Optic Pit Maculopathy: A Review of Literature and Suggested Treatment Algorithm

Dr. Nagesha C. K. MS, Dr. Rajiv Raman MS DNB, Dr. Laxmi Gella MPhil Opt, Dr. Tarun Sharma MD, FRCS Ed, MBA

Introduction
Optic disc pits (ODP) are congenital excavations of optic nerve head, usually seen in association with other abnormalities of the optic nerve and peripapillary retina. These pits are caused due to faulty closure of embryonic fissure that extends along the inferior aspect of the globe which also results in diversity of other cavitory disc lesions.

Furthermore, histopathological examinations demonstrate similarities between ODP, morning glory syndrome and typical coloboma; suggesting these cavitory disc anomalies probably represent a spectrum of disease. In each case dysplastic retina is herniated posteriorly through a defect in lamina cribrosa and/or juxtapapillary area, ranging from a focal defect in ODP to a circumpapillary defect as seen in morning glory anomalies. On the other side, ocular and systemic associations with each anomaly often differ, which suggests that each anomaly is better considered as a distinct disease entity; probably molecular genetics in the future may help us answer this intriguing question.

ODP was described for the first time by Weith in 1882 in a 62year old woman. They occur in 1 in <10,000 eyes, although there is considerable variance among studies and are bilateral in 10 to 15% of cases, approximately two third of patients with ODP develop serous macular detachment. These may occur during childhood or later in life but most common between age group of 20 and 40.

ODP are usually located in temporal side of the disc in 70% of cases and 20% are situated centrally; the remaining found inferiorly, superiorly, and nasally. Central ODP behave differently from temporal pits. They are full thickness defects in lamina cribrosa centre with no serous retinal detachment or herniation of neural tissue and they do not develop maculopathy. Moreover, majority of disc with central ODPs are found to have glaucoma with gaucomatous neuroretinal thinning, RNFL loss and corresponding field defects.

ODP are reported to have presented with other ocular and systemic associations in few isolated case reports. Co-existence of keratoconus, papilledema and disc coloboma are reported in the literature. Acquired ODP in myopia has been reported and the eyes with ODP are more myopic, have significant larger axial length, larger optic discs than highly myopic eyes without pits. Mechanical expansion of papillary region was thought to be primary cause of ODP in these cases.

Although spontaneous resolution of maculopathy has been reported, most eyes with ODP associated with macular detachment have poor visual prognosis if left for its natural course. Cystic retinal degeneration, macular hole formation and retinal pigment epithelial atrophy often limits visual recovery in these cases. However, there are reports that ODP associated maculopathy remains stable for a long time, Jonathan et al reported six patients with pit maculopathy having vision > 20/200 observed on presentation and remained unchanged at an average follow-up of 19.5 months.

Pathomechanism of maculopathy

Cause of structural changes and source of fluid

Role of Vitreous traction: In many case series, maculopathy was treated successfully by standard vitrectomy with or without ILM peeling claiming equal surgical outcomes. The role of laser in cases undergoing vitrectomy is less, suggesting PVD induction with vitrectomy is essential step to flatten the macula. These clinical based evidences stress the importance of transverse and antero-posterior traction exertion by vitreous as main pathogenic event.

Vitreous fluid is speculated source of fluid implicated in ODP maculopathy. John et al studied optic pit architecture using scanning laser microscope and serial histopathological sections. They found holes in the diaphanous membrane overlying the disc at the edge of the pit and believe that
these membranous defects provide access for passage of vitreous fluid into adjacent neurosensory retina.

Brown et al\textsuperscript{20} conducted experimentation on collie dogs injecting Indian ink into vitreous cavity and observing it in subretinal space. Intracranial migration of silicon oil,\textsuperscript{21} subretinal migration of gas bubble\textsuperscript{22} from vitreous cavity suggests indirect evidence of connection between vitreous cavity and subretinal space and vitreous as a source of fluid. Successful attempt to endoaspirate the subretinal fluid at the optic disc site intraoperatively in many cases\textsuperscript{16,23,24} also suggests the communication between subretinal fluid and the pit.

On the contrary, many critical reviews of optical coherence tomography (OCT) images in the ODP failed to show any vitreous traction causing tenting of the macula or traction over the disc.\textsuperscript{16,25,26,27} Development of maculopathy in young children long before the development of partial PVD and traction also remains unanswered\textsuperscript{28} if the vitreous theory holds true.

**Role of Cerebrospinal Fluid (CSF):** Irvine\textsuperscript{29} and Gass\textsuperscript{30} suggested CSF may leak from the optic nerve arachnoid space into ODP and eventually into intra and subretinal space. However, intrathecal injection of dye failed to demonstrate any connection in experimental animals.\textsuperscript{20} However in a recent analytical study by Nieraj et al,\textsuperscript{31} the author postulated the role of translaminar pressure in causation of ODP maculopathy. The dynamic translaminar (intraocular - intracranial) pressure gradient fluctuates throughout the day and sometime becomes large enough to drive the CSF into pit and by repeated small aliquots of fluid driven into retinal stroma causes progressive schisis like retinal edema. Fluid ejected from the pit sac in a given eye could be liquid vitreous, CSF, or even a mixture of the two fluids.

**Clinical presentation:** Optic pits are usually incidental findings on fundus examination and remain asymptomatic unless complicated by macular lesions such as edema, schisis or serous detachment. A patient with macular involvement generally presents with visual acuity of worse than 20/70 in the affected eye, and 80 percent of these eyes lose visual acuity to 20/200 or worse.\textsuperscript{14} It has been suggested that these patients have a greater propensity to develop normal/high tension glaucoma.\textsuperscript{33-35} although the arcuate visual field defects may be caused by the optic pit itself rather than by glaucomatous damage.

Morphology of the optic pit: Congenital pits of the optic nerve head vary in size, shape, depth and location. They appear as small, hypopigmented, greyish, oval or round excavated depressions in the optic nerve head. They are usually about 500 \( \mu \)m in size and may be bilateral in 10 to 15 percent of cases. Optic pits are most commonly located on the temporal side of the optic disc, but they may be situated centrally or anywhere along the margin of the optic disc.\textsuperscript{8}

**Macular changes:** Optic pits along the rim of the optic disc are most likely to lead to serous detachments of the retina, with associated full-thickness or laminar retinal holes, retinal pigment epithelium mottling and general cystic changes. The retinal detachments are usually confined between the superior and inferior vascular arcades and are contiguous with the optic disc, sometimes through a visible isthmus of subretinal fluid. The elevated retina contains cystic cavities in the outer plexiform layer. The symptomatic maculopathy is most commonly seen at age around 30 years; probably the age related vitreous changes may have a role.

**Optical coherence tomography:** OCT of an optic pit usually shows a schisis like separation between the inner and outer retina and a larger retinal detachment.

**Visual field testing:** Optic pits may be associated with visual field changes, which can be due to one or both of the following mechanisms.\textsuperscript{36}

- An optic pit, especially if large, may displace nerve fibers to produce an arcuate scotoma or may lead to an enlarged blind spot.
- Associated serous macular detachment may manifest as metamorphopsia or blurred vision, and visual fields may demonstrate central scotoma. However, unlike degenerative or reticular retinoschisis, there is no absolute scotoma in optic pit maculopathy.

Fluorescein angiography: Fluorescein angiography is usually unremarkable in cases of optic pit.\textsuperscript{37} There is no dye accumulation in the area of the serous detachment, although there may be late hyperfluorescence of the optic pit. It has been suggested that vitreopapillary traction in this area may cause leakage from optic disc blood vessels.\textsuperscript{8,18,38}

Electrophysiological testing: An electroretinogram (ERG) may show poorly defined and low-amplitude waveforms, consistent with schisis and serous detachment. Preoperative evaluation of macular function is important for predicting the likelihood of central vision recovery after successful macular reattachment but cannot be used alone as prognostic
factor. Patients with a poor ERG response are less likely to experience visual acuity improvement even after anatomical reattachment.

**Structural alterations in Optic pit maculopathy based OCT:** Bilaminar model: Lincoff et al in pre-OCT era proposed a bilaminar structure in which retinal elevation that communicates with ODP is frequently a schisis like separation of intraretinal layers of retina and separation of outer layers of retina is secondary phenomenon that starts in macula. Separate case series by Rutledge et al, Krivoy et al and Akito Hirakata et al have supported this bilaminar model using OCT.

**Fluid movement:** The recent studies showed wide variation and different architecture of maculopathy contradicting bilaminar model. Immamura et al in his series of 17 patients, characterised the architecture using high resolution OCT concluding that the fluid from pit can go directly to the subretinal internal limiting membrane space, ganglion cell layer, inner nuclear layer, outer nuclear layer or subretinal space.

Similar recent study including 32 eyes, author opine that collection of fluid in ORL is the first step in optic pit maculopathy and explained the possible movements of fluid. Fluid from outer retinal layers could follow bidirectional seepage either into subretinal space or through inner retinal layers into subretinal space or just into inner retinal layers with no involvement of subretinal space.

Gaurav Sangli et al opine that intraretinal fluid may split any of the inner and outer retinal layers suggesting bilaminar structure proposed by Lincoff may not be appropriate. An outer layer hole could be demonstrated in 73% of cases with schisis and OLD (outer layer detachment) suggesting origin of SRF could be an extension of fluid from schisis like cavities into subretinal space.

**Disc in Optic pit is crowded; should present with Glaucoma and not maculopathy?**

Anton et al studied the planimetry in 23 patients with optic pit and compared with age matched controls and found optic pit patients have generally bigger areas in their optic nerve heads compared to normals of their age group. Also they have thicker RNFL thus making the glaucoma susceptibility akin to normal population.

**Management:**

Patients with asymptomatic optic pits need regular monitoring for the onset of any macular involvement. The management of optic pits with associated macular involvement is not well defined; various treatment modalities have been tried with variable success. Less-invasive treatments like laser photocoagulation should be tried initially, followed by a combination of vitrectomy, complete posterior vitreous detachment (PVD) induction and internal gas tamponade if symptoms persist.

When the optic pit is asymptomatic, the patient should be advised about the importance of regular comprehensive eye exams, including dilated retinal evaluations and threshold visual fields. Patients should be educated about the use of home visual acuity assessment and Amsler grid testing to monitor for the onset of maculopathy. They should be made aware of the signs and symptoms (e.g., blurred vision and metamorphopsia) of macular complications.

**Laser photocoagulation**

This is used to produce one or several rows of laser burns between the area of the serous retinal detachment and the optic disc. The objective is to achieve a very light white laser burn with little collateral damage to the nerve fiber layer. This presumably creates a wall of scar tissue to block the passage of fluid from the optic pit to the inner retinal schisis cavity and subretinal space (although the scarring may also involve peripapillary retinal tissue). While studies have reported successful resolution of the serous detachment in eyes that have been treated with photocoagulation, this does not always translate into improved final visual outcome.

Laser treatment alone had very poor results in previous studies that led to use of lasers as additional procedure to improve the post-op outcomes before or after standard vitrectomy. Most authors used Argon blue green laser, 532nm, red or infrared lasers and none of them noted any significant field defects except enlarged blind spot in few.

Lincoff theorized that primary communication with ODP is inner layers of retina. However laser energy primarily absorbed by RPE and choroid may account for overall low success rate. OCT imaging has clarified that laser photocoagulation alone is typically ineffective because it fails to produce a barrier to intraretinal fluid migration.

**Pneumatic displacement with or without vitrectomy**

The principle of surgery is to place a large air gas bubble in the vitreous cavity that will dry and compress the retinal layers in the juxta papillary area and facilitates displacement of OLD. Lincoff et al studied C3F8 gas tamponade in 3 of his patients. 2/3 had reappearance of OLD which was
confirmed by OCT at 1 month and 5th year; 3rd case had no improvement from the beginning. Author opines that the effect is temporary because the reservoir created by gas displacement flattens and closes with time while from disc pit remains constant.

Justice et al\textsuperscript{23} reported case of vitrectomy and endodrainage of intraretinal fluid causing flattening of schisis which maintained well during post-operative period without tamponade and face down position. Similar studies have shown ineffectiveness of tamponade to flatten the elevation in cases which had already developed PVD.\textsuperscript{48} In contrast, gas tamponade successfully flattened macula which had previous vitrectomy with non-resolving macular elevation.\textsuperscript{49}

Alute Hirakata\textsuperscript{17} observed persistent subretinal fluid in patients who had undergone vitrectomy with tamponade for long time post-operatively. Eventually the fluid resolved long after disappearance of gas tamponade doubling its actual role and suggested not to contemplate additional surgical procedure too early for macular attachment. Hideo et al\textsuperscript{50} used 0.3% SF6 in his 8 Japanese patients. Four out of eight cases had complete resolution and none of them had recurrence but remaining 4 were managed subsequently with vitrectomy and ILM peeling. He observed that eye may require upto 1 year to reattach. He recommends that the patients should be treated initially with intravitreal gas.

**Vitrectomy with adjuncts**

Vitreous traction is believed possibly to induce a small tear in diaphanous tissue overlying disc, additionally vitreous traction on peripapillary retina and/or macula is thought of having the potential to facilitate accumulation of fluid in the macula.

This assumption was clinically proved in large case series where vitrectomy with Posterior vitreous detachment resulted in resolution of maculopathy in majority of cases.\textsuperscript{17,18,43}

Supporting above theory, Bonnet\textsuperscript{18} found no clinical evidence of PVD in all 25 cases and 2 of the 4 untreated eyes that exhibited spontaneous retinal reattachment later developed PVD.

Pars plana vitrectomy with tamponading without laser or ILM peeling resulted in complete resolution in 10/11 cases but it took long time for visual recovery implying vitrectomy has major role than short living tamponade in flattening the macula.\textsuperscript{17} Recently, the trend towards vitrectomy with additional procedures is gaining wide acceptance in managing pits with good outcomes. Importance of ILM peeling as an additional step to eliminate tangential traction for good surgical success has been suggested by few case series\textsuperscript{16,51,52}.

Wisdom of peeling ILM over extremely thin retina ending in full thickness macular hole has been questioned by few and have argued that vitrectomy along with tamponade alone had shown good results. Shukla et al\textsuperscript{16} had 4 cases of full thickness macular hole out of 7 which had vitrectomy with ILM peeling, among which 3 holes closed completely during post-operative period. The author observed that the final visual acuity appeared unaffected by the macular hole in entire group (mean BCVA 20/30 and 20/25 respectively) in eyes with or without macular hole. On other side, macular hole which developed postvitrectomy was successfully treated with revision surgery involving ILM peeling and tamponade.\textsuperscript{17}

**Additional procedures**

In a case report by Richard et al,\textsuperscript{15} partial thickness fenestrations were made over schitic retina adjacent and temporal to pit which created alternate outflow path for SRF. He suggested that the redirection of flow seems to offer a rationale alternative to block passage of fluid through tamponade or laser irrespective of the origin of SRF.

Makoto Inoue et al\textsuperscript{53} noted glial tissue over optic disc intraoperatively and that the glial tissue might have developed after continuous vitreous traction attached to the ODP. Mechanical separation of the posterior hyaloid may relieve anteroposterior forces on the peripapillary retina, over that removal of condensed vitreous and glial tissue may remove additional traction on the retina around or within the pit. However, Gregory et al\textsuperscript{25} observed two of the three cases where vitreous was separated but fibrous tissue was not peeled from the pit; OCT imaging showed complete resolution of the retinal detachment at the most recent follow-up visit.

Endodrainage of SRF has been tried intraoperatively resulting in flattening of macular elevation which was later stabilised with\textsuperscript{16} or without tamponade.\textsuperscript{23} The endodrainage is not an essential step when combining laser and tamponade and its effectiveness for long term is not known. Drainage retinotomies\textsuperscript{54} temporal to macula without post-op complications have also been reported previously.

**Conclusion**

Maculopathy caused by optic pits has an overall poor prognosis, and long-term studies involving large groups of these patients are lacking. Given that the exact pathophysiology is still a matter of debate, management
should be tailored to the visual disability and macular changes of the specific patient.

Case studies

Case 1:
A 25-year-old male came for routine check-up. VA was OU 6/6. Examination showed disc with pit but no maculopathy (Fig 1a&1b).

Highlights:
1. Despite large pit there was no macular changes
2. Maculopathy is unrelated to size of pit

4. Case 2:  A 37-year-old male presented with sudden loss of vision in the left eye since 3 months (Fig-2a&2b). VA was 6/36, N36. He gives past h/o laser treatment in OS. He was advised Pneumatic displacement. After, intravit C3F8 injection, post operatively (Fig-2c)the fluid reduced but the Vision was 6/36, N18.

Highlights:
5. Nearly 1/4th of the eyes with macular involvement have outer lamellar hole
6. Progressive reduction of vision despite laser and OCT progression are indications of other interventions.
7. There may be slight or no improvement in Vision after pneumatic displacement. However, in this case there was an improvement of near vision.

Case 3: A 19-year-old male presented with sudden loss of vision in the right eye since 3 months. VA was 6/36, N12 (Fig 3a). He underwent laser to the margins of pit. After 2 months he was symptomatically better, VA improved to 6/12, N8 (Fig3b).

Highlights:
8. Treatment of laser alone is effective in selected group of patients.
9. Though there may be improvement in Vision and OCT characteristics, still OCT changes of maculopathy may persist.

**Case 4:** A 31-year-old male presented with sudden loss of vision in the left eye since 1 months (Fig 4a). VA was 2/60, < N36. He underwent Vit + ILM peeling + Glial tissue removal + endoaspirate + EL(diode) + C3F8. Post operatively (Fig 4b) after 2 months vision improved to 3/60, N36.

**Highlights:**
- Fibrous traction over the disc pit requires vitreoretinal intervention.
- Poor vision and large NSD are indications of vitreoretinal surgery.
- There is a high risk of postoperative macularhole formation especially if there is presence of inner layer schisis.

Fig 4a: Pre-operative OCT images showing traction at the disc (Right image) and Elevated retina with thinned inner & outer layers (left image).

Fig 4b: Post-operative OCT images showing Relief of traction at the disc(right image) and Full thickness macular hole at fovea (left image).

Suggested treatment algorithm for optic pit maculopathy:
References


Amniotic Membrane Transplantation

Dr. Anjana Devi R. MS

Introduction
Amniotic membrane (AM) is the innermost layer of the fetal membranes. It has a stromal matrix, a thick collagen layer, and an overlying basement membrane with a single layer of epithelium. In the field of ophthalmology, amniotic membrane transplantation was initially used in the 1940s for conjunctival defects. De Ro’tth1 reported the first use of amniotic membrane in ophthalmology for symblepharon correction and Sorsby2 used amniotic membrane as a biological bandage in the treatment of caustic burns to the eye. In 1995, Kim and Tseng3 reintroduced the use of amniotic membrane in an experimental model of chemical injury. Since then, amniotic membrane transplantation (AMT) has been used in the treatment of several ocular surface diseases, such as cicatricial keratoconjunctivitis (Stevens–Johnson syndrome [SJS], ocular cicatricial pemphigoid [OCP], and ocular burn), corneal epithelial defect, recurrent pterygium, and symblepharon4-12.

Anatomy
The amnion of the human placenta varies in thickness from 0.02 mm to 0.5 mm in thickness. It contains no blood vessels and has no direct blood supply. Bourne13 described the amnion as consisting of five layers from within outward: (a) epithelium; (b) basement membrane; (c) compact layer; (d) fibroblast layer; and (e) spongy layer. The epithelial layer consists of a single layer of amniotic membrane epithelium. These cells are polygonal in shape and vary from columnar over the placenta to cuboidal or flat away from the placenta. The basement membrane is a thin layer composed of reticular fibers. It is closely adherent to the amniotic epithelium from which multiple processes interdigitate into it. The compact layer is a dense layer almost totally devoid of cells and consists mainly of a complex reticular network. The fibroblastic layer is the thickest layer of the amnion and consists of fibroblasts embedded in a loose network of reticulum. The outermost spongy layer forms the interface between the amnion and chorion and consists of wavy bundles of reticulum bathed in mucin.

Method of preparation
Human placenitas are obtained from consenting mothers who undergo cesarean sections and are negative for hepatitis B and C, syphilis, and human immunodeficiency virus. These tests are mandatory and are carried out in the third trimester of pregnancy, as close to the date of cesarean section as possible. All the above tests, especially HIV, are repeated six months after delivery and the tissue used for surgery only if all tests, on both occasions, are negative or non-reactive.

Processing and preparation of the membrane is carried out under sterile conditions. Under a lamellar flow hood, the placenta is first washed free of blood clots with sterile saline. The inner amniotic membrane is separated from the rest of the chorion by blunt dissection (through the potential spaces between these two tissues), and rinsed in sterile saline (2 litres). Samples are taken for microbiology to assess sterility. An antibiotic cocktail to cover Gram-negative and Gram-positive bacteria and fungi is used in washing and storage solutions. In the method popularized by Tsuboto’s group5 wherein the membrane is cut into pieces measuring 10 cm × 10 cm and rinsed sequentially for five minutes in each of 0.5M dimethyl sulfoxide (DMSO) (4%w/v in 0.01M phosphate buffered saline PBS), 1.0M DMSO (8%w/v in 0.01M PBS), and 1.5M DMSO (12% w/v in 0.01M PBS). The second method was popularized by Kim and Tseng3,14. The membranes are washed with phosphate-buffered saline containing 50 mg/ml penicillin, 50 mg/ml streptomycin, 100 mg/ml neomycin, and 2.5 mg/ml amphotericin. The amniotic membrane is flattened onto a sterilized nitrocellulose filter paper with the epithelial side up. The paper with the adherent membrane is then cut into pieces 3 × 3 and 4 × 4 cm. The HAM is then stored in 50% Dulbecco’s modified Eagle’s medium and 50% glycerol at -80°C. The membrane is defrosted immediately before use by warming the container to room temperature for 10 minutes, and rinsed three times in saline. Recently storage of amniotic membrane in sterile vials containing RPMI media at -80°C has been reported15.
The tissue is stored frozen at -80°C. In UK it is released for use only after the second serological screening test, carried out six months after delivery. Tissue has been stored and used for up to 2 years post-delivery. Due to the risk of infection with HIV and hepatitis C, tissue transplantation laws in different countries require different protocols for preservation, testing, and storage. Several workers have used fresh membrane for clinical use. There may be some theoretical advantages of fresh membranes over preserved membranes. But the risk of HIV infection can be there despite seronegativity, due to the window period between infection and sero-conversion.

Properties of amniotic membrane

The amniotic membrane is a thin, semitransparent tissue from the inner part of the placenta. The amniotic membrane has a thick basement membrane and an avascular stromal matrix. The basement membrane facilitates migration of epithelial cells, reinforces adhesion of basal epithelial cells, and promotes epithelial differentiation. The basement membrane also plays a role in preventing epithelial apoptosis. Collectively, these are the possible actions by which the amniotic membrane permits rapid epithelialization. Amniotic membrane is also found to have anti-inflammatory and antiscarring effects. It reduces, cicatricial and angiogenic reactions, and seems to be immunologically inert.

Amniotic membrane has unique properties including antiadhesive effects, antibacterial effects, wound protection, pain reduction, and epithelialisation effects. Its antiadhesion property can be striking enough to cause severe symblepharon lysis completely. Amniotic membrane is composed mainly of a thick collagen layer and overlying basement membrane components including laminin and type IV collagen. The probable mechanism of this effect is contact of the unhealthy ocular surface with normal substrates; contact with healthy tissue induces an arrest in tissue proliferation. In addition, the amniotic membrane transplant may also function as an anatomical barrier to fibrous tissue proliferation.

The membrane, especially the epithelium, also produces various growth factors including basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor. Studies on human amniotic membrane preserved at -80°C for 1 month revealed the presence of EGF, TGFβ, KGF, HGF, bFGF, TGF-81, and -82 by RTPCR for the mRNA and by ELISA for the protein products. TGF-83 and growth factor receptors KGFR and HGFR were also detected by RT-PCR. A higher level of various growth factors were found in amniotic membrane with epithelium than without epithelium indicating an epithelial origin for these growth factors. The epithelium of the amniotic membrane has been found to survive for up to 70 days after preservation. The growth factors may modulate the differentiation and proliferation of conjunctival and corneal cells. Its unique characteristics make the amniotic membrane a suitable material for treating subconjunctival fibrosis. It has been shown that amniotic membrane induces a downregulation of transforming growth factor signaling responsible for fibroblastic activation in wound healing. It causes reduced expression of TGFβ-1, β-2, and β-3 isoforms in addition to reduced expression of TGF-Receptor II. This had the subsequent effect of preventing fibroblast activation into myofibroblasts. Tseng et al. maintain that this mechanism is primarily responsible for the anti-scarring properties of amniotic membrane. Similar results were found by Lee et al. when human conjunctival fibroblasts and pterygial fibroblasts were cultivated on the matrix side of amniotic membrane. In a study in rabbits, Choi and Tseng demonstrated that corneal epithelial cells induce differentiation of keratocytes into myofibroblasts and this effect could be prevented by placing amniotic membrane as a “barrier” between the epithelial sheet and corneal stroma/keratocytes, both in vivo and in vitro.

The stromal matrix of the amniotic membrane excludes inflammatory cells, contains various forms of protease inhibitors and prevents myofibroblast differentiation of normal human corneal and limbal fibroblasts. Amniotic membrane was found to reduce inflammatory cell infiltration and loss of keratocytes and thereby reduced corneal haze in rabbit eyes undergoing excimer lasers. Hao et al. identified the presence of mRNA for cytokines IL-1RA (receptor antagonist) and IL-10 in both amniotic epithelial and mesenchymal cells. These cytokines are potent inhibitors of inflammation. Shimmura et al. showed trapping of inflammatory cells in the matrix of amniotic membrane. They also showed apoptosis of trapped inflammatory cells and suggested that this might explain some of the anti-inflammatory effects of amniotic membrane.

Hao et al., using the reverse transcriptase polymerase chain reaction, demonstrated messages for several anti-angiogenic chemicals like thrombospondin-1 and endostatin expressed by both amniotic epithelial and mesenchymal cells. In addition mRNA expression of all four tissue inhibitors of metalloproteases (TIMP-1, -2, -3 and -4) was demonstrated and these proteases are known to have a potent antiangiogenic effect. They suggest that these findings may explain the anti-angiogenic properties of amniotic membrane. In addition they suggest that the
anti-inflammatory properties of amniotic membranes further dampen the stimulus to angiogenesis.

Another unique characteristic of amniotic membrane is its lack of immunogenicity; the tissue does not express the usual major histocompatibility antigens—for example, HLA-A, B, or DR. As a result, amniotic membrane does not induce immunological rejection after its transplantation. Antibacterial effects of both amnion and chorion have been demonstrated against a wide range of bacteria, including Hemolytic streptococcus group A, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa.

The amniotic membrane may promote nerve regeneration by maintaining nerve growth factor (NGF) signalling. The amniotic membrane contains a large amount of NGF, and preferentially maintains the NGF signalling system for human limbal epithelial cells in culture thereby causing healing of neurotrophic ulcers.

Surgical methods

Amniotic membrane transplantation (AMT) is generally performed with peribulbar anaesthesia using 2% lidocaine. Bed for amniotic membrane is prepared by conjunctival peritomy and superficial keratectomy leaving the adherent epithelia intact. Symblepharon release and fornix reconstruction is done in case of cicatricial ocular surface diseases. Amniotic membrane is peeled from the nitrocellulose paper and the membrane of the measured size is sutured to the corneal or conjunctival surface with the epithelial side up with interrupted 10-0 monofilament nylon radial sutures. The epithelial side of the amniotic membrane is determined by identifying the opposite side to which the chorion was attached. The orientation of the amniotic membrane is confirmed by touching a surgical sponge to the amniotic membrane—the “stickier” side being the stromal side and the less sticky side the basement membrane side. Bandage contact lens is applied. In case of TLD, after the amniotic membrane and limbal transplantation, lateral tarsorrhaphy can be performed in the most severe cases to prevent desiccation.

Postoperatively, topical antibiotic and steroid eyedrops are started with artificial tear supplements. On subsequent follow-ups, steroid medication is tapered, and the patient is continued on artificial tears. Bandage contact lens is removed after one month when all the sutures are removed.

Patch or overlay Technique: One layer of AM is placed over the entire cornea and limbus. When used as a “patch” amniotic membrane will eventually fall off or is removed.

When used as a patch, epithelialization is expected to occur beneath the membrane, with the membrane acting as a bandage.

Sandwich technique: the graft and patch techniques are combined. Sutureless amniotic membrane transplantation has been performed using fibrin glue in various conditions like partial LSCD, corneal ulcers or perforations, scleral melt, conjunctivochalasis and pterygium. When sutures are used to secure the membrane to rabbit or human corneas, epithelialization may occur both over and under AM. It has been shown that in contrast to suturing, epithelial growth takes place only over AM when fibrin glue is used for central epithelial defects in rabbit corneas. In the procedure using fibrin glue, the amniotic membrane is placed on the denuded ocular surface with the stromal side facing down. Half of the amniotic membrane is flipped to disclose the denuded surface. Both components of the fibrin glue are applied to this surface and the membrane is then flipped back. After waiting for five to 10 seconds, a muscle hook is used to spread the fibrin glue under AM. The same procedure is then applied to the other half of the membrane. The excessive membrane and fibrin gel are trimmed off to flush with the surrounding corneal, limbal, and conjunctival edges. The standard method of applying fibrin glue for fixing amniotic membrane has certain limitations. The membrane will bulge forward if it is not pressed into the glue clot within seconds and this is difficult to achieve because of the properties of the membrane. The short drying period of the glue does not allow sufficient time for a precise manipulation.
as a graft. In fornix reconstruction, fornix-deepening sutures may need to be placed and tied on the skin surface over bolsters.

**Total ocular surface cover:** In severe ocular surface burns when extensive areas of the corneal and conjunctival epithelium have been destroyed, the membrane can be used to cover the entire ocular surface. A large patch of membrane is placed over the lids and with a blunt instrument such as a squint hook, the membrane is tucked into the fornices so that a double layer is formed, one covering the palpebral surface and one covering the bulbar surface and cornea. Fornix-deepening sutures are placed and tied on the skin over bolsters, superiorly, inferiorly, medi ally, and temporally. Excess membrane is then trimmed at the lid margin and the edge tacked to the lid margins.

The orientation of the membrane can be epithelial side up or epithelial side down or combined approach\(^1^6\). When required as a substrate for migrating cells, that is, when used as a graft, the membrane has to be sewn in place with the basement membrane or epithelial side up. When the membrane is supplied, spread on a filter paper, the epithelial side is usually up, with the stromal side applied to the surface of the paper. The membrane is used with the epithelial side against the ocular surface when it is used as a biological bandage, primarily to contain the inflammatory reaction while epithelialisation is occurring beneath the membrane. The stromal side of the membrane traps inflammatory cells and induces apoptosis reducing inflammation. In the combined approach two membranes can be used, one epithelial side up and the other down. The inner membrane applied to the ocular surface is sutured with the epithelial side up, to act as a graft. The other, usually larger membrane is sutured on top of the first, with the stromal side applied to the surface of the paper. They report that this application technique prevents development of foam and leads to a thin fibrin film, which minimizes any irregularities of the fixed membranes and creates extra time to adjust the membranes’ position.

Amniotic membrane may be used either to cover the cornea partially or completely, or to cover the bulbar and fornical conjunctiva or for total ocular surface cover\(^1^6\).

Partial or subtotal corneal cover: The membrane may be used to partially cover the cornea when a small non-healing area is covered by a membrane of appropriate size and held in place with a few sutures. It is usually trimmed manually to a size and shape to fit the defect. Subtotal corneal cover is usually required in bullous keratopathy or when it is used as a graft in association with auto or allo-limbal transplant. Complete corneal cover: In large corneal epithelial defects or in association with limbal transplant operations, it may be necessary to suture the membrane 360° around the limbus to peritomized conjunctiva. It may act either as a patch or a graft depending on the state of the underlying corneal stroma once the fibrovascular membrane has been removed.

Bulbar and fornical or palpebral cover: In lid surgery and conjunctival surgery, especially after release of symblepharon or excision of pterygium, the membrane may be used as a patch or graft to cover areas of denuded sclera or episclera. In such situations it is usually applied as a graft. In fornix reconstruction, fornix-deepening sutures may need to be placed and tied on the skin surface over bolsters.

**Multiple Layers:** Multiple layers of amniotic membrane, stacked one on top of the other, can be used to fill in an area of corneal melt or thinning. The final layer is slightly larger...
as corneal epithelium covering only the superficial surface of the AM. This is the most common integration pattern.

Intrastromal Integration: The term intrastromal integration is used for cases in which AM stroma is surrounded by corneal stroma, without any contact with the corneal epithelium. This is observed only after multilayered AMT, the technique indicated for deep stromal lesions.

Superficial Localization (Disintegration): Superficial localization means lack of real integration; that is, the AM is attached to the corneal surface, but is not covered by any corneal tissue.

Although the integration process depends on many factors, the classification of integration patterns after AMT may be useful in understanding the fate of AM on (or in) the cornea in the context of different diseases, and may aid in choosing the most appropriate technique of AMT and (where necessary) the proper timing of PK after AMT.

The application technique of AMT seems to have a great impact on the morphology of AM integration. The surgical technique depends on the type and severity of ocular surface disease. In general, authors state that a patch disappears most often during the first 1 or 2 weeks after AMT without remnants, so it is preferred for central corneal lesions, especially shallow stromal defects, due to optical reasons. For peripheral lesions, graft AMT might be preferred. If layers of corneal epithelium grow between AM layers, they may help to integrate the AM into the cornea and to guarantee some degree of stabilization, especially in the presence of very deep ulcers or even descemetoceles. Using the sandwich technique, the patch is typically lost early after AMT (similar to single patch), but all layers of grafts may be integrated into the corneal stroma and stay there for many months, potentially reducing vision. Connolly et al showed that amniotic membrane, once transplanted into the corneal stroma, can remain intact within the cornea for many months postoperatively without being broken down or dissolved by the host tissue. They showed that its continued presence within the eye does not result in inflammation, rejection, or a loss of transparency and therefore, amniotic membrane is highly suitable for the surgical reconstruction of the corneal stroma.

Kruse et al found out using vital staining that no viable AM epithelial cells remain after cryopreservation. Resch et al found by TEM, that AM epithelial cells which are present in cryopreserved amniotic membrane, showed intracellular signs of degeneration. After transplanting fresh AM
immediately after preparation (without cryopreservation), a longer survival of the AM epithelium could be expected, but proliferation of AM epithelium does not seem to occur. Anderson et al. found calcification of the cornea in 12.8% of cases after graft AMT, but never after patch.

After amniotic membrane transplantation in limbal deficiency, successful ocular surface reconstruction is defined on the basis of corneal epithelialization, decrease in corneal neovascularization, and improvement in visual acuity. Corneal epithelialisation is based on 3 criteria: a clear appearance without epithelial defect on slit-lamp examination, the absence of abnormally high fluorescein permeability, and the absence of conjunctiva-derived goblet cells on impression cytology. If all 3 criteria are fulfilled, that is an indication that the epithelium is of corneal origin and that surgery has been successful.

Indications for amniotic membrane transplantation

Chemical injury

Several surgical techniques have been proposed for ocular surface reconstruction in chemical burn with limbal dysfunction. Simple excision of fibrous tissue and conventional keratoplasty are not sufficient to avoid recurrence of the fibrovascular pannus in severe cases. Success rates of ocular surface reconstruction with limbal allograft or autograft transplantation were reported to range from 70% to 90% during a follow-up of 2 years. However, a decrease in these rates to 50% was reported after a follow-up of 5 years, probably as a result of limbal graft failure caused by persistent perilimbal stromal inflammation. Amniotic membrane transplantation has been found to successfully reconstruct the ocular surface epithelia in eyes with chemical and thermal burns. Amniotic membrane seems to function as a substrate, promoting proper epithelialization while suppressing excessive fibrosis. Repeat amniotic membrane transplantation may have to be performed in patients with chemical injuries. When there is severe conjunctival involvement, amniotic membrane transplantation with limbal autograft transplantation, appears to be effective. It is believed that amniotic membrane restores a noninflamed perilimbal stromal environment to support the transplanted limbal epithelial stem cells, which seems to increase the success of subsequent corneal surface reconstruction.

Acute stage

Ophthalmic interventions during the acute stage of chemical and thermal injury and acute Stevens-Johnson syndrome have traditionally been supportive in nature, like aggressive lubrication, prophylactic topical antibiotics, and lysis of adhesions. But these are not effective to improve the poor ophthalmic prognosis associated with this condition. Amniotic membrane has been used as a temporary patch graft in acute phase of chemical and thermal injury and acute Stevens-Johnson syndrome. Amniotic membrane transplantation is reported to enhance epithelialization, reduce inflammation, reduce scarring, improve visual acuity, and prevent the occurrence of conjunctivalization in such cases. In the majority of patients, the entire ocular surface, that is, the cornea, the bulbar and palpebral conjunctiva, and the eyelid margins, needs to be covered with amniotic membrane. During the procedure, the eyelids are retracted with a lid speculum and a sheet of cryopreserved amniotic membrane is placed on the ocular surface with the basement membrane side facing away from the corneal surface and secured to the corneal surface with sutures. A variable number of additional 10-0 nylon sutures are placed more posteriorly to the limbus to further secure the amniotic membrane to the bulbar conjunctival surface. A large-diameter bandage contact lens is then applied to the eye. Then lid speculum is removed. A second sheet of cryopreserved amniotic membrane is placed on the eyelid, again with the basement membrane side facing away from the corneal surface and secured to the corneal surface with sutures. A variable number of additional 10-0 nylon sutures are placed more posteriorly to the limbus to further secure the amniotic membrane to the bulbar conjunctival surface. A large-diameter bandage contact lens is then applied to the eye. Then lid speculum is removed. A second sheet of cryopreserved amniotic membrane is placed on the eyelid, again with the basement membrane side facing away from the corneal surface. One end of the sheet of amniotic membrane is sutured to the eyelid skin, close to the eyelid margin. A muscle hook is then used to push the amniotic membrane into the fornix. Two double-armed 5-0 or 6-0 prolene sutures are then passed through the amniotic membrane, passed through the eyelid, and then secured over the skin with a bolster. The same procedure has to be performed for both upper and lower eyelids. Amniotic membrane coverage of the ocular surface in its entirety coupled with the use of intensive short-term topical corticosteroids during the acute phase of SJS and TEN is associated with the preservation of good visual acuity and an intact ocular surface. Partial amniotic membrane coverage of the ocular surface may not serve to minimize the cicatrising ocular sequelae of SJS and TEN as effectively as complete coverage.
In a study evaluating the efficacy of AMT for treating moderate to severe ocular burns in the acute stage, Meller et al. showed that AMT can be considered an early, if not immediate, surgical procedure to promote epithelialisation and suppress inflammation so that scarring-induced sequelae can be prevented in the chronic stage. Amniotic membrane transplantation rapidly restores the ocular surface, especially in mild to moderate (grades II and III) chemical or thermal burns. In severe (grade IV) burns, AMT alone reduces limbal stromal inflammation, restores the conjunctival surface, and prevents symblepharon formation, but cannot prevent the development of limbal stem cell deficiency. The latter requires additional stem cell transplantation to restore the corneal surface integrity.

Persistent inflammation with leukocyte infiltration, a key characteristic of acute burns, is known to prevent epithelialization and contribute to the melting process in the acute stage and to formation of granuloma and scar in the chronic stage, also leads to limbal stem cell deficiency in humans and failure of autologous limbal conjunctival transplantation in rabbits. Without effective measures to suppress inflammation in the acute stage, the remaining population of the epithelial stem cells declines, paving a difficult way to recovery. Furthermore, leukocyte infiltration comes in two waves in burns, the first within 12 to 24 hours and the second starting at day 7; the first wave is crucial for the recruitment of the second. Thus AMT performed at the early stage may help suppress the gradual recruitment of more inflammatory infiltration, and collectively may shorten the duration and extent of inflammation further. The action of AM to suppress acute inflammation alone may not explain its entire efficacy because various medical therapies to suppress acute inflammation had limited success. Early epithelial replacement has been regarded as essential in the management of ocular burns. AMT is an effective surgical measure to promote epithelialization and to restore normal epithelial phenotype by expanding the remaining epithelial stem cells. For epithelialization to take place on its basement membrane side, the basement membrane of AM may be substituted for the damaged basement membrane of the normal conjunctiva. One other important action of AM is to help preserve and expand the slow-cycling property of the epithelial progenitor cells. Scarring in the lid margin causing cicatricial entropion and inward turning of lashes, and symblepharon, which obliterates the formation of tear meniscus and interferes with eyelid blinking, generate a vicious cycle leading to more ocular surface failure in the chronic stage and present difficulties for subsequent ocular surface reconstruction. Amniotic membrane prevent scarring directly and indirectly by reducing inflammation. Low incidence of symblepharon formation is noted when AMT is performed in the acute stage, a finding also noted by Sorsby and colleagues.

**Limbal stem cell deficiency**

Dysfunction of the stem cells of the corneal epithelium is identified by the presence in the central cornea of goblet cells derived from the conjunctiva (“conjunctivalization”), persistent epithelial defects or completely keratinized epithelium accompanied by an absence of palisades of Vogt. Partial limbal deficiency (PLD), can be treated with AMT alone. Total limbal deficiency (TLD) requires AMT and conjunctival and limbal stem cell transplantation. This results in complete epithelialisation and reduced inflammation and vascularization of the ocular surface. Amniotic membrane transplantation expands remaining limbal stem cells and corneal transient amplifying cells during the treatment of partial limbal deficiency. However, this specific action is not effective when there are no stem cells, and then limbal stem cells needs to be transplanted. Amniotic membrane has been safely used to inhibit neovascularization before limbal stem cell transplantation. It is believed that the amniotic membrane restores a noninflamed perilimbal stromal environment to support the transplanted limbal epithelial stem cells, which seems to increase the success of subsequent corneal surface reconstruction. AM transplantation using fibrin glue appears to be a safe and effective method of restoring a stable corneal epithelium for cases with partial LSCD. Santos et al. showed that conjunctival limbal grafts associated with AMT are useful for restoring corneal epithelium phenotype in eyes with total limbal stem cell deficiency. However, the cumulative survival declined substantially over a 2-year period. Dry eye was found to be the most important prognostic parameter.

**Pterygium**

Shimazaki et al. reported that the combination of an amniotic membrane transplant to inhibit subconjunctival fibrosis, and a limbal autograft to restore limbal function is an effective surgical procedure for treating patients with...
Ang et al. studied the efficacy of autologous cultivated tissue at the limbal region. Vascularization from host necrosis and secondary infection might aid in preventing serious complications associated with simple denuded HAM transplantation, such as scleral necrosis and secondary infection.

Anterior segment ICGA is useful to monitor graft vascularization after pterygium surgery. Conjunctival autograft health may be demonstrated by early graft vascularization and perfusion; however, there is a delay in graft vascularization after AMT that may be related to the antiangiogenic effects of the membrane. In a study by KÜÇÜKERDÖNMEZ et al., in contrast with the early vascularization of the conjunctival autografts, there was no vascularisation in the AMT group, and the grafts remained avascular, showing hypofluorescence during ICGA one month after surgery. Except for the underlying vascular network of the episcleral bed, the grafts showed no hyperfluorescence or isofluorescence as a sign of reperfusion, as is seen in conjunctival autografts. This study results seem to support the barrier phenomenon suggested by Tananuvat and Martin in AMT, which could be demonstrated as a lack of vascularization from host tissue at the limbal region.

Ang et al. studied the efficacy of autologous cultivated conjunctival epithelial sheet in the management of pterygium. Conjunctival biopsy was done from the eye with pterygium 2 weeks prior to surgery and ex vivo expansion of these conjunctival epithelial cells was done over amniotic membrane. They reported that transplantation of autologous cultivated conjunctival epithelial sheet facilitated early postoperative epithelialization and recovery, and may aid in preventing serious complications associated with simple denuded HAM transplantation, such as scleral necrosis and secondary infection.

**Persistent epithelial defect**

Treatment of persistent epithelial defects consists of correction of the underlying cause and tissue lubrication. In cases refractory to medical treatment several options can be considered, including AMT. Lee and Tseng used amniotic membrane to treat persistent epithelial defects. AMT is useful in patients with persistent epithelial defects, although most cases require further surgery for visual and ocular surface rehabilitation.

**Severe corneal and scleral ulcers**

Azuara-Blanco et al., in a study of eyes with severe ulceration and impending perforation found that AMT failed to stabilise the cornea, and additional urgent tectonic procedure had to be undertaken, but they had used AMT as a patch and not as a graft. In case of graft, as proposed by Lee and Tseng, epithelialisation occurs over the membrane, which is incorporated into the corneal tissue. This alternative might be more helpful to build up the corneal thickness and to provide a healthy substrate in cases of severe ulceration and impending or recent perforation.

In a study by Hanada et al., amniotic membrane transplantation was done for severe corneal ulcers with perforation or descemetocele and scleral ulcers. In the surgical method they have described, first, the bottom of the ulcer is debrided, and poorly attached epithelium at the edge of the ulcer is removed as bluntly as possible. After the ulcer surface is treated and healthy corneal or scleral stroma is exposed, the first segment of amniotic membrane is transplanted as filling material in the stromal layer (amniotic membrane filling). The amniotic membrane is cut into small pieces and stuffed into the ulcer. In the scleral ulcer cases, the ulcer is filled with autotenon’s capsule tissue. The second amniotic membrane is transplanted as a basement membrane (amniotic membrane graft) with epithelial side up and the third amniotic membrane is transplanted as a cover (amniotic membrane patch). The amniotic membrane patch is placed on the entire wound and corneal limbus with epithelial side up to protect the area of re-epithelialization. They used layered amniotic membrane transplantation to achieve the following goals. Amniotic membrane filling provides a substitute for collagens, the amniotic membrane graft provides basement membrane for proper epithelialization, and the amniotic membrane patch protects the wound. In their study eight out of 11 eyes (72.7%) were successfully treated by this method with a mean epithelialization period of 16.5 ± 8.0 days.

**Neurotrophic ulcers**

Interruption of the corneally derived sensory afferent nerve anywhere along its course of V1 may result in a disease state termed neurotrophic keratopathy, which is characterised by corneal anesthesia and epithelial breakdowns leading to persistent and progressive neurotrophic ulcers. Common causes of neurotrophic keratopathy include herpetic infection (simplex or zoster), alkali burn, diabetes mellitus, tumours affecting the trigeminal ganglion or sensory routes, radiation, and anterior segment surgeries. Chen et al. did a study of amniotic membrane transplantation in severe neurotrophic ulcers. Amniotic membrane was fitted to fill up the ulcer and cover the defect by trimming off the excess edges. More than one layer of amniotic membrane was used if the ulcer was deep, and in those instances the bottom layers were left unsutured and only the top layer was sutured. Depending on the aqueous tear status and the eyelid blinking function, a bandage contact lens, amniotic membrane as a temporary patch, or temporary tarsorrhaphy was added. Amniotic membrane was used
as a patch was when the stromal thinning was minimal and
the ulcer bed appeared to be non-necrotic. The rationale
of using AM as a patch in addition to using it as a graft
was to prevent surface exposure and dryness and promote
epithelial healing in these cases with poor blinking reflex.
They reported that constant protection and wetting of the
ulcerated area by the membrane is also beneficial and AM
used as a patch may function like tarsorrhaphy to minimise
exposure. Chen et al suggested that amniotic membrane
also provides protective effect by preventing eyelid blinking-
mediated microtrauma, resembling therapeutic soft contact
lens (TSCL) and tarsorrhaphy as speculated by Baum54.
Baum proposed that sufficient oxygen supply, enough
hydration of epithelium, and less external friction to fragile
corneal epithelium are important for treating persistent
corneal epithelial defect. Yoshita et al 55 found that AM has
a higher water content and a higher Dk than TSCL which
helps explain why AM is also effective in treating persistent
defects.

Glaucoma
Leakage of aqueous humor from conjunctival filtering blebs
may occur as an early or late complication of glaucoma
filtration surgery especially in trabeculectomies performed
with 5 Fluorouracil or mitomycin c. Bleb leaks have been
repaired successfully with bleb function maintained with
amniotic membrane graft56. The leaking area is covered
by amniotic membrane graft without excision of the
leaking bleb. This technique has several advantages. The
membrane can be measured accurately to cover the cystic,
leaking area, thereby eliminating the risk of developing a
too small or tight flap. And because of the antimicrobial
properties, amniotic membrane graft seems to have fewer
risks of postoperative infection. Amniotic membrane graft
enhances the healing of the bleb leaks while maintaining the
bleb function, because of it’s strong epithelialisation effect.
But in a study conducted by Budenz et al57 where amniotic
membrane transplant was compared with conjunctival
advancement in patients with leaking glaucoma filtering
blebs, they found that after an average follow-up of 19
months, there were seven failures in the amniotic membrane
transplant group out of 15 eyes and there were no failures
in conjunctival advancement group. The cumulative survival
rate for amniotic membrane was 46% at 2 years whereas it
was 100% for conjunctival advancement. This suggests that
this material may not be a suitable substitute for conjunctiva
in the repair of leaking filtering blebs.

Permanent repair of an extruding glaucoma drainage device
(GDD) may be difficult to achieve in eyes with extensive
conjunctival scarring from ocular surface disease or
previous surgery such as scleral buckling. Donor sclera may
melt if inadequately covered with conjunctiva, yet adequate
mobilization may be hampered by the above factors. Failure
to repair an exposed GDD may necessitate removal or risk
endophthalmitis. Rai et al58 suggested that AMT may be
used satisfactorily to cover donor sclera in eyes with an
extruding GDD. Where extensive scarring or conjunctival
shortening hampers closure, AMT acts as a scaffold for
regrowth of conjunctival epithelium and after a period of
time appears indistinguishable from other conjunctiva that
has undergone surgery.

Symblepharon
The cause of cicatricial diseases includes Stevens-Johnson
syndrome, chemical burn, chronic cicatricial conjunctivitis
of unknown cause, multirecurrent pterygium, mucous
membrane pemphigoid, pseudopemphigoid, multiple
previous surgeries for lid tumours etc. Several reports
showed that fornix reconstruction could be accomplished
by AMT with or without intraoperative MMC8,11,59,60. Keirkhah
et al61 studied surgical strategies for fornix reconstruction
based on symblepharon severity. For grade I symblepharon
where the residual and recessed conjunctiva was sufficient
to cover the entire palpebral surface, and for grade II
symblepharon where the residual and recessed conjunctiva
was sufficient to cover the tarsal surface but not the
entire palpebral surface, cicatrix lysis and AMT alone was
successful. For grades III and IV symblepharons where the
residual and recessed conjunctiva was not sufficient to cover
the tarsal surface, the procedures of cicatrix lysis, AMT, and
anchoring sutures were not as effective, but overall success
increased to 100% if included with either oral mucosal graft
or conjunctival autograft to the tarsus.

Bullous keratopathy
Amniotic membrane transplantation to the corneal surface
has been reported as effective in reducing the formation
of epithelial bullae and associated ocular discomfort in patients
with painful aphakic bullous keratopathy and pseudophakic
bullous keratopathy52. Patients with intolerable pain
preoperatively become pain-free postoperatively. However,
regression of epithelial bullae and recurrence of pain and
discomfort can occur depending on the use of different
modifications of the AMT technique (inlay or overlay)
or different side of AMT coming above. Recurrence of
symptoms can also occur after dissolution of the AMT.
The AMT is performed as inlay (or graft) or overlay (or
patch) technique in most cases with bullous keratopathy.
Graft allows the migrating epithelial cells to grow over the
membrane, whereas the patch acts a biological contact
lens protecting the healing surface beneath and as a barrier
to the chemical mediators in the tear film and reducing inflammation.

Various suturing techniques have been described for amniotic membrane in bullous keratopathy like suturing into peripheral cornea, suturing beneath conjunctival peritomy and a modified technique by Espana and associates where amniotic membrane is sutured into lamellar pockets. The modified technique may be more advantageous by providing a new basal membrane more resistant to bullae formation but it is recommended to leave the limbus out, so that re-epithelialization must be over and not under the AMT. This is in contrast to direct suturing of AM on the cornea, which may allow some epithelialization to take place under AM if the suturing method does not ensure tight approximation. Altiparmak et al reported a modification of Espana’s technique by creating more regular contours of the stromal pockets, using a corneal trephine and cutting the AM with a punch trephine to fit into this pocket. This is theoretically more advantageous by ensuring that epithelialization will take place on the top of AM.

Infectious keratitis

Amniotic membrane transplantation has been used successfully in infectious keratitis. Studies have shown that human AM transplantation promotes rapid epithelialization and reduces stromal inflammation and ulceration in herpes simplex virus (HSV)-1 keratitis. When used with antifungal agents as adjunctive treatment, AM transplantation can enhance epithelialization and prevent corneal perforation in acute fungal keratitis.

Kim et al did a study of amniotic membrane transplantation in infectious keratitis with causative organisms including Staphylococcus species, Pseudomonas species, Acanthamoeba species, fungus, and herpesvirus. Sufficient antibacterial, antifungal, or antiviral agents were applied to eradicate causative organisms before permanent or temporary amniotic membrane transplantation, or a combination of the two in few patients. The amniotic membrane was soaked in antinfective agents before transplantation in all cases. The corneal surface was healed successfully and recurrences of microbial infection were not noted in any case. Visual acuity was improved in cases that were nonscarring or after additional penetrating keratoplasty.

Gicquel et al did a study on patients with severe bacterial keratitis and reported immediate pain relief and epithelial healing that they attributed to early AM transplantation combined with topical corticosteroids. 12 patients with severe microscopically-proven BK were treated with immediate maximal topical antibiotics followed by AMT at 48 hours (single-layer epithelial side-down or multilayer epithelial side-up), plus topical steroid treatment at 72 hours. The organisms causing the keratitis included Pseudomonas aeruginosa, Klebsiella pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, Staphylococcus epidermidis, and Streptococcus pneumoniae.

In an experimental study of staphylococcus aureus keratitis in rats by Barequet et al, the group treated with antibiotic drops and amniotic membrane transplantation was found to have the best clinical results compared to the groups treated with antibiotic drops alone and amniotic membrane transplantation alone. They reported that AM transplantation is a useful adjunctive treatment after bacterial keratitis. The transplanted AM improved the healing process, resulting in decreased corneal haze and less neovascularization.

Miscellaneous indications

Amniotic membrane has been used to cover bare sclera in oral mucous membrane grafting for total limbal stem cell deficiency and also to cover the mucous membrane graft. Ocular motility defects and diplopia are well recognized problems after operations to treat retinal detachment. The fat adherence syndrome plays a significant role in causing restriction. Conventional extraocular muscle surgery has shown only limited success. Yamada et al described an alternative treatment, the transplantation of amniotic membrane onto the extraocular muscle, to prevent regrowth of restrictive scar tissue. Here the therapeutic effects of amniotic membrane like suppression of recurrent subconjunctival fibrosis and lysis of symblepharon was utilised.

After resection of neoplasms like conjunctival melanoma, amniotic membrane can be used to replace the large conjunctival defect avoiding the need for extensive repair with a conjunctival or oral mucous membrane graft.

Ligneous conjunctivitis, a rare form of membranous conjunctivitis particularly resistant to treatment, is characterized by woodlike indurated membranes on the upper and lower tarsal conjunctiva. Barabino et al reported that amniotic membrane transplantation in ligneous conjunctivitis provides a favorable outcome.

In the treatment of large conjunctival defects that may follow strabismus surgery, Mocan and Azar reported the use of AMT. Conjunctival dehiscence is a potential complication of strabismus surgery. It can lead to exposure
of sclera and the recessed muscle. Previous surgeries of the conjunctiva make tissue manipulation difficult and increase the probability of conjunctival rips or uncontrolled tears. In the treatment of large conjunctival defects that may follow strabismus surgery, AMT may be an alternative to conservative management or primary conjunctival closure.

Conjunctivochalasis (cch), defined as a redundant, loose bulbar conjunctiva interposed between the globe and the eyelid, tends to affect both eyes of older populations. CCh is a common cause of ocular surface irritation and its clinical significance is often overlooked. No treatment is needed if patients with CCh remain asymptomatic. For symptomatic cases, medical therapies are directed to suppressing ocular surface inflammation, and when they fail, surgical removal of the redundant conjunctiva becomes necessary. Surgical technique usually includes excision of the bulbar conjunctiva or suture fixation of the conjunctiva to the sclera. Amniotic membrane transplantation (AMT) has been shown to be successful in reconstructing the conjunctival surface after removal of CCh. Previously, focal inflammation of host conjunctiva, scar formation, and suture-induced granuloma were noted in some cases, after AMT using sutures for CCh. Using fibrin glue, similar incidence of focal inflammation of host conjunctiva is noted only adjacent to the border of AM in the fornix.

Amniotic membrane can be used to reconstruct the ocular surface after excision of the invading granulation material typical of LOGIC syndrome (laryngeal and ocular granulation tissue in children from the Indian subcontinent). Moore et al. reported a case where after 24 operations to treat the ocular complications induced by LOGIC syndrome, amniotic membrane transplantation was the first effective treatment. In the early follow up period (2-3 months), there was complete cessation of the proliferation of granulation tissue and reepithelialization of the corneal surface. Longer follow up (10 months) demonstrated limited recurrence, which required retreatment.

Complications and drawbacks

Despite the widespread use of amniotic membrane in ocular surgery, very few complications have been reported. The complications which are reported are suture granuloma and persistent inflammation, and these are not specific to the membrane. Gabler and Lohmann reported hypopyon after repeated amniotic membrane transplantation in a case of neurotrophic ulceration who developed a hypopyon 2 days after the second and again after the third amniotic membrane transplant. On both occasions it responded to topical steroids. The authors suggest that use of membrane from different donors (when required repeatedly) may help minimize the risk since it is likely to be an immune reaction. Accumulation of blood (hematoma formation) under the membrane in the immediate postoperative period or during suture removal has been reported.

Failure to achieve the intended effect with amniotic membrane is perhaps the most significant drawback. Another one important drawback is the loss of membrane, either by degradation or by cheese wiring of the sutures, in the immediate postoperative period. Another less significant undesirable effect is the residual subepithelial membrane that persists in some cases. According to Dua et al. this is more likely to happen if the membrane used is from the relatively thicker portion of the amnion, near the umbilical cord. When this occurs in the visual axis, it can affect the visual acuity. Then there is the potential danger of spread of virus and bacteria. As membrane from a single donor can be used in several patients, a single donor can cause infection to multiple recipients. Adequate donor screening to cover the window period, proper handling, processing, and storage, and frequent microbiological tests on used and stored membranes should minimize this risk.

Recently, temporary amniotic membrane patching has successfully been used for acute chemical burns of corneas, a devastating and emergency condition in ocular surface management. In the acute phase of ocular burns, intraocular pressure (IOP) may increase significantly and should be monitored carefully. However, once amniotic membrane is patched onto the eye, measurement of IOP through the amniotic membrane becomes difficult using conventional methods, such as Goldmann applanation tonometer. The accuracy of IOP measurement by the Tono-
Pen XL over a single layer of amniotic membrane patching was demonstrated using rabbit eyes. IOP by Noncontact Tonometer (NCT) was found to be reliable through a combination of a single-layer AM/TSCL (therapeutic soft contact lens) on normal human eyes. However, IOP measured by NCT over a combination of a double-layer AM/TSCL was inaccurate and tended to be an underestimation.

**Modifications**

Cryopreserved AM requires some processing and the storage of cryopreserved AM requires space-consuming freezers. Furthermore, there is no reliable method of sterilizing the cryopreserved AM. To resolve these problems, a hyperdry AM was developed by Kitagawa et al. that is processed using far-infrared rays and microwaves, then is sterilized by y-ray irradiation; it is capable of being stored at room temperature.

PROKERA is a device, in which a cryopreserved amniotic membrane is clipped into a dual polymethyl methacrylate ring set that acts like a symblepharon ring. The conformer fits snugly over the cornea and under the eyelids. PROKERA is stored at -80 °C before use. After thawing at room temperature for five to 10 minutes, PROKERA is rinsed with physiological saline before insertion with the aid of an eye speculum and topical anaesthetic. PROKERA is found to affect visual functions in normal human eyes. Both distant and near visual acuities but not color vision deteriorated after insertion. Significant annoying symptoms were also noted after insertion of PROKERA, presumably owing to the contact of rigid polymethyl methacrylate skirt with the eye like a symblepharon ring. Such discomfort was not noticeable in eyes with neurotrophic conditions, and was markedly reduced by a softer skirt made of polycarbonate material. However, it is conceivable that a softer skirt may present some disadvantages, especially in cases that may benefit from a symblepharon ring.

Although some of the activity of amniotic membrane can be attributed to a mechanical effect of the basement membrane, the biologic role of amniotic epithelium is increasingly recognized. Parmar et al. assessed the effect of tissue-cultured human amniotic epithelial cells (AECs) in restoring the ocular surface, transplanted using a collagen shield seeded with AECs supported by a soft contact lens. AECs were isolated from serologically screened donor human placenta, seeded onto collagen corneal shields, and incubated in tissue culture medium for 7 days. These collagen shields were placed over the persistent epithelial defect (PED) and supported by an overlying soft contact lens. The collagen shields dissolved by 72 hours, and the contact lenses were removed after this time. This cycle was repeated every week until healing of PED was achieved.

**Conclusion**

Amniotic membrane is a useful tool in many ocular surface diseases. But the success of the procedure differs in different patients. The efficacy of the procedure depends on a lot of factors like the severity of the ocular surface disorder, the technique used (patch or graft), the orientation of the membrane (epithelial or stromal side up), the suturing technique, inter and intra donor variations in the membrane and the depletion or alterations in its constituents subsequent to processing and storage. The exact mechanism of action of the membrane causing healing of the various surface disorders is still not clear and there is plenty of scope for future research. The risk of infection and the unpredictable results after the amniotic membrane transplantation has led to the search for a more standard synthetic membrane using collagen or polymer matrices impregnated with putative beneficial ingredients, such as growth factors and antimicrobials.

**References**


A bout 50% of cases of RP belong to one of the three basic mendelian genetic types, establishing the family tree is an essential component of the clinical interview and work up of any new RP case. And the same applies to all the allied inherited retinal disorders. Determining the particular inheritance pattern of a patient’s RP is an essential step in providing genetic counseling to the patient and his family so that they can be informed of the genetic risks associated with the particular genetic subtype in their family.

In the case of autosomal dominant (ad) RP a typical family tree is often obtained on interviewing the proband. However a typical pedigree may not be obtained because some affected individuals [ie carrying the RP gene] do not exhibit the RP phenotype either because of incomplete penetrance, or because of very late onset. The variable expression of many adRP genes makes diagnosing the disease from family tree analysis somewhat difficult.

Sibs of a proband: In adRP in which one of the parents has the disease (genotype G G*) the risk to sibs (brothers and sisters) of the affected is 50%. In other words each sib has a 50% chance of inheriting the mutant RP gene [G*] from the affected parent.

Children of a proband: Each child of a patient affected with adRP (ie having the genotype GG*) has a 50% chance of inheriting the disease gene and RP.

Note: Because of the variable expression of many adRP mutations the development of the disease may not follow the same course as in the case of the proband.

In the case of autosomal recessive (ar) RP the disease is only manifest in an individual when both copies of a particular autosomal RP gene are mutated or defective, ie when the patient has the genotype G*G*. If an individual carries one defective RP gene (and the other is normal or wild type, G) the individual does not exhibit RP but is a carrier of the disease gene (genotype GG*). Each parent of the proband carries a single copy of the mutant RP gene and a single copy of the ‘wild type’ RP gene (GG*). Both parents are termed obligate heterozygotes.

Sibs of a proband: Each sib has a one in four chance (25%) of receiving two copies of the mutated RP gene (one copy from each of his parents and having the disease genotype GG*, and hence of developing the disease; a one in two chance (50%) chance of inheriting one defective gene and one good gene and being an asymptomatic carrier having genotype GG*, a one in four chance (25%) of receiving two wild type genes (one from each parent) and being unaffected and NOT a carrier having genotype GG.

Children of a proband: All children of an affected who has married a non affected are obligate carriers having genotype GG*. The sibs of obligate carriers (such as the proband’s parents i.e., his maternal or paternal uncles and aunts) have a 50% chance of also being obligate carriers (GG*).

In the case of arRP the counselor should point out that the chance of either the proband or his sibs having a child with RP is greatly increased by marrying a close relative. For estimates of risk involved in such marriages the proband should be referred to a genetic specialist. Where the proband is intending to marry an unrelated individual then the risk of having an affected child is greatly reduced.

In X-linked recessive (XL) RP the disease affects males who inherit a defective RP gene G* on their X chromosome they inherit from their mothers. Affected males pass on the defective X chromosomal gene, G*, to their daughters all of whom are obligate carriers. Female carriers have one copy of the good gene, G, on one of their X chromosomes and a copy of the defective gene G* on their other X chromosome (ie. genotype G G*). They are unaffected because the wild type RP gene, G, provides sufficient healthy gene product to ensure normal function. Any women in an established X linked RP pedigree who has an affected son is an obligate carrier having the bad gene, G*, on one of her X
chromosomes.

Sibs of proband: If there is a family history which implies that the mother is a carrier [genotype, GG*] then all female sibs of the proband have a 50% chance of inheriting the bad gene from their mother. Each brother of the proband has a 50% chance of being affected.

Children of affected: The daughters of affected males are all obligate carriers as each receives her father’s X chromosome and one of her mother’s two X chromosomes. There is no male to male transmission as a son does not inherit his father’s X chromosome carrying the bad gene G*. Sons of affected fathers are generally unaffected unless their mother is a carrier.

As well as risk estimates knowing the genetic subtype can provide useful prognostic information for the family. For example the chances of preservation of good visual acuity (central vision) beyond 60 are greater in adRP compared with arRP and XLRP.

Molecular diagnosis: Carrier detection and prenatal testing: The next logical stage in the work up of a new RP case, after having established the genetic subtype, is to identify the RP gene responsible for the disease by genetic screening using DNA analytical techniques. There are many benefits to knowing the actual mutation.

In the case of arRP and XLRP while risk of carrier status may be assessed from knowledge of the family history only by testing at risk individuals for the actual mutation itself can the actual carrier status of those at risk be definitively determined. For example in families with XLRP DNA can reveal the actual genetic status of at risk females i.e., sisters of boys with XLRP and the sisters of women with an affected son. As XLRP is a severe form of the disease this information may be vital for female relatives intending to marry or planning to start a family. In the case of adRP although non carriers are non affected, because of incomplete penetrance and variable onset of the disease, the only safe way to establish carrier status is by detecting the actual RP mutation.

A married couple at risk of conceiving an affected child with RP may request prenatal diagnosis. Female carriers of an XLRP mutation may wish to abort an affected fetus as many cases of XLRP are relatively severe. The early onset forms of arRP and LCA are also very severe leading to blindness from infancy or birth and many couples at risk of conceiving a child afflicted with such severe retinal diseases may also request prenatal diagnosis. In the case of adRP which tends to be less severe than arRP or LCA there may be less demand for prenatal diagnosis.

Carrier detection may be important in regions of the world such as south Asia where consanguineous marriages are common and where relatives of an affected with arRP may wish to marry another closely related family member.

There are other benefits from DNA testing detection. Knowing the underlying mutation can establish definitely the diagnosis before clinical symptoms have become apparent. This can be important in the case of childhood dystrophies where the clinical prognosis may differ greatly depending on the gene implicated. DNA diagnosis can also be important in confirming the diagnosis and determining the genetic subtype of isolated cases.

Moreover although heterogeneity and variability of expression is one of the hallmarks of RP knowing the mutation does have a certain predictive value. In the case of RHO mutations it is well established that certain mutations tend to be more severe than others. For example as mentioned above the P23H causes a relatively mild and late onset form of RP. It is also widely considered that XLRP and arRP tend to be more severe than adRP.

Finally as Daiger et al (2007) point out “ in the not to distant future knowing the gene responsible will be important as increasingly gene specific therapies become available. .. and knowing the gene responsible will be important in the enrollment of patients into clinical trials.”

There are two major problems which must be overcome if the RP gene mutation responsible for the disease is to be detected in the majority of RP cases presenting at the retinal clinic.

One problem is that only approximately 50% of all RP cases are caused by mutations in known genes. However as mentioned above some authors expect that in about 10 years time most of the mutations causing RP and allied retinal disorders such as LCA may have been identified at least for north American and European populations. This will allow the widespread application of genetic testing of RP sufferers at least in western countries. A second problem is the great complexity of the genetics of RP and of the allied retinal disorders. Moreover in the case of many RP genes, RHO and RPGR for example, a great number of different mutations have been identified. Clearly identifying the mutation in every RP patient that presents for counseling at an ophthalmic clinic is a huge technological challenge.
However this challenge is lessened to some extent by the finding that mutations in only a small number of RP genes are responsible for nearly one half of all RP cases⁷. Remarkably already mutations in only 9 known RP genes are responsible for nearly 17% of all RP cases (table) and 17% of non syndromal RP [assuming that 65% of RP is non syndromal⁷. Indeed mutations in only 2 genes RHO and RPGR are responsible for more than 10% of all cases of RP (table) and 17% of non syndromal RP [assuming that 65% of RP is non syndromal⁷. Because of the high prevalence of RHO and RPGR mutations in RP, mutational analysis of these two gene has considerable diagnostic relevance.¹ The fact that relatively few RP genes cause a significant proportion of RP greatly lessens the complexity and burden of providing a useful DNA diagnostic service.

Moreover developing technologies including large throughput direct sequencing machines and the development of microarrays² is making possible the detection of an ever increasing inventory of RP mutations. The fact that perhaps only a relatively small number of genes will be found to be responsible for most cases of RP in conjunction with the rapid technological advances in mutation detection, raises the prospect of providing eventually and perhaps within 10 years for US and European populations a molecular diagnostic service for most families with RP⁸. Indeed Daiger et al (2007) speculate that “high −throughput sequencing methods will make genetic testing of all genetic diseases affordable and efficient, at least in the developed world, within 10 years.”
Note
1. The RPGR gene has proved difficult to screen for mutations because of the high purine content and unusual structure of exon ORF15 (Shu et al 2006). However there is great clinical utility in screening this gene. The clinical utility lies in the value of RPGR mutation identification for (i) carrier testing, (ii) prenatal diagnosis and (iii) clarifying the mode of inheritance for genetic counselling. A significant proportion (29%) of males with early onset and severe RP with no family history were found in one study to have RPGR mutations, so that this test will clarify the mode of inheritance in this subgroup of patients (Shu et al 2006).

2. Chip technology - Microarrays: A major challenge for the future is to develop techniques that provide a means of rapid detection of RP mutations for carrier detection [in families with recessive disease and X ] and for early diagnosis of individuals in adRP pedigrees. Zernant et al (2005) used arrayed primer extension (APEX) technology to design a genotyping microarray for early-onset, severe retinal degenerations [early onset RP and Leber congenital amaurosis] that includes all of the >300 disease-associated variants currently described in eight genes already known to cause this class of retinal degenerations. The resultant array allows simultaneous detection of all known disease-associated alleles in any patient with early-onset RP Other workers are aiming at similar arrays for later onset arRP (Nawajes et al 2005). Ultimately it may be possible to detect thousands of RP mutations routinely applying various chip technologies. From Pagon and Daiger (2005). Percentages from Daiger et al (2007: Table 3) and assuming that 20% of cases of RP are adRP, 13% are arRP [not including early onset recessive RP which is usually diagnosed as LCA] and 8% XLRP (Daiger et al, 2007).

Reference