Amniotic membrane transplantation

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In 1910 Davis was the first to report the use of fetal membranes as surgical material in skin transplantation. Since then the use of amniotic membrane in surgery has been expanded.\(^1\) It is now utilised as a biological dressing for burned skin, skin wounds, and chronic ulcers of the leg.\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\) It is now used as an adjunctive tissue in surgical reconstruction of artificial vagina,\(^8\)\(^9\)\(^10\)\(^11\)\(^12\) and for repairing omphalocoeles.\(^13\)\(^14\)\(^15\) It has also been used to prevent tissue adhesion in surgical procedures of the abdomen, head, and pelvis.\(^16\)\(^17\)\(^18\) In the 1940s several authors reported the beneficial role of amniotic membrane in treating a variety of ocular surface disorders.\(^19\)\(^20\)\(^21\)\(^22\) However, its use was abandoned for decades until recently, when it was reintroduced to ophthalmologists. Several studies have addressed this subject and the scope of the application of amniotic membrane transplantation (AMT) in the management of ocular surface disorders is ever increasing.

Certain characteristics make the amniotic membrane ideally suited to its application in ocular surface reconstruction. It can be easily obtained and its availability is nearly unlimited. The tissue can be preserved at \(-80^\circ\text{C}\) for several months, allowing sufficient time to plan surgery or consider a trial of other options. Amniotic membrane does not express HLA-A, B, or DR antigens and hence immunological rejection after its transplantation does not occur.\(^23\)\(^24\) It is also believed to have antimicrobial properties, reducing the risks of postoperative infection.\(^25\) Antifibroblastic activity\(^26\)\(^27\)\(^28\)\(^29\) and cell migration/growth promoting activity\(^30\)\(^31\) have also been demonstrated with regard to the amniotic membrane.

The purpose of this paper is to review the characteristics of amniotic membrane that make it potentially useful to treat ocular surface abnormalities and to discuss the current indications, the surgical technique, and the outcome of AMT.

Histology and physiology

Mammalian embryos lie within a fluid filled sac (fetal membranes) that arises from extraembryonic tissues. At full term of gestation, the fetal membranes are composed of two principal layers. The outer layer or chorion which forms the outer aspect of the sac and is in contact with maternal cells. It consists of compressed trophoblastic tissue of chorion laeve and mesenchymal tissue. The inner layer or amniotic membrane consists of a single layer of ectodermally derived columnar cells firmly fixed to an underlying layer of mesenchyme which contains large amounts of collagen.\(^32\)\(^33\) The amniotic membrane is bathed by amniotic fluid. According to Shimazaki et al,\(^34\) the epithelium of the amniotic membrane survives for up to 70 days after preservation. We have noted that after freezing the amniotic membrane at \(-70^\circ\text{C}\) for 6 months to a year the epithelial cells appear very vacuolated but remain attached to the underlying basement membrane and mesenchyme (unpublished data).

The apical surface of amniotic cells have many microvilli (Fig 1). At the base, cell processes or pedicels extend into the basement membrane in podocyte fashion. The basal cell processes have a hemidesmosome type of attachment to the basal membrane with tonofilaments, and the subjacent basement membrane substance is partly amorphous and partly microfibrillar. The cytoplasm contains many pinocytic vesicles, abundant organelles including cisternal endoplasmic reticulum, and Golgi apparatus. The nucleus has a very irregular configuration, with a number of indentations of the nuclear membrane. The nucleolus is often large and homogeneous suggesting nucleolar activity. Overall, the ultrastructure of the epithelium suggests that the amnion has multiple specialised functions. It has been specifically adapted to perform three major functions—as a covering epithelium, as an active secretory epithelium, and for intense intercellular and transcellular transport.\(^35\)\(^36\)

Amniotic membrane in ophthalmology

MECHANISM OF ACTION

Promoter of epithelialisation

The presence of a normal substrate in the cornea is essential for normal proliferation and differentiation of epithelial cells. Basement membrane facilitates migration of epithelial cells;\(^37\)\(^38\) it also reinforces adhesion of basal epithelial cells;\(^39\)\(^40\) promotes epithelial differentiation,\(^41\)\(^42\) and prevents epithelial apoptosis.\(^43\) The amniotic membrane, by serving as a “transplanted basement membrane”, acts as a new healthy substrate suitable for proper epithelialisation. Additionally, the amniotic membrane produces various growth factors such as basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor \(\beta\), that can stimulate epithelialisation.\(^44\)\(^45\) However, it has been demonstrated that cryopreservation of amnion results in a decrease of growth factors.\(^46\) Amniotic membrane also inhibits protease activity.\(^47\)\(^48\) It has also been shown that in some instances the amniotic membrane, rather than providing a substrate, acts as a “bandage contact lens” allowing epithelialisation to occur under its cover.\(^49\)

Inhibitor of fibrosis

Several factors are involved in the antifibrotic effect of the amniotic membrane.\(^50\)\(^51\)\(^52\) It has been shown that amniotic membrane induces a downregulation of transforming growth factor \(\beta\) signalling, responsible for fibroblastic activation in wound healing.\(^53\) The amniotic membrane may also function as an anatomical barrier, keeping the potentially adhesive surfaces apart. The stroma of the amniotic membrane is normally avascular and is believed to inhibit the incursion of new vessels.

Indications for AMT in ophthalmology

Amniotic membrane transplantation has been successfully used in patients with persistent epithelial defects unresponsive to medical treatment,\(^54\)\(^55\) and as an alternative to conjunctival flaps, botulinum toxin injection, or tarsor-
The frequency of success in two recent series was 10 of 11 cases and four of five cases, respectively. In our experience, the amniotic membrane, by virtue of its transparency, allows the patient navigational vision and is particularly useful if the affected eye is the better seeing eye. The use of more than one layer may be effective in covering ulcers with substantial stromal depth. However, the use of AMT as a tectonic procedure in cases with impending or recent perforation appears to be unsatisfactory, and failure was reported in five consecutive cases.

Amniotic membrane has been used as an alternative to conjunctival autograft during the removal of pterygia. The recurrence rate of pterygium after AMT (10.9% for primary pterygia) was lower than the bare sclera technique (45%), but higher than autologous conjunctival graft (2.6%). Multiple surgical approaches have been used to treat pterygium. Although conjunctival autograft is considered to be the most efficient, AMT appears to be a reasonable option in cases with diffuse conjunctival involvement and patients in whom the bulbar conjunctiva must be preserved for a prospective glaucoma filtering procedure.

AMT has been successfully used in the treatment of recurrent pterygium associated with severe symblepharon and diplopia. In Shimazaki’s series, all four patients had a favourable functional and anatomical outcome after AMT. Similarly, AMT has been used successfully in 13 of 16 eyes in the reconstruction of conjunctival defects created during surgical removal of large conjunctival lesions.

Corneal stem cell deficiency is associated with conjunctivalisation of the cornea and can be complicated with persistent epithelial defects, vascularisation, scarring, calcification, ulceration, melting, and perforation of the cornea. Patients with these abnormalities are poor candidates for conventional corneal transplantation. Lamellar or penetrating keratoplasty provides only a temporary replacement of the host’s corneal epithelium and does not permanently restore limbal function. In cases with diffuse corneal stem cell deficiency, limbal transplantation (allo or auto) is now considered essential for corneal surface reconstruction. AMT combined with limbal transplantation has been successfully used in patients with diffuse limbal stem cell deficiency and severe ocular surface disease, including Stevens–Johnson syndrome, advanced ocular cicatrical pemphigoid, chemical and thermal burns. Alternatively, autologous limbal-corneal epithelium can be cultured on amniotic membrane and used for corneal surface reconstruction.

Notwithstanding the encouraging and successful results reported thus far it is important to caution against the overenthusiasm in the use of amniotic membranes that is beginning to emerge of late. The beneficial effect of amniotic membrane in the management of ocular surface disorders has not always been validated with controlled clinical trials. In some series the favourable outcomes could well be attributed to concurrent surgical procedures. Shimazaki et al stated that “we do not know exactly in which case the amniotic membrane should be used and how much the current procedure (AMT and limbal transplantation) is superior to the simple limbal autograft transplantation.” Similarly, Tseng et al recently reported successful management of patients with sector limbal stem cell deficiency treated with removal of conjunctiva-like epithelium from the corneal surface combined with AMT. They did not, however, have any controls, where amniotic membrane was not used. In a similar group of patients Dua and Dua et al also reported excellent outcome following removal of conjunctiva-like epithelium, without AMT, suggesting that AMT is probably not required in such patients. Following observations on the healing of corneal epithelial defects involving the limbus, Dua and Forrester had observed the migration of conjunctival epithelium on to the corneal surface and reported that mechanical debri-dement could prevent the manifestation of conjunctivalisation of the cornea (partial stem cell deficiency).

Fujishima et al recently used amniotic membrane in guarded filtration procedures supplemented with mitomycin C to inhibit scarring and promote filtration. Amniotic membrane was placed underneath the scleral flap. In this...
small series the mid term outcome of trabeculectomy was satisfactory in 13 of 14 eyes. After filtering operations the extracocular changes (that is, subconjunctival fibrosis) account for the majority of failures. Anti-fibrotic agents, mitomycin C and 5-fluorouracil, are currently used to improve the chances of success, although the complication rate also rises. A controlled clinical trial will be needed to evaluate whether amniotic membrane helps to improve the outcome of filtration procedures.

Bleb leaks after filtration surgery can be associated with hypotony, shallow flat anterior chamber, and choroidal detachment and may increase the chances for bleb infection and subsequent endophthalmitis. Leaking filtering blebs usually require prompt treatment. Therapeutic options include bandage contact lens, Simmons’ shell, injection of autologous blood, cryopexy, thermal Nd:YAG laser, cyanoacrylate glue, fibrin tissue glue, and surgical revision. Recently reported favourable use of human AMT for revision of leaking blebs after glaucoma surgery in five patients. AMT compared favourably with conjunctival advancement.

Anecdotally, AMT has been successfully used in three patients to treat myopic regression with corneal opacity after photorefractive keratectomy (PRK) in high myopia. Excessive corneal haze and myopic regression are associated with excessive healing response, which might be inhibited by amniotic membrane. In rabbits the corneal haze was reduced by AMT in excimer laser photoablation. Safety criteria applied to organ transplantation should be applied even more strictly to tissue transplantation such as amniotic membrane.

Others will use a suture, with the knot as the marker or intermediate to the recipient and from the recipient back to the donor. A controlled clinical trial will be needed to evaluate whether amniotic membrane helps to improve the outcome of filtration procedures.

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Figure 3 Surgical technique. The amniotic membrane is sutured to perilimbal episclera and to the edge of the conjunctiva (after peritomy) covering the whole corneal surface.

indelible marker pen, to mark one side of the membrane. We have developed a method that we find useful. After spreading the membrane on the corneal surface we apply the tips of a blunt fine forceps to one surface of membrane and pinch lightly with the forceps and lift. A fine strand of “vitreous-like” substance can usually be drawn up from the mesenchymal but not the epithelial (basement membrane) side of the amniotic membrane.

The amniotic membrane is spread on to surface of the eye and cut to appropriate size and shape, keeping the final piece slightly larger than the size of the defect to be covered. It is usually sutured to the cornea with 10-0 nylon sutures and to the episclera/conjunctiva with 9-0 vicryl sutures. After surgery a bandage contact lens is put in place, and topical steroids and antibiotics are used. Sutures can be removed at 3 weeks. The membrane stains with fluorescein stain and like the cornea, attracts ciprofloxacin deposits, if the drug is used topically, with fluorescein stain and like the cornea, attracts in place, and topical steroids and antibiotics are used.

Vicryl sutures. After surgery a bandage contact lens is put on. It is usually sutured to the corneal surface we apply indelible marker pen, to mark one side of the membrane. We have developed a method that we find useful. After spreading the membrane on the corneal surface we apply the tips of a blunt fine forceps to one surface of membrane and pinch lightly with the forceps and lift. A fine strand of “vitreous-like” substance can usually be drawn up from the mesenchymal but not the epithelial (basement membrane) side of the amniotic membrane.

In cases of persistent epithelial defects, the base of the ulcer and loose epithelium adjacent to the edge of the ulcer are debrided before applying the membrane. The amniotic membrane is trimmed and fitted to cover the epithelial defect and sutured to the edge of the defect (Fig 2). If the epithelial defect is large, a 360 degree peritomy is done and the membrane sutured to cover the cornea from limbus to limbus (Fig 3). In pterygium or symblepharon surgery, the membrane sutured to cover the cornea from limbus to conjunctiva. It is also e

Figure 4 Surgical technique. The amniotic membrane can be used to cover a conjunctival defect after releasing adhesions during symblepharon surgery, and in a similar manner (nasally or temporally) after excision of pterygium.

be related to collagenases present on the ocular surface as, in our experience, it occurs more often in cases with intense inflammation (unpublished data). The amniotic membrane will not remain attached to the ocular surface if the mesenchymal surface is not facing the host.

Summary

Amniotic membrane has unique properties that can be helpful to treat different corneal surface diseases. AMT is useful in promoting normal epithelialisation of cornea and conjunctiva. It is also effective in preventing excessive fibrosis during ocular surface reconstruction. Future possible indications are being investigated. Because of the potential risk of infection strict safety criteria must be applied. The procedure is still evolving and, not surprisingly, is being tried in a variety of diverse conditions. Some of these may prove inappropriate. Controlled clinical trials will be needed to establish the role of AMT in ocular surface reconstruction.

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