. The pattern of components of type IV collagen subchains in AM was identical to that in the conjunctiva, but different from that in the cornea. No difference in the distribution pattern of other components was observed. These results demonstrate that the basement membrane of AM and the conjunctiva might resemble each other suggesting that AM might provide the suitable basement membrane for conjunctival epithelial cells.

The Key Issue is: Role of amniotic membrane basement membrane?

[Note]:

Amniotic Membrane as a Substrate for Cultivating Limbal Corneal Epithelial Cells for Autologous Transplantation in Rabbits

Presenter: Noriko Koizumi, M.D.

Associates: Tsutomu Inatomi, M.D., Ph.D., Andrew J. Quantock, Ph.D.,
Nigel J. Fullwood, Ph.D., Atsuyoshi Dota, M.S.
and Shigeru Kinoshita, M.D., Ph.D.

Affiliation: Department of Ophthalmology, Kyoto Prefectural University of Medicine, Japan

Purpose. To examine the viability of using human amniotic membrane (AM) as substrate for culturing corneal epithelium and transplanting them onto keratectomized rabbit corneas. **Methods.** A small biopsy of limbal epithelium was taken from the eyes of 5 rabbits and cultured on acellular AM. Three weeks later, sheets of cultured epithelium with AM were transplanted to keratectomized cornea of 5 rabbits. Three rabbits received grafts of acellular AM alone. **Results.** A confluent primary culture of limbal epithelium was established on acellular AM after 14 days. Cells were partially stratified and fairly well attached to the underlying AM, though a fully formed basement membrane was not evident. All three rabbits that received AM transplantation alone had total epithelial defects on the graft in the early post-operative period. All eyes that were grafted with AM that contained cultivated epithelium, however, were partially epithelialized up to 5 days after surgery. **Conclusion.** Autologous transplantation of cultivated corneal epithelium is feasible using AM as a carrier.

The Key Issue is: Amniotic membrane may be used for cultivating corneal epithelial cells for transplantation

[Note]:

Suppression of Epithelial Expression of IL-1 β by the Amniotic Membrane

Presenter: Abraham Solomon, M.D.

Associates: Dagoberto Monroy, Zhonghua Ji, M.D.
Scheffer C. G. Tseng, M.D., Ph.D., Stephen Pflugfelder, M.D.

Affiliation: Ocular Surface and Tear Center, Department of Ophthalmology, Bascom Palmer Eye Institute, Miami, Florida

The IL-1 gene family is a group of potent cytokines, responsible for altered host responses to various inflammatory, infectious, and noxious stimuli. IL-1 β , which is secreted by the corneal epithelium, is the most inducible form of the IL-1 family. To determine the mechanism underlying the anti-inflammatory property of the amniotic membrane, human corneal epithelium, derived from the limbocorneal ring explant, was cultured either on the basement membrane side of the amniotic membrane or on plastic, and the expression of IL-1 α , IL-1 β and IL-1 receptor antagonist (IL-1 RA) was evaluated at the protein and mRNA levels. Following 14 days of incubation, the cultures were switched to a serum-free medium, and then incubated with bacterial lipopolysaccharide (LPS) for 24 hours. A dramatic decrease of the mature form of IL-1 β protein was demonstrated in the conditioned medium from cells cultured on the amniotic membrane, when compared to cells cultured on plastic. Suppression of IL-1 β mRNA and up-regulation of IL-1 RA were evident using the RNase protection assay. These data suggest that part of the anti-inflammatory effect of the amniotic membrane may be mediated through the suppression of the IL-1 signaling system.

The Key Issue is: (1) Possible mechanisms responsible for the anti-inflammatory properties of the amniotic membrane? (2) How can IL-1 β be suppressed by the amniotic membrane, and at what level? (3) Which side (stroma versus basement membrane) of the membrane exerts such an anti-inflammatory activity?

[Note]:

Expression of IL-8, Gro-alpha and ENA is Down-Regulated in Keratocytes in vitro by Amniotic Membrane

Presenter: Friedrich E. Kruse, M.D.

Associates: Lingtao Yau, M.D., Ulrich Spandau

Affiliation: Department of Ophthalmology, University of Heidelberg Medical School, Heidelberg, Germany

Purpose. Clinical observations have consistently shown that amniotic membrane transplantation (AMT) can reduce ocular inflammation. Here we investigate the effect of amniotic membrane on the expression of proteins, which recruit leukocytes (chemokines) by stromal keratocytes in vitro. **Methods.** Corneal keratocytes were cultured either on plastic or on amniotic membranes both without and in the presence of the inflammatory mediator lipoteichoic acid (LTA)(10 ng/ml). Chemokine expression was quantified by dot and Northern blot. **Results.** Consistent with our earlier findings LTA can induce the expression of the following chemokines: growth regulated oncogene-alpha (Gro-alpha), interleukin-8 (II-8) and neutrophil activating peptide-78 (ENA). In contrast, culture on amniotic membrane significantly reduced the increase in expression of II-8, Gro-alpha and ENA, while other chemokines like IP-10 were only slightly down regulated. **Conclusion.** Culture of corneal keratocytes on amniotic membrane can modulate the pattern of chemokine expression in response to inflammatory stimuli such as LTA (which is a component of bacterial cell walls). Since chemokines recruit inflammatory cells into the cornea, this might be a mechanism by which AMT can counter ocular inflammation.

The Key Issue is: Can this new evidence help explain the anti-inflammatory effect of amniotic membrane?

[Note]:

Suppression of TGF- β Signaling in Normal and Pterygial Fibroblasts by Amniotic Membrane Stromal Matrix

Presenter: De-Quan. Li, M.D., Ph.D.¹

Associates: Sao-Bing Lee, M.D.^{1,2}, Donald.T. H. Tan, M.D.²
Scheffer C. G. Tseng, M.D., Ph.D.¹

Affiliation: ¹Ocular Surface and Tear Center, Bascom Palmer Eye Institute,
University of Miami School of Medicine
²Singapore National Eye Center, Singapore

When cultured human corneal, limbal and conjunctival fibroblasts and pterygial body fibroblasts were seeded on the stromal side of amniotic membrane, they quickly (within 8 hours) turned off mRNA expression of TGF- β 2, TGF- β 3, TGF- β RI, TGF- β RII, TGF- β RIII. Associated with this downregulation were marked suppression of CD44, and mild suppression of β 1 integrin, α -SM actin, and FGFR1/*flg*, while no change in expression of TGF- β 1 and PDGFR. These results provide for the first time in vitro evidence that amniotic membrane transplantation exerts an anti-scarring effect during ocular surface reconstruction and pterygial surgeries, and explain in part why fetal wound healing is known to be scarless.

The Key Issue is: (1) Is such a suppressive effect specific to TGF- β signaling or other genes as well? (2) Further delineation of the signaling mediated by the amniotic membrane matrix should yield useful information to suppress unwanted scarring during wound healing.

[Note]:

Inhibition of Epithelial Cell-induced Corneal Stromal Scarring by Intrastromal Implantation of Amniotic Membrane

Presenter: Tae Hoon Choi, M.D.

Associate: Scheffer C. G. Tseng, M.D., Ph.D.

Affiliation: Ocular Surface and Tear Center, Bascom Palmer Eye
Institute, University of Miami School of Medicine

We will present experimental data to show that human amniotic epithelial cells or cultured rabbit corneal epithelial cells, when transplanted into the rabbit corneal stroma, could induce keratocytes to express α -smooth muscle actin (α -SM), a marker of myofibroblast differentiation. This finding has direct implications on the phenomenon of epithelial ingrowth noted in some patients receiving LASIK. It further supports the notion that epithelial cells migrating into the corneal stroma as a result of disrupted basement membrane can induce fibrosis. Furthermore, such epithelial cell-induced scarring by corneal keratocytes can be suppressed by amniotic membrane stromal matrix when transplanted into the rabbit corneal stromal pocket.

The Issue is: (1) Can TGF- β s be the epithelial-derived signal(s) that activate keratocytes into myofibroblasts? (2) What is the exact signal that amniotic membrane stromal matrix send to inhibit such scarring?

[Note]:

DIFFERENTIAL GENE EXPRESSION BY HUMAN CULTURED UMBILICAL VEIN ENDOTHELIAL CELLS ON AMNIOTIC MEMBRANE

Presenter: ¹Akira Kobayashi, M.D.

Associates: ¹George Inana, M.D., Ph.D., ²Daniel Meller, M.D., ²De-Quan Li, M.D., Ph.D., and ²Scheffer C. G. Tseng, M.D., Ph.D.

Affiliation: ¹Laboratory of Molecular Genetics, and ²Ocular Surface and Tear Center, Department of Ophthalmology, Bascom Palmer Eye Institute, Miami, FL

Amniotic membrane-reconstructed conjunctival surfaces frequently show less neovascularization. We report here that human umbilical vein endothelial cells (HUVEC) were round, arrested in proliferation, and a subpopulation underwent apoptosis revealed by TUNEL when cultured on the stromal side of amniotic membrane, but remained flat, mitotic and devoid of apoptosis when growing on the basement membrane of AM or on plastic. Using cDNA differential array HUVEC grown on the stromal side showed down-regulation of MAP kinase, guanine nucleotide-binding protein G-s alpha subunit, c-myc, nuclease-sensitive element DNA-binding protein, and macrophage-specific colony-stimulating factor (CSF-1), and up-regulation of angiogenin inhibitor, BAX, caspase-10, IL-13 as compared to those grown on plastic. These data support the hypothesis that amniotic membrane is effective in inhibiting angiogenesis, an important property for ocular surface reconstruction.

The Key issues is: What is the signal in the stromal matrix inhibiting vascular endothelial growth?

[Note]:

Identification of Anti-neovascularization Proteins in Human Amniotic Membrane

Presenter: Fen Zhang, Ph.D.

Associates: Yanxia Hao, M.D., David H.K. Ma, M.D., David G. Hwang, M.D.

Affiliation: Ocular Cell and Gene Therapy Laboratory
Department of Ophthalmology
University of California San Francisco, San Francisco, California

Purpose. To identify the potential anti-neovascularization proteins existed in human amniotic membrane. **Methods.** Human amniotic epithelial cells and stromal cells were separated from human amniotic membrane. The presence of potentially anti- neovascularization proteins was identified primarily by RT-PCR. The presence of TIMPs was further studied by immunohistochemistry. **Results.** RT-PCR results show that human amniotic cells express all the TIMPs, collagen-18a, thrombospondin, and IL-1ra. Immunohistochemistry show that all the TIMPs are present in epithelial and stroma cells. In addition, there is abundant deposit of TIMPs in the stroma of the amniotic membrane. **Conclusion.** Human amniotic membrane cells express assorted anti-angiogenic and anti-inflammatory proteins. Those proteins are potentially deposited in the stroma and the basement membrane of the amniotic membrane, which may contribute its anti-neovascularization property in ophthalmologic applications.

The Key Issue is: Anti-neovascularization, anti-inflammation

[Note]:

Fornix Reconstruction with Amniotic Membrane

Presenter: Jonathan J. Dutton, M.D., Ph.D.¹

Associates: William J. Lipham, M.D.¹, Alexander K. Dastgheib, M.D.²
Christopher M. Debacker, M.D.¹, Maureen K. Lundergan, M.D.²

Affiliation: ¹Duke University Eye Center, Durham, North Carolina
²Moram Eye Center, University of Utah, Salt Lake City, Utah

Amniotic membrane has been used for ocular surface reconstruction in eyes with conjunctival scarring resulting from ocular cicatricial pemphigoid (OCP), Stevens-Johnson syndrome (SJS), and chemical injury, as well as following pterygium and tumor excision. We have used amniotic membrane for forniceal reconstruction in conjunction with other oculoplastic procedures where mucus membrane grafting is typically employed. In this study, amniotic membrane transplantation was performed on five eyes with forniceal ablation secondary to OCP, SJS and chemical injury. With a mean follow-up of six months (2 to 14 months), we achieved successful forniceal reconstruction in four out of five eyes. The single failure occurred in a case of OCP that responded well following a second amniotic membrane reconstruction procedure. Amniotic membrane transplantation can be considered an excellent alternative substrate for fornix reconstruction in cases where mucus membrane grafting has been traditionally employed, especially in instances where the surrounding conjunctival tissue is relatively normal. It provides excellent cosmetic as well as functional results.

The Key Issue is: Fornix reconstruction.

[Note]:

Punctum Patch with Amniotic Membrane in Dry Eyes

Presenter: Lucia Zhuo Chen, M.D.

Associate: Juan Murube, M.D., Ph.D.

Affiliation: Rizal Foundation for Ophthalmic Research, University of Alcala, Madrid
Hospital Ramon y Cajal

Patching the lacrimal punctum with amniotic membrane or with autologous conjunctiva is an easy way to prevent the lacrimal outflow in patient with dry eyes. This method is very easy, efficient and is perfectly tolerated. If compared to the pre-existent methods of long-term canalicular occlusion (i.e., cauterization and plugs), the punctal patch appears to be better tolerated, presenting no complications. Moreover, this method is reversible by piercing the transplanted patch, thus restoring the functioning of the lacrimal pump and drainage of tears.

The Key Issue is: A new alternative for punctal occlusion

[Note]:

Amniotic Membrane Transplantation as an Alternative to Mucous Membrane Grafts

Presenter: Eva Dafgard Kopp, M.D., Ph.D.

Associates: Gabor Koranyi, M.D., Skold Peter Matthis, M.D.

Affiliation: St. Eriks Eye Hospital, Department of Ophthalmology, Karolinska Institutet, Sweden

Amniotic membrane has been used as an alternative to buccal mucous membrane grafts in oculoplastic surgery. In the first case a patient enucleated in childhood and in spite of repeated buccal mucous membrane grafts the lower and upper fornices were too small for the prosthetic eye to fit. New fornices were reconstructed with amniotic membrane transplantation. In the 2nd patient a blind eye with keratitis caused by a pseudomona infection was enucleated. During the operation there was not enough conjunctiva for primary closure. Amniotic membrane was used to close the wound. In the 3rd case a patient with an unsightly buccal mucous membrane graft after a large conjunctival melanoma. The graft was replaced by amniotic membrane. Though the series is small we conclude that amniotic membrane grafts generate smooth and thin surfaces without inflammatory signs and are a good replacement for mucous membrane graft.

The Key Issue is: Amniotic membrane transplantation in oculoplastic surgery

[Note]:

The Effect of Amniotic Membrane on the Healing Process of Human Eyelid Skin After Carbon Dioxide Laser Ablation

Presenter: Ivan P. Hwang, M.D.

Affiliation: University of Utah, Department of Ophthalmology, John Moran
Eye Center, Salt Lake City, Utah

Purpose. Currently, the treatment of human skin after thermal injury is limited. After severe thermal injury to the skin, the patient is often left with cicatricial changes that is severely debilitating. This is especially true around the eyelid region because the eyelids serve to protect and replenish the eye with lubrication that is essential for its survival and its function. It is feasible that amniotic membrane may be beneficial in reducing the cicatricial changes to the human eyelid skin by decreasing inflammation while promoting healing after thermal injury.

Methods. Full thickness human eyelid skin after blepharoplasty were soaked in sterile saline solution and transplanted onto the backs of eight CD-1 mice. At four weeks, each mice received carbon dioxide laser ablation to the human skin graft at a laser energy setting of 500 millijoules to simulate controlled thermal injury. The mice then was randomly selected into two groups of four animals: the control group receiving petroleum jelly dressing and the treatment group receiving amniotic membrane dressing. Each animal was observed daily for epithelial healing and specimens sent from each group for histological examination. **Results.** The rate of epithelial healing and the amount of inflammation in the human eyelid skin tissue were observed. Of the observed animals, the amniotic membrane treated skin demonstrated complete epithelial healing at day two. The petroleum jelly treated skin demonstrated complete epithelial healing at day five. On histology, the amniotic membrane treated skin demonstrated a decrease in inflammatory cells when compared to the petroleum jelly treated skin at the same time points. **Conclusion.** These findings suggest that amniotic membrane may be useful in decreasing the cicatricial changes in the human eyelid skin after thermal injury. Future studies which evaluate the effectiveness of amniotic membrane in the treatment of thermal injury to the eyelid skin in patients are warranted. Support: Research to Prevent Blindness, Inc.

The Key Issue is: What is the action mechanism?

[Note]:

Repair of Leaking Glaucoma Filtering Blebs Using Preserved Human Amniotic Membrane Graft

Presenter: Donald L. Budenz, M.D.¹

Associates: Keith Barton, M.D.², Scheffer C. G. Tseng, M.D., Ph.D.¹

Affiliation: ¹Bascom Palmer Eye Institute, Department of Ophthalmology,
University of Miami School of Medicine, Miami, Florida
²Moorfields Eye Hospital, London²

Purpose. To determine the safety and efficacy of human preserved amniotic membrane graft (AMG) for repair of leaking glaucoma filtering blebs. **Methods.** Randomized prospective trial of patients undergoing filtering bleb revision for leak comparing AMG with conjunctival advancement. **Results.** Indications for revision included Seidel (+) leak (12), previous blebitis or endophthalmitis (4), hypotony maculopathy (2), decreased vision from corneal edema (1), and choroidal effusion (1). Five patients were randomized to AMG and 7 to conjunctival advancement. Six months post-revision, all blebs were Seidel (-) and IOP was equivalent (AMG 14.7 mmHg, conjunctival advancement 14.2 mmHg, P=.84). Patients in each group required similar numbers of glaucoma drops (AMG 0.7, conjunctival advancement 1.2). However, 2/7 patients in the conjunctival advancement group required repeat glaucoma surgery (glaucoma drainage implants) for uncontrolled IOP by 6 months compared to 0/5 in the AMG group. In addition, patients in the conjunctival advancement group had a higher incidence of postoperative ptosis and diplopia. **Conclusion.** Preserved human amniotic membrane graft may be a useful alternative to conjunctival advancement in the management of leaking glaucoma filtering blebs.

The Key Issue is: Does amniotic membrane repair of leaking glaucoma filtering blebs provide advantages over conventional technique?

[Note]:

Amniotic Membrane Transplantation in Glaucoma Surgery: Pre-clinical Studies

Presenter: ^{1,2}Keith Barton, M.D.

Associate: ¹Donald L. Budenz, M.D., ¹Scheffer C. G. Tseng, M.D., Ph.D.

Affiliation: ¹Bascom Palmer Eye Institute, Miami, Florida
²Moorfields Eye Hospital, London, England

Purpose. To investigate the potential use of preserved human amniotic membrane transplantation (AMT) in the reconstruction of glaucoma filtering blebs. **Methods.** 24 NZ albino rabbits underwent trabeculectomy. In 12, the conjunctival flap was excised and replaced with AMT at the conclusion of the procedure. IOPs were measured at 3-4 day intervals. 12 animals were euthanized after day 14 and the remainder after day 36. Impression cytology, frozen and formalin-fixed tissue specimens were obtained and conjunctival biopsies explanted in tissue culture. **Results.** Bleb formation was observed in all eyes although in 2 AMT cases the membrane was not adequately secured, with aqueous leak and failure of complete epithelialization. Throughout the study IOPs were significantly lower in both groups than in unoperated eyes. From days 11-16 the percentage IOP reduction in AMT eyes was significantly greater than in controls ($p=0.014$). Only minimal fibroblast outgrowth occurred from AMT explants when compared with control conjunctiva ($p=0.01$) and conjunctiva adjacent to AMT ($p=0.0005$). AMTs remained intact on histological examination after 14 days but were associated with considerable granulomatous inflammation. After 36 days, the area remained clinically intact, but beneath the epithelium, lysis of AMTs was noted histologically. **Conclusion.** AMT provides an alternative tissue for the construction of filtering blebs, but its survival in the rabbit was limited by the xenogenic response, a finding not encountered in human use of AMT. This tissue may be used as an alternative substrate for bleb revision.

The Key Issue is: In a rabbit model does amniotic membrane provide an adequate alternative tissue for glaucoma filtration bleb formation?

[Note]:

Problem Solving in Glaucoma Surgery with Amniotic Membrane

Presenter: Francisco E. Fantes, M.D.

Associates: Brian Cavallaro, M.D., Jean-Marie Parel, Ph.D. D.Ing. ETSG
Richard K. Parrish, M.D., Scheffer C. G. Tseng, M.D., Ph.D.

Affiliation: Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami,
Florida

Control of wound healing is a major challenge in any type of glaucoma surgery. There are a number of complications that are related to poor or excessive healing. Amniotic membrane modulates wound healing response, and it can be of significant help in glaucoma surgery, or solving some of its complications.

Selected cases will be presented where the use of amniotic membrane was of help in solving some of these problems. They include use in glaucoma implants, giant blebs, conjunctival scarring blebitis, and scleral necrosis.

Currently, we are studying in rabbits, the possible role of amniotic membrane as a wound healing modulator in glaucoma implants. Short-term results will be reported.

The Key Issue is: How can amniotic membrane be used to treat difficult problems in glaucoma surgeries?

[Note]:

Subretinal Implantation of Human Amniotic Membrane: A Rabbit Model for the Replacement of Bruch's Membrane during Submacular Surgery

Presenter: Philip J. Rosenfeld, M.D., Ph.D.

Associates: Jenifer Merritt, Eleut Hernandez, Daniel Meller, M.D.
Robert H. Rosa, Jr., M.D., Scheffer C. G. Tseng, M.D., Ph.D.

Affiliation: Bascom Palmer Eye Institute, University of Miami School of Medicine,
Miami, Florida

Purpose. Submacular surgery for the removal of choroidal neovascularization in age-related macular degeneration (AMD) disrupts the retinal pigment epithelium (RPE) and Bruch's membrane. This damage to Bruch's membrane may prevent the re-epithelialization by RPE and contribute to the poor visual outcomes often reported in these patients. Using a rabbit model system, we have investigated the subretinal implantation of human amniotic membrane as a Bruch's membrane replacement. **Methods.** Sixteen pigmented rabbits underwent vitrectomy in one eye. A retinotomy was performed followed by the creation of a localized retinal detachment. Preserved human amniotic membrane was implanted under the retina through the retinotomy site. Weekly dilated examinations were performed, and fundus photos were obtained. Rabbits were sacrificed for histological examinations beginning 4 weeks after implantation. **Results.** The implantation of amniotic membrane was achieved using standard subretinal surgical techniques and instrumentation. No evidence of inflammation, infection, or cataract formation was detected after observing these eyes for over 120 days. The retina reattached over the amniotic membrane, and the amniotic membrane remained flat. Histological evaluation revealed RPE proliferation over the basement membrane side of the amniotic membrane without inflammation. **Conclusions.** Human amniotic membrane implanted under the rabbit retina appears to be well tolerated without evidence of infection or inflammation. This membrane may serve as a useful replacement for Bruch's membrane following the removal of choroidal neovascularization during submacular surgery. Support: V. Kann Rasmussen Foundation, Research to Prevent Blindness, and Florida Lions Eye Bank.

The Key Issue is: Amniotic membrane as a Bruch's membrane replacement to facilitate re-epithelialization by the RPE following submacular surgery.

[Note]: