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REVIEW

Amniotic Membrane Transplantation in Ophthalmology: An Updated Perspective

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Aim: The aim of this paper is to provide a succinct literature review of the different clinical applications for AMT usage in an ophthalmic setting, ranging from commonly used applications to less mainstream approaches. The hope is that this review enables the reader to have a better understanding of the biological properties of amnion as well as the indications and scenarios in which AMT can be used, whilst presenting relevant evidence from within the literature which may be of interest. We also provide an update on the methods of preservation of amniotic membrane and the application methodologies.

Methods: Literature search. A PubMed search was performed using the search terms "amniotic membrane transplant", "amnion AND cornea", amnion AND ophthalmology", "amnion AND ocular surface" and "Amnion AND eye". A full review of the literature using the PubMed database was conducted up until 01/05/20. The articles used were written in English, with all articles accessed in full. Both review articles and original articles were used for this review. All full publications related to ophthalmology were considered.

Keywords: amnion, amniotic membrane transplant, amniotic membrane graft, amniotic membrane

Introduction

The placenta is usually discarded as a waste material following birth. However, amnion, the innermost layer of the placental sac can be harvested as a transplant material. AMT is useful clinically due to its unique structure, biocompatible composition and subsequent biological functions.[1,2] Amnion was first introduced into clinical medicine as a substrate for skin transplantation[3] and has since been adopted into a wide range of surgical applications including adhesion reduction,[4] restoration of hearing,[5] and replacing the vaginal[6] and urethral[7] mucous membranes.

The role of an AMT in an ophthalmic setting is usually to support damaged tissue, protect and shield defects from further degeneration or breakdown from external factors and to promote re-cellularisation.[1] This is possible due to a myriad of biological properties, including a lack of immunogenicity, thus reducing the risk of inciting an immune response.[8] AMT also preserves and supports stem cells[9] whilst inhibiting neoplastic,[10,11] inflammatory,[12,14] angiogenic and fibroblastic cells.[1,12] When applied in combination these biological properties support and facilitate wound healing.[8,15] Amnion has also been shown to improve pain management[16,17] in conjunction with delivering anti-microbial benefits.[18,20]

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The Historical Background of Amniotic Membrane Transplantation

In 1910, AMT was first utilised surgically as a skin graft substitute material.[3] The first ophthalmic usage was in the 1940s as a conjunctival substitute after removal of fibrotic tissue,[21] with good outcomes reported when compared with the widely used alternative of rabbit peritoneum. Its usage then fell out of favour, potentially as in this era only fresh amniotic membrane was available which was difficult to obtain, and carried a risk of bloodborne virus infection. By the early 1990s, alternative applications began to gain popularity amongst ophthalmologists for a second time. After different preservation methods were developed and refined, better storage and distribution techniques increased tissue accessibility.

The Wide-Ranging Properties of Amniotic Membrane

General Structure

Amnion is composed of five layers, usually between 20–500µm thick in total[22] (see Figure 1). It consists of an epithelial monolayer supported by a basement membrane, and an extracellular matrix (ECM) stromal layer, consisting of an acellular compact layer and sparsely populated fibroblast layer. The innermost layer, called the spongy layer, acts as the interface between the fibroblastic layer of the amnion and the reticular layer of the chorion.[23,26] By the second month of gestation, the mesenchymal cells separate from the epithelium by a layer of tissue containing loosely packed collagen fibrils and occasional fibroblastic cells. It is predominantly the collagen component of the mesenchymal layer that provides additional tensile strength.[8] Structural proteins such as laminin, fibronectin and collagens in the amnion ECM and basement membrane provide a scaffold with which cells can interact to promote epithelial regeneration.[27,29] At full term, a single layer of amniotic cells exists, firmly adherent to a mesenchymal layer usually six to eight cells in thickness.[30,31] Unusually it is avascular with no direct blood supply.[8,32,33] Although the exact role that AM performs in the homeostasis of amniotic fluid currently remains uncertain, it has an exceptional metabolical activity during pregnancy.[34] As it does not have a blood supply of its own, it derives its nutrition and oxygen supply from the surrounding chorionic fluid, amniotic fluid, and fetal surface blood vessels. Energy is derived primarily through anaerobic glycolysis pathways.[8]

Anti-Angiogenic, Anti-Inflammatory, Anti-Scarring and Anti-Fibrotic Factors

The tissue's anti-inflammatory properties have been well documented within the literature.[17,35,36] Although the exact mechanism of action is not yet understood, evidence points towards a range of properties that summate to this, such as the expression of anti-inflammatory and regulatory mediators coupled with the added ability to remove infiltrating inflammatory cells.

The amnion ECM complex contains protease inhibitors of heavy chain 1 of inter-α-trypsin and hyaluronan/pentraxin 3 (HC-HA/PTX3), which is thought to contribute to the anti-inflammatory, anti-scarring and anti-angiogenic therapeutic action.[37,39] In addition, inhibition of the ever recognised TGF-ß signal transduction within fibroblasts by mediators from the stromal layer provides additional anti-scarring properties by up-regulating matrix metalloproteinases,[12] with reduced numbers of fibroblasts shown in the cornea, limbus and conjunctiva after the application of AMT stromal matrix.[12] IL-10 is also present, which is known to reduce IL-6 and TNF alpha levels. This blocks the process of fibrosis formation by removing the activation of the profibrogenic cytokines. Authors have also shown the glycoprotein Lubricin to be present in AM, which is a naturally occurring boundary lubricant with anti-inflammatory, anti-adhesive, and anti-friction properties and this is likely to play a role in the wide-ranging functions of AMT.[40]

Fibrosis development is driven by the formation of myofibroblasts, which form following the activation of keratocytes, as a response to the disruption of the ocular

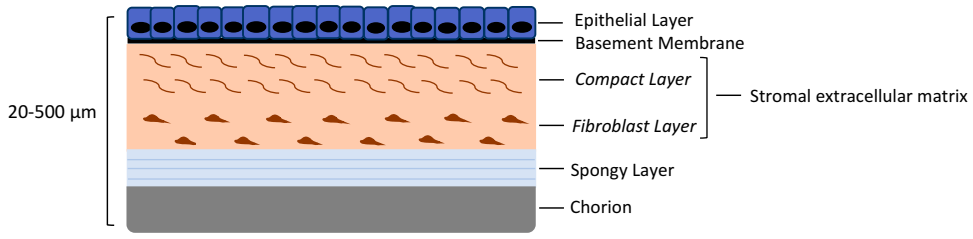

Figure 1 A diagram of the general structure of an amniotic membrane.

surface. The anti-fibrotic features of amniotic membrane act in a complementary fashion to the anti-inflammatory nature of the tissue.

Anti-Microbial Action

The antibacterial effects of both amnion and chorion are active against a range of bacteria, including Hemolytic streptococcus group A, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.[41,42] Several factors have been demonstrated to be present in amniotic fluid including bactricidin, beta-lysin, lysozyme, transferrin, and immunoglobulins. Progesterone hormones present in the amniotic fluid are also said to be bacteriostatic against some gram-positive organisms.[41,43]

Amnion secretes elafin (peptidase inhibitor 3) and secretory leukocyte proteinase inhibitor, both of which have antimicrobial action and are components of the innate immune system.[44] Amnion is also reported to have antiviral properties due to the analogue of a cystine proteinase inhibitor, cystatin E.[44]

Promotion of Epithelisation

Amnion's ECM and epithelial layers are interspersed with a complement of trophic components, in particular, epidermal growth factor, keratocyte growth factor and neurotrophic substances that support the wound healing properties of the matrix mentioned above. This complex interplay of healing components uniquely regulates and promotes regenerative healing.[45,46] The structure and especially the collagen composition of AM's basement membrane closely resemble that of the conjunctiva and cornea. This allows it to act as a substrate on which epithelial cells can easily replicate. AM is said to have multiple effects on the regenerating corneal epithelium, including facilitation of migrating epithelial cells[47,48] and reinforcing basal epithelial cell adhesion.[49] It also plays a role in the promotion cell differentiation[50,51] and blockade of apoptosis.[52,53] These features mean it can be useful in cases of non-healing or persistent epithelial defects, as will be discussed later on in this review.

Amniotic Membrane Procurement and Manufacture

Procurement of Amniotic Membrane

Amnion is procured from consenting mothers who are undergoing elective caesarean sections. All donors answer a lifestyle questionnaire, which is used to minimise the hazard of any transmissible diseases by removing potentially "high risk" donors. For all consenting donors, blood samples are extensively tested for any viral infections or disease markers to ensure the risk of transmission from transplantation is minimised.

The physical processing of amniotic membrane is conducted under sterile conditions and the tissue is washed using antibacterial and antifungal agents. Following this the chorion is usually removed prior to processing,[54,55] but this does vary between amnion manufacturers. Amnion is then preserved to allow long-term storage of the tissue; this can be conducted in multiple ways as discussed below.

Methods of Amniotic Membrane Preservation

It must be noted that all preservation methods cause some degree of compromise to integrity of the tissue.

Cryopreservation

Cryopreservation is the most common technique of preservation. Cryopreservation involves adding a storage medium to the tissue, such as Dulbecco's Modified Eagle Medium containing cryoprotectants such as glycerol, and freezing to −80°C. When cooled to these temperatures, degradation through unregulated enzymatic reactions is said to be limited. Some reports within the literature suggest that freezing may lead to ice crystal formation which potentially damages cellular integrity, and the thawing process prior to application may result in a loss of soluble proteins that are important to the wound healing process[55,56] such as the aforementioned angiogenic factors.[56]

Freeze-Drying/Lyophilisation

Freeze-drying involves cooling the amnion to −80°C and then a process of sublimation follows in order to remove the water from the tissue. Gamma irradiation is used in order to sterilise the tissue. This process can inflict similar ice crystal damage to the tissue as cryopreservation, but unlike cryopreserved products, these do not have cold chain storage logistical issues and do not require thawing prior to use.[57] Lyophilisation is a method that consists of removing water by a sublimation process. This results in the inhibition of chemical reactions that lead to adverse alteration of tissues.[57] Lyophilised tissue can then be stored at room temperature for long periods facilitating transportation, thus resolving the two main disadvantages of cryopreservation. Authors comparing cryopreserved and lyophilized samples have shown the presence of type IV collagen throughout the basement membranes, in both cryopreserved

and lyophilized samples.[57] Growth factors and total protein content were not significantly different in either preservation method, other than certain fibroblast growth factors were higher in cryopreserved samples.

Dehydration and Low-Temperature Vacuum Evaporation

Air or heat can be utilised to remove the moisture and dehydrate the tissue. Dehydrated amniotic membrane does not have the issues related to lyophilised or cryopreserved amnion, as it does not involve a step where the tissue is frozen. Gamma irradiation is used to sterilise the tissue.

This evaporation process uses a sugar protectant, such as trehalose,[58] before undergoing a low-temperature vacuum process to remove any water content from the tissue in a controlled process. Sugar protectants such as trehalose replace intracellular water during dehydration or freezing to form a glassy matrix, therefore preventing the major disruption of internal cell organelles, keeping the tissue viable.[56,59] Similar to dehydrated amnion, this process does not include any freezing steps throughout. Some authors suggest that this method provides a superior substrate compared to conventional cryopreserved AM,[56] although there are some conflicting reports.[54] In addition, this product is stable allowing it to be transported globally for use in clinical and military sectors.[56]

Application Methods

The application method for transplantation will depend on the aetiology of the disease. The depth and size of the wound and the area of the ocular or mucosal surface that has been affected, are all contributing factors. Publications available from within the literature have excellent images detailing the different forms of transplant methods.[60] In addition, please see Figure 2.

Inlay Transplantation (Graft)

Inlay transplantation involves the amnion being grafted into the damaged ocular surface after the defective tissue has been removed from the site. Amnion acts a replacement for the lost tissue. A graft is permanently incorporated and remodelled into the host corneal matrix over time. Dependent on the depth of the wound, single or multilayer grafts may be used. Amnion's ability to integrate into the corneal stroma is possible due to the formation of hemidesmosomes and desmosomes.[61] These keratinocytes provide both stability and anchorage, which allow for amnion to help improve the structural quality of the tissue.[62,63] Amnion is placed epithelial side up, as the membrane is able to act as a substrate for epithelial regeneration. Usually, a rim of epithelium is removed from the periphery of the stromal defect to ensure that no overlapping epithelium remains, reducing the chances of proliferation underneath the graft. This helps the epithelium to grow over the inlay graft, acting as a basement membrane.[60] At times, residual membrane may remain visible as wavy white lines or superficial "scar" tissue has been incorporated into the cornea.

Onlay Transplantation (Patch)

Onlay or "patch" amnion transplantation is the addition of the tissue epithelial side down over the periphery of a superficial wound.[8,55] The transplant acts as a temporary biological dressing, which is able to protect the wound by providing a physical barrier against: environmental damage; the formation of symblepharon and ankyloblepharon; and any further physical insult. Proteins are secreted from the amniotic membrane onto the ocular surface. These proteins limit fibrosis and scarring, allowing for an environment for effective wound healing. Onlay transplantations will remain on the surface of the eye until removed or the product self-degrades. The transplant is not intended to become integrated into the cornea.

Combinatorial (Sandwich) Transplantation

Combinatorial transplantation utilises both the inlay and onlay methodologies of transplantation. The graft provides structural integrity, whilst the patch allows for the protection of the graft.[1,8] Both of the transplants are able to deliver anti-inflammatory and pro-epithelisation factors.[61] The epithelium is expected to grow between the two layers[8] if the orientation of the graft (epithelial side up) and patch (epithelial side down) is used in this manner.

Ocular Surface Reconstruction: Graft

Corneal Ulceration and Persistent Epithelial Defects

AMT can be incorporated into the treatment of corneal ulcers by any of the methods of application mentioned above: i) Inlay, ii) Onlay, iii) Combinatorial Technique. The chosen method of application will depend on the depth of the ulceration, desired effect from AMT and surgeon preference.

Schuerch et al[64] 's retrospective analysis studying non-epithelialising corneal ulcers refractory to standard medical

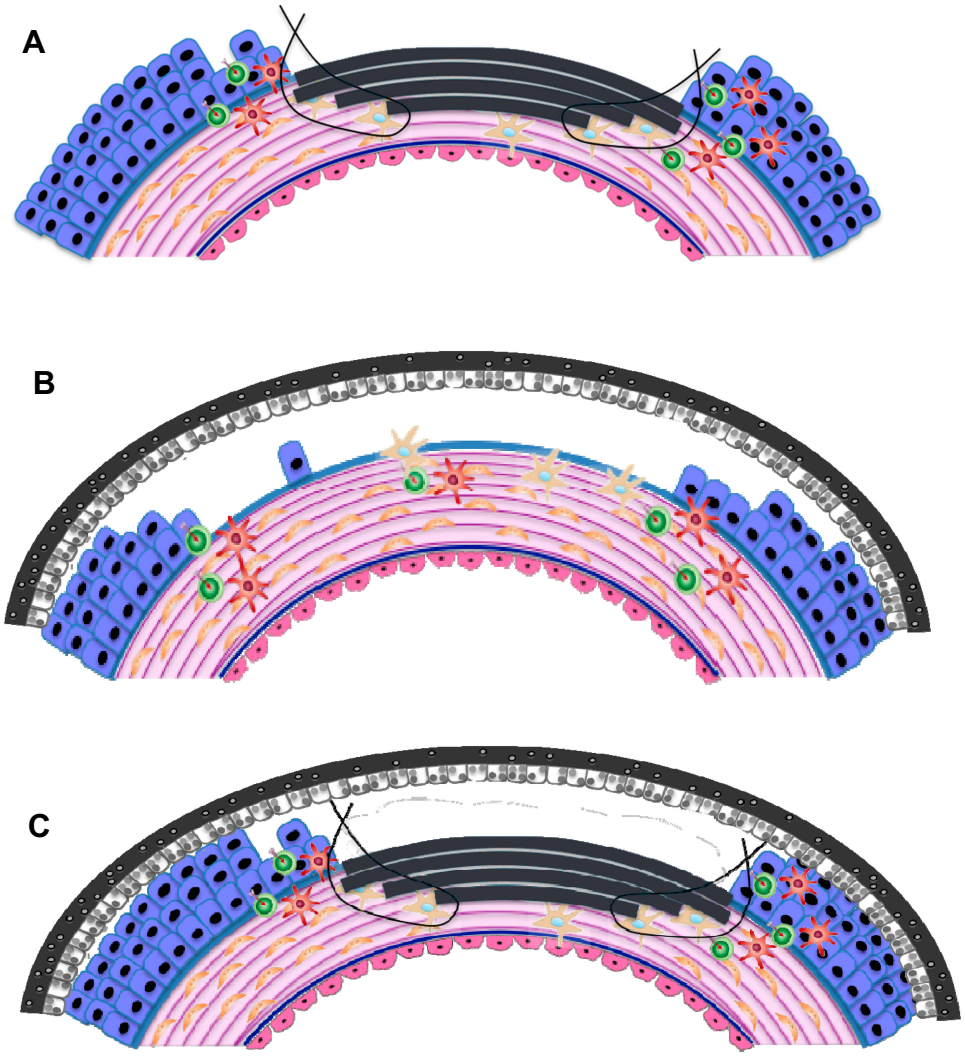

Figure 2 Schematic depicting potential applications and orientation of AMT (depicted in grey) on the ocular surface. (**A**) Inlay (graft) amnion transplantation. Epithelial-side-up: amnion replaces lost stromal tissue, up to the basement membrane. (**B**) Onlay (patch) transplantation where amnion is placed epithelial-side-down over the wound periphery as a temporary biological dressing. (**C**) Combinatorial/Sandwich AMT.

therapy included 149 eyes. It is important to note that a variety of ulcer aetiologies were included, with the majority being herpetic infections or patients that had undergone previous penetrating keratoplasty. In brief, an amniotic membrane was placed with the epithelium up within the corneal epithelial defect and fixed using nylon 10–0 interrupted sutures. A second amniotic membrane with the epithelium down was placed overlapping the first layer and fixed with a continuous nylon 10–0 suture to the conjunctiva. The mean duration from the diagnosis of the corneal ulcer to AMT was 42 (range 6–46 days).[64]

Ulcers due to bullous keratopathy, bacterial ulcers, herpetic ulcers and neurotrophic ulcers had the highest overall closure rates (79%, 80%, 85%, and 93%, respectively) and achieved epithelial closure mainly within the first 3 months after AMT. Of note, those ulcers related to rheumatic disease only epithelialised in 52.5% of cases.[64]

A meta-analysis by Lui et al[65] studied three different methodologies for the application of amniotic membrane in ulcer treatment (as mentioned above). The meta-analysis pooled 18 eligible studies and found the highest rate of epithelial healing was amongst the "sandwich application" group, although this technique resulted in the smallest visual improvement outcomes, making technique selection difficult.[65]

Tabatabaei et al[35] presented the largest of the trials, studying 99 eyes, all of which were diagnosed with bacterial keratitis. After 2–5 days of topical antibiotic treatment, AMT was performed in the treatment group and standard care was continued in both groups. The tissue transplantation was

deemed safe and showed better outcomes in regard to visual acuity at 6 months ($p<0.001$). Other studies show favourable outcomes in terms of pain management and epithelial healing when early AMT intervention (at 48 hours) is combined with topical steroid application (at 72 hours).[66,68]

Further evidence of AMT efficacy is detailed by the findings of Fuchsluger et al[69] and Khokhar et al,[70] the latter specifically examining the use of AMT in corneal ulcers relating to neurotrophic keratopathy in a randomised control trial. AMT was found to be as effective as conventional tarsorrhaphy or bandage contact lens placement for refractory neurotrophic corneal ulcers.

Similarly, other authors have shown that usage for symptomatic, painful bullous keratopathy can improve pain scores and epithelialisation,[71] although it has been suggested that given the ease and reduced cost of anterior stromal puncture techniques, these may be preferred in certain scenarios to AMT.[72] Similar findings are seen when used in conjunction with the surgical removal of band keratopathy.[73] It should be noted that these studies all have relatively small patient numbers and a variety of methodologies utilised amongst them, making direct comparison of outcomes difficult.

It is also worth noting that AMT can be used in eyes that have undergone penetrating keratoplasty to treat PEDs. Seventy percent of eyes showed successful epithelial closure within 4 weeks of AMT and, interestingly, the success rate appeared to be inversely proportional to the number of previous transplants.[74]

Limbal Stem Cell Deficiency (LSCD)

Progenitor stem cells for the conjunctiva and cornea have been shown to reside in the conjunctival fornices and limbal area, respectively, they then migrate onto the ocular surface and differentiate into daughter cells that continuously regenerate into the conjunctival and corneal surface epithelia. Corneal epithelial cells have been shown to migrate rapidly when limbal explants are placed on AM denuded of amniotic epithelial cells but with an intact basement membrane. Interestingly, they migrate relatively slowly when the amniotic epithelium is left intact, and at their slowest when they are grown on the stromal surface. Culturing limbal explants on an intact AM with devitalized epithelium has been shown to be conducive to the expansion of an epithelial phenotype that closely resembles limbal stem cells.[75]

Amnion can be used in conjunction with limbal stem cell transplantation (LSCT) for the treatment of LSCD. Two differing in-vivo expansion surgical procedures have been developed to tackle the issue regarding the conjunctival-cornea epithelial cell admixture that forms following LSCT procedures. Currently, sector sequential conjunctival epitheliectomy (SSCE) is used to mechanically remove any conjunctival epithelial cells which are migrating onto the cornea.[76] However, this procedure requires multiple clinic appointments following surgery and is unpleasant for patients. Both of the in-vivo stem cell expansion techniques aim to prevent conjunctival epithelial admixture contamination and therefore increase the success of LSCT.

Simple Limbal Epithelial Transplantation (SLET)

In 2012, Sangwan et al[77] reported a novel surgical technique for the treatment of limbal stem cell deficiency. Simple limbal epithelial transplantation technique involves harvesting limbal stem cells from a donor area of 2x2 mm from the superior limbus using sub-conjunctival dissection. In the recipient eye, a 360° peritomy is performed, and any vascular pannus removed. A human amnion graft was placed over the bare ocular surface and tucked under the conjunctiva, then secured in place with fibrin glue. The donor tissue was cut into small pieces and these explants placed epithelial side up on top of the amniotic membrane in a circular manner around the cornea. This technique has been further explored by a number of studies, such as Shanbhag et al[78] and Borroni et al.[79] Both these studies show SLET surgery to be an effective and safe surgical technique. One retrospective multicentre study using 68 eyes showed the success of SLET in 57 cases (83.8%) with a survival probability of greater than 80% at one-year post operatively.[80]

Amnion-Assisted Conjunctival Epithelial Redirection (ACER)

ACER utilises an amnion patch to redirect the conjunctival cells and allows for the corneal epithelial cells to regenerate with potentially less dilution from any invading conjunctival epithelium. Dua et al[81] reported the surgical procedure using a 360° peritomy, with donor conjunctivo-kerato-limbal grafts sutured at the six and twelve positions with an inlay amnion graft sutured into the wound bed. Following this, a large AMT is tucked under the edge of the recessed conjunctiva between the ends of the limbal explants. This outer AMT patch is held in place through the use of fibrin glue and is large enough to cover the cornea and adjacent sclera.

The aim is to ensure the conjunctival epithelial cells are redirected to only grow over the amnion, meaning the conjunctival cells are theoretically prevented from mixing with the limbal explant-derived epithelial cells; conjunctival

cells expand over the outer amnion, whilst the regeneration of the corneal epithelial surface is nurtured beneath, unhindered. Authors have shown that patients undergoing ACER require less outpatient appointments and can hopefully avoid the painful SSCE procedures.[81]

Cultivated Stem Cell Transplantation

In situations where there is significant bilateral limbal damage without residual LSCs, or if there is not enough healthy limbal tissue in an unaffected eye to harvest sufficient LSCs, one must consider the transplantation of ex vivo cultured and expanded cells. The usual source of cells is usually human limbal[82] (CLET) or oral epithelium[83] (COMET). The cells can be taken either from the patient (autologous), a living relative or cadaver (allogenic). The reported advantage is that less than 1mm^2 of donor tissue is required for transplantation.[84,85]

AM is used as described as in other conditions to act as a basement membrane scaffold for cultured cells and as a cell carrier substrate in CLET or COMET procedures. Although both de-epithelialized and intact AM has been used, de-epithelialized AM is said to be superior to intact AM because as it preserves the properties of LSCs whilst promoting the migration of LSCs.[86]

Indeed, there is some evidence that using intact AM allows some LSCs to undergo epithelial–mesenchymal transition and invade the limbal stroma.[87,88] AM has been shown to preserve limbal epithelial cells in an undifferentiated state, maintaining its naturally occurring slow cycling.[88,91] Evidence suggests that AM provides a unique stromal microenvironment beneficial for LSC survival and expansion, whilst acting as an IL-1 antagonist to prevent cell apoptosis.[92] Many authors show CLET to be successful, with grafts remaining stable one year post operatively with low rejection rates.[93,100]

It is important to note however that many variables exist within the different reported studies, with success rates depending on confounding factors such as age, donor source, and cell quality.[85,88,98,101] Fibrin glue techniques have been shown to be just as effective, although AM has wider accessibility in many countries.[88]

COMET procedures have been shown to have good outcomes two years post operatively.[102,108] There currently exists no comparative trial comparing CLET and COMET procedures so it is difficult to directly compare the clinical outcomes from different studies, although some reports show substrate-free oral mucosal cell sheet transplants to have higher success rates.[88,106,107] Some authors would dispute this as although a stable phenotype is achieved post operatively with this method, CK12 the corneal epithelial marker and the PAX6 eye specific transcription factor, is not expressed in transplanted oral mucosal cells,[109] indicating that they do not undergo a true cellular transdifferentiation.[88]

Glaucoma

AMT may be used in conjunction with bleb revision for persistent leaks following trabeculectomy surgery or aqueous shunt revision. The amnion can be grafted onto the epithelial surface over the bare sclera to replace lost or damaged conjunctiva.

In 2012, Bochmann et al[110] published a meta-analysis examining various interventions involving AMT for late-onset bleb leaks. The meta-analysis searched for a range of techniques but only a single study by Budenz et al[111] met the required criteria. In this review, amnion was not shown as an effective alternative to conjunctival advancement for reducing bleb leaks. Other reports from the literature agree with this finding.[112] Authors also report success from using AMT to repair bleb leaks[113,115] across a range of post operative time points, incorporating both early and late post operative bleb leaks.

Published meta-analysis data[116] suggest that that amnion usage intraoperatively was effective at reducing IOP when compared with trabeculectomy alone. Amnion application during trabeculectomy is the most well-documented indication in glaucoma patients, and Sheha et al[117] report that amnion combined with mitomycin-c increased the surgical success rate of secondary trabeculectomies in refractory surgical cases (93.7%). The authors describe the technique in their publication, using amnion sutured stromal side down under the trabeculectomy flap. AMT augmented trabeculectomies have been shown to increase success in primary trabeculectomy surgery.[117] There are reports to suggest it can be used as a patch graft during drainage device insertion, providing good tectonic support and allowing direct visualisation of the tube,[118] despite not improving clinical success.[119,120] AMT has also been used to repair conjunctival defects causing bleb leaks and hypotony following GDDs and MIGS device insertion.[121,122]

Neoplasia

Amnion is used to act as a substrate for conjunctival migration and reconstruction following excision of both malignant and benign tumours. Qin et al[123] studied 24 eyes with intraepithelial epithelioma in a randomised trial,

assessing whether a concave-convex (sandwich technique) AMT had better post operative outcomes when compared with standard AMT. Epithelialisation rates were similar in each group, although the tumour recurrence rate at one year was significantly lower in the concave-convex AMT treated group ($p<0.05$).

Similarly, Agraval et al[124] report improved local surgical outcomes following conjunctival melanoma excision combined with AMT improved conjunctival healing, resulting in minimal symblepharon, granuloma or scar formation. Other authors have also commented that the transparency of amniotic membrane allows for monitoring of tumour recurrence in deeper tissues, and may also provide a superior cosmesis when compared with thicker (for example, buccal) mucous membrane grafts.[125]

Although the anti-neoplastic mechanism of amnion is not well understood,[10] it is attributed to the secretion of anti-angiogenic, pro-apoptotic and immune-modulatory factors.[11,126,129] Amnion contains interleukins (IL-2, IL-3 and IL-4) and can express cytotoxic cytokines, these are known to enhance the cytotoxicity of natural killer cells, which are able to attack mitotic cancer cells.[11]

Oculoplastics

As mentioned briefly above, AMT has been suggested as a potential alternative to mucosal membrane transplantation in lid revision, orbital linings or symblepharolysis. Reports exist describing forniceal reconstruction utilising AM,[130,132] with other authors stating that AM can be a viable alternative to established mucous membrane grafting, causing less patient morbidity, faster recovery times and better prosthesis fitting in anophthalmic-contracted sockets.[132,134] It can also be particularly useful if a patient has an insufficient amount of mucosal membrane available, producing effective results when combined with antimetabolites such as MMC.[135]

Cicatricial eyelid abnormalities can be difficult to treat as tissue loss needs to be overcome. Good outcomes have been reported using a gray line lid split procedure with vertical anterior lamella repositioning, in patients with moderate to severe cicatricial entropion.[136] AMT was used to cover the bare tarsus up to the lid margin and secured with 7–0 Vicryl sutures. Good cosmetic outcomes were reported, with rapid epithelialisation of the previously bare tarsus. No lashes were abrading the globe in 88% of cases 12 months post operatively. In addition, it has been used following conjunctival reconstruction in advanced mucous membrane pemphigoid. After scar tissue was carefully excised, AMT was placed over the cornea, bulbar and tarsal conjunctiva, and secured with 8–0 Vicryl sutures to the conjunctival edges and the deep fornices with double-armed 6–0 silk sutures.[137] Immunosuppressive systemic therapy and topical steroids were administered to all patients for at least 6 months following surgery. Post operatively the forniceal depth was significantly improved up to 28 weeks following surgery, although the effect was said to deteriorate over time.[137] Comparable outcomes have been shown in Stevens–Johnson Syndrome patients, using a similar surgical technique.[138]

There are reports of authors using AMT in conjunction with an Hughes procedure to restore tissue loss after excision of conjunctival melanoma involving the fornix. In addition, despite being a rare condition, AMT has been used effectively to manage cases of cryptophthalmos. One centre reports surgical success in terms of both acceptable functional and cosmetic outcomes in 20 of 24 patients receiving repair procedures from a single centre over a 12-year period. The authors conclude that a one-stage reconstruction of both the eyelid and fornix with scleral and amniotic grafts is an effective strategy to correct abortive cryptophthalmos.[139]

Pterygium

The use of AMT in pterygium is one of the most well-documented indications. However, it is also one of the most controversial areas for its usage, with the current treatment standard for pterygium excision being a repair of the defect with a conjunctival autograft. A Cochrane systematic meta-analysis review[140] compared the use of autograft with AMT following pterygium excision. Conjunctival autograft was associated with a lower risk of recurrence at six months' following surgery than AMT. Participants with recurrent pterygia, in particular, were found to have a lower risk of recurrence when they received conjunctival autografts. The obvious advantage AMT has in this setting is no donor site defect is created. There are few studies comparing the two techniques with respect to visual acuity outcomes, and no studies report on vision-related quality of life or direct or indirect costs.[140] Unfortunately, an insufficient number of studies have used adjunctive mitomycin C to be able to draw conclusions on the effects on pterygium recurrence following conjunctival autograft or AMT.

Prajna et al[141] studied double pterygium excision (combined nasal and temporal) comparing autograft to AMT. Again, the autograft group was shown to have statistically significant lower recurrence rates than AMT, which has also been reported by other studies.[142]

Strabismus

Cryopreserved AMT has been utilised during strabismus surgery either to cover defects or to reduce muscle fibrosis and adhesion. Kassem et al[143] reported a randomised controlled study of revisional strabismus patients, where one group received amnion wrapped around the rectus muscle. Although no adverse events were reported from the amnion group, the authors concluded that no clinical benefits were shown from this technique. The patients in the treatment group were followed up on a long-term basis (up to 85 months) in a further study,[144] with the conclusion that the effect of amniotic membrane transplantation on the success of strabismus reoperations was moderate in terms of ocular alignment, but more pronounced in terms of ocular motility range, likely due to a reduction in fibrosis and subsequent muscle restriction.[143,145]

There are also reports of AMT usage in complex strabismus cases, with successful outcomes in patients that suffer diplopia following vitreoretinal surgery.[146] It has also been used with adjunctive MMC in cases with congenital fibrosis of the extraocular muscles.[147]

Fresh human AMT has been used successfully in secondary strabismus cases, where authors report the placement of two AM sheets, one between the operated muscle and tenon's capsule, with the stromal side facing tenon's capsule. The other AMT is placed between the muscle and sclera with the stroma facing the sclera. Areas of bare sclera were covered using AMT with the stroma against the sclera. The authors concluded that AMT placement around the extraocular muscle improved ductions and reduced the residual deviation angle due to less post operative scar formation.[148] This was attributed to the prevention of adhesions by the AMT as it formed a temporary biological barrier between the layers of perimuscular connective tissue. Despite these outcomes, the use of fresh AMT is discouraged due to the risk of communicable diseases, which is significantly reduced in other forms of AMT. Interestingly, dried AMT has resulted in poor outcomes in strabismus cases[145,149,150] apart from one case report.[151]

The orientation of AMT varies throughout these studies and conclusions are hard to draw from the outcomes. One histopathological study using rabbits compared three groups; one with stroma orientated towards the muscle, another with the epithelium towards the muscle, and one with folded AM where the epithelium was in contact with muscle and sclera. The authors concluded that any AMT orientation was effective in preventing adhesions, with no advantage seen in either group.[152] Although there is limited evidence within the literature to support a sutured or sutureless technique, one review article recommends the use of a sutureless technique as it induces less of an inflammatory reaction and results in a shorter operating time.[145]

Ocular Surface Wound Healing: Patch

Chemical/Thermal Injury

Amnion has a range of biological properties that make it attractive for use in the treatment of chemical burns, such as its epitheliotrophic; anti-inflammatory and anti-neovascular effects.[153] As well as offering increased oxygen permeation and reducing mechanical epithelial trauma from eyelid friction,[8] as previously discussed, it can reduce pain whilst increasing patient comfort.[8,154]

As mentioned above, AMT can be utilised in LSCD, which can occur as a sequelae of chemical and thermal injuries. However, AMT has a role to play in the treatment of the acute phase of these injuries. Tandon et al[36] presented the first randomised controlled trial, where amnion was transplanted epithelial side down within the first seven days of injury for chemical injuries of Roper Hall grading II–IV.[155] The study recruited 100 patients, split between moderate (grade II–III) and severe (grade IV) burns. AMT significantly increased the rate of epithelial healing in the moderate burns patients ($p = 0.0004$). However, no difference was seen in the secondary long-term outcome measures, such as final visual acuity, symblepharon formation, corneal clarity or neovascularisation. Patients with severe burns showed no improved rate of epithelial healing. In 2012, Clare et al[156] published a meta-analysis, which analysed only the Tandon et al paper as this was the only available randomised control trial. It concluded that the evidence for amnion use in chemical burns is equivocal.

Sharma et al[17] compared three treatment groups: standard care (SC) alone, SC with amniotic membrane transplantation and SC with umbilical cord serum. Both the umbilical cord serum group and amniotic membrane transplantation group promoted epithelisation faster than SC. However, at three months' there was no difference between the three groups in terms of visual outcome, symblepharon formation, tear film status, and lid abnormalities. The AMT and serum group saw a reduction of pain

for patients at day seven of treatment, although the serum group had the largest reduction in pain scores at all measured time-points.

Most recently, Eslani et al[157] enrolled 60 eyes with grade IV Roper Hall chemical injuries. Patients were randomised to receive medical management or AMT in conjunction with medical management. No significant difference was found between the rates of corneal epithelisation between the two groups (p=0.610), with the authors concluding that medical therapy with AMT does not accelerate corneal epithelialisation or improve final visual acuity in patients with severe chemical injuries. Although the authors suggest that in milder cases AMT may be beneficial based on the outcomes of other studies, it may not actually be a necessary treatment. It is suggested that the LSC function may be so poor in these severe injuries that the anti-inflammatory properties of AMT are not able to overcome the extensive damage. Moreover, its anti-angiogenic effect may reduce the effect of the recovering LSCs.

The current data suggests that the success of amnion transplantation in chemical burns is dependent on the severity of the burn, especially when considering the rate of epithelisation. However, other outcome measures, such as reduction of pain and inflammation, can be improved with amnion usage, with most of the studies indicating the need to apply the AMT within the first week of any injury.

Dry Eye Disease (DED)

The DEWS II report[158] proposes the use of AMT for use in severe dry eye, as a 'step 4' therapeutic intervention. Reports from within the literature suggest that sutureless AMT (PROKERA®) can result in a sustained symptomatic improvement for four months in dry eye subjects when worn for approximately five days on average,[159] demonstrating reduced corneal and conjunctival staining and improved visual acuity. Other studies show similar findings.[160,161] One retrospective review by Macdonald et al[162] applied AMT for 5.4 (±2.8 days) on average with a sustained reduction in the DED marker (3.25±0.5 to 1.47±0.6) three months post operatively with improvement in both patient reported symptoms and clinical signs. Finally, other authors report how corneal nerve density can be significantly improved following AMT application,[163] potentially accelerating the recovery of the ocular surface health in patients with DED.

Cicatrising Conjunctivitis – Steven's Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN), Graft versus Host Disease and Pemphigoid

The only published randomised controlled trial examining AMT in SJS patients, comparing medical management and AMT with solely medical management, is by Sharma et al.[154] In terms of outcomes, the AMT groups showed significantly improved visual outcomes, longer tear break up times and improved Schirmer test outcomes. Importantly, no case in the AMT group demonstrated corneal haze, limbal stem cell deficiency, symblepharon, ankyloblepharon, or lid-related complications. Other authors have shown amnion to be safe and effective in paediatric populations with similar conditions.[164]

Ma et al[165] published a novel technique for amnion application in SJS and TEN patients, which involved the use of 10cm x 5cm amnion rectangle combined with a custom-made forniceal ring to provide coverage of the full mucosal surface and eyelids. Amnion is placed on the upper eyelid and secured with two nylon sutures, laid across the ocular surface, and the forniceal ring is placed onto the eye. The technique minimises the manipulation of ocular tissues and decreases surgical time.

Following Ma's study, Shanbhag et al[166] published data using cyanoacrylate glue instead of suturing the AMT into position, making it easier to perform at a patient's bedside, providing clinicians with the potential to avoid the risk of visiting theatre with a medically unstable patient.

Recurrent Corneal Erosion (RCE)

RCE has been highlighted as a therapeutic indication in which amniotic membrane could be utilised. In a case series by Huang et al,[167] 11 eyes of 9 patients, each treated with epithelial debridement followed by sutureless AMT. During the follow-up period (average 12 months), only one eye suffered symptoms of recurrent erosions. A comprehensive review by Miller et al[168] in 2019 listed amnion as an effective treatment for acute RCE attacks and suggested its use once conservative treatment of topical lubrication and punctal occlusion has failed, instead of inserting a bandage contact lens.

Vitreoretinal – Amnion Plugs

Following Pars Plana Vitrectomy: Macular Holes and Tears

Amnion plugs can be implanted in the subretinal space with the chorion layer facing the RPE in an attempt to close

macular holes.[169] Evidence suggests that human retinal pigmented epithelial cells seed over amnion within 24 hours of implantation. Moreover, these cells maintain epithelial features and can proliferate over epithelium-free amnion, resulting in a tight monolayer with well-defined intercellular and cell–substrate interactions, secreting several growth factors important for maintaining retinal homeostasis.[170] Recently, Caporossi et al, 2019[169] studied 16 eyes of 16 patients with recurrent macular holes in a prospective case series of highly myopic eyes. All patients underwent a 23-gauge par plana vitrectomy with AMT implantation, with either 20% SF6 or air tamponade. Mean post operative BCVA was 0.94±0.24 logMAR, which improved to 0.67±0.26 logMAR post operatively . All patients experienced complete closure anatomically, although one hole reopened, requiring a repeat amniotic membrane implant two weeks post operatively.

Similarly, Caporossi et al[171] presented a case series of two eyes of two patients with retinal detachment and large macular tears. Pars plana vitrectomy was performed in conjunction with an AMT, and standard silicone oil tamponade. For both patients, BCVA improved from light perception to 1.3 logMAR. At three months, both patients' optical coherence tomography showed neuroretinal ingrowth over the amniotic membrane plug.[171]

The use of amnion in vitreoretinal surgery is a novel and innovative technique that requires greater understanding and research but certainly appears encouraging.

Potential Risks of Amniotic Membrane Transplantation

As amnion is an allogenic tissue from a single donor, there will be an implicit risk of acquiring infectious diseases from its usage. Adequate donor screening, testing, handling, processing and storage, should be employed in order to minimize this risk. Legislation should stipulate that HIV, hepatitis B, C, and HTLV tests are undertaken on the donor serum at the time of membrane procurement, with a repeated HIV test 6 months later to cover the latent infectious period of the virus. In order to safely achieve this, the membrane is quarantined for 6 months. Additionally, as with any human-derived product, the risk of prion infection must also be considered. Very few complications have been reported despite AMT's use in a wide range of clinical applications. Hypopyon formation has been reported in one case following repeated applications to a neurotrophic ulcer.[172] This was resolved with topical steroid treatment. Haemotomas may form post operatively, which may be need to be drained if they cause discomfort or dislodge the transplant. Granulomas can also form around sutured membranes, and dislocated membranes can be frustrating. The authors also report persistent subepithelial membranes that are thought to be from thicker membranes harvested near the umbilical cord. This can reduce the visual potential if they reside on the visual axis.[8]

Conclusion

We have shown the wide-ranging applications of AMT that exist within ophthalmic settings, alongside a discussion of the relevant literature. We hope this review gives the reader greater understanding of the clinical scenarios in which AMT may provide benefit. Given its widespread use, there are likely to be scenarios where usage will be beneficial, but similarly, there will be times when it is not required. Our understanding of AMT is not yet complete, but it is improving. It is clear that AMT is beneficial in some conditions, and, as discussed above, offers a good option in some challenging situations. With time, our understanding will improve, along with clarifying the clinical scenarios for which it is required.

Abbreviations

AM, amniotic membrane; AMT, amniotic membrane transplant; LSC, limbal stem cell; LSCD, limbal stem cell deficiency; SLET, simple limbal epithelial transplantation; CLET, cultivated limbal epithelial transplantation; COMET, cultivated oral mucosal epithelial transplantation; ACER, amnion-assisted conjunctival epithelial redirection; SSCE, sector sequential conjunctival epitheliectomy.

Disclosure

The author reports no conflicts of interest in this work.

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