Variations in amniotic membrane: relevance for clinical applications

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Br J Ophthalmol 2010 94: 963-964
doi: 10.1136/bjo.2009.157941

Variations in amniotic membrane: relevance for clinical applications

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The amniotic membrane (AM) has found several clinical applications for ophthalmic indications, in particular, those related to ocular surface (OS) diseases. Successful results have been reported after use in treatment of persistent corneal epithelial defects, bullous keratopathy, acute and late stages of chemical burns and OS inflammatory diseases such as Stevens Johnson syndrome and after excision of conjunctival lesions, besides others.[1–3]

The AM has a complex structure, and several layers have been described. Essentially, it is composed of a metabolically active epithelium, which rests on a basement membrane and an avascular stroma. The epithelium and the stroma contain several growth factors, cytokines and other metabolically active substances. The transforming growth factor (TGFb) and the epidermal growth factor (EGF) are major and important growth factors. Proinflammatory and anti-inflammatory cytokines, such as interleukin 6 (IL-6), IL-8, IL-10 and IL-1ra, metalloproteases and tissue inhibitors of metalloproteases, and others have also been described.[3 4]

The mechanism of action of the membrane is not precisely known. Much of its beneficial effect can be attributed to its role as a substrate or scaffold supporting cell growth, migration and adhesion.[5] The actions of several of its chemical constituents are also invoked, although their exact concentrations and bioavailability in processed membrane is unknown. The membrane has been variously described as promoting wound healing, preventing scarring, inhibiting vascularisation, arresting corneal stromal melts, facilitating re-epithelisation and maintaining stemness of corneal epithelial stem cells.[2 6] The term 'biological bandage' is often used to indicate several or all of the above effects.

The membrane has been used fresh (includes storage at 4°C for up to a week or two), frozen/cryopreserved (after processing using at least two different protocols and storage at −80°C: in phosphate-buffered saline and dimethyl sulfoxide or in Eagle minimum essential medium with glycerol for up to 2 years) and freeze dried. Despite the variations induced by these modalities of processing and preservation, equal success has been reported with all of them. TGFb plays an important role in wound healing. Hopkinson *et al*[7] have demonstrated variations in TGFb content in different regions (placental, mid and apical) of the membrane and also in relation to manipulations undertaken during processing and storage. Similarly, EGF can influence epithelial cell regeneration and migration. Gicquel *et al*[8] have reported similar regional and processing variations in EGF content of the membrane. Interdonor variations in the membrane too have been reported in relation to age, race, maternal health and diet of the donor. Fetal sex, health, gestational age and proximity to labour also affect the composition and the physical structure of the membrane. In this issue, Connon *et al*[9] report the variations in thickness, transparency and refractive index of freeze-dried and freeze-thawed AM samples depending not only on the method of processing but also on the site of the sample, whether close to the placenta or distal to it (***see page 1057***). This adds further to the list of variations reported in AM used in ophthalmic surgery.

If one believes that the chemical constituents of the membrane contribute to its mechanisms of action, then intuitively, Fresh membrane would be better than processed and stored membrane. Its ability to act as a viable substrate, however, would remain relatively stable in both fresh and preserved samples. Opinion is divided on whether denudation or retention of amniotic epithelium before transplantation is advantageous or detrimental to OS epithelial regeneration. Viable amniotic epithelium would continue to secrete the beneficial chemical substances but be less conducive to OS epithelial cell migration over it.

Although success and failure have been reported with use of the membrane, the overall impression one gets from reading the numerous published papers is that the membrane has good efficacy. However, recent publications related to randomised controlled trials have demonstrated that in many instances, the membrane is no better and can be worse than existing alternative options. For example, in patients with glaucoma, when an AM was used as an antiscarring agent, the results of trabeculectomy were no different with or without an AM[10] and when used to treat leaking blebs, AM-treated eyes were prone to early releakage.[11] In acute alkali burns, the AM did not convey any benefit in visual improvement. However, there was a reduction in acute pain and more rapid epithelisation in moderate burns[7 12] but not in severe burns. Similarly, no difference was found in the treatment of neurotrophic ulcers compared with conventional therapies.[13]

In a recent meta-analysis paper (submitted Suleman *et al*), it was shown that for all indications, the AM efficacy was just inferior or as good as other options, but for pterygium surgery, as a substitute for autoconjunctival graft, it was definitely inferior.

Given the many interdonor and intradonor variations in the membrane, further compounded by the variations introduced by procedures adopted in processing and storage, it is not surprising that different outcomes have been reported. Having said that, it remains to be conclusively demonstrated that the constituents of the membrane, which are more prone to variations, are indeed essential to its mechanism(s) of action. The non-standard nature of the AM product used across the world makes comparisons of different applications and indications difficult.[3]

The potential for epidemic infections, such as human immunodeficiency virus and hepatitis B and C, is a serious issue because in many parts of the world, fresh unquarantined membrane, at times with no tests for the above infectious agents, is being used. High standards of good medical practice in donor testing, processing and storage of the membrane are sadly not replicated everywhere in the world.

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Competing interests None.

Provenance and peer review Commissioned; externally peer reviewed.

Accepted 18 April 2010

Br J Ophthalmol 2010;**94**:963—964.
doi:10.1136/bjo.2009.157941

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Cover illustration

'Tell-ing Eyes' of Sumer

A TELL is an artificial mound built of debris left from an earlier habitation.[1] 'Tell Asmar' is an ancient mound located in the Diyala plain of Iraq, with important deposits of the Mesopotamian civilisation (2700BC).[2] Among other things it contained statuettes made of marble and clay, of different sizes representing in order of height, gods and goddesses, priests and worshippers.[1] The gods were worshipped mainly to bring fertility to women and crops. The figures have simple torsos but powerful faces dominated by huge eyes giving the face a staring 'open-eyed' appearance. It is likely that the eyes were coloured with inlays of stone or enamel. Many of the statuettes represented 'stand-ins' left as a religious ritual on behalf of a dead person, the large-eyed faces representing supplication to the gods.

The figures depicted on the cover are specifically of the Sumerian civilisation, an ancient Iraqi civilisation. The two statuettes represent the king and queen of Ashnunnak (an old province) praying to the gods with the huge eyes, staring out from a head sunken between the shoulders, focused in the distance. Sumer means 'the land of the civilised lords'. The Sumerian artists mixed human expressions with those of the gods in order to depict the sensations primitive man experienced in the presence of the numinous.[3]

The Sumerians had developed many things and became one of the most important ancient and technologically advanced civilisations through their discoveries. The Sumerians practiced application of eye makeup for eye protection rather than for cosmetic reasons.[4] They used pastes made from charred frankincense resin and from powdered antimony or lead compounds (*kohl*); all of which have antibacterial properties, as a protection against eye disease and to avoid the glare of the sun.[4]

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Br J Ophthalmol 2010;**94**:964. doi:10.1136/bjo.2010.190983

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