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FULL LENGTH REVIEW

Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting—a review

Katerina Jirsova · Gary L. A. Jones

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Abstract The use of amniotic membrane in ophthalmic surgery and other surgical procedures in the fields of dermatology, plastic surgery, genitourinary medicine and otolaryngology is on the increase. Furthermore, amniotic membrane and its epithelial and mesenchymal cells have broad use in regenerative medicine and hold great promise in anticancer treatment. Amniotic membrane is a rich source of biologically active factors and as such, promotes healing and acts as an effective material for wound dressing. Amniotic membrane supports epithelialization and exhibits anti-fibrotic, anti-inflammatory, anti-angiogenic and anti-microbial features. Placentas utilised in the preparation of amniotic membrane are retrieved from donors undergoing elective caesarean section. Maternal blood must undergo serological screening at the time of donation and, in the absence of advanced diagnostic testing techniques, 6 months postpartum in order to cover the time window for the potential transmission of communicable diseases. Amniotic membrane is prepared by blunt dissection under strict aseptic conditions, then is typically transferred onto a nitrocellulose paper carrier, usually with the epithelial side up, and cut into multiple pieces of different dimensions. Amniotic membrane can be stored under various conditions, most often cryopreserved in glycerol or dimethyl sulfoxide or their mixture with culture medium or buffers. Other preservation methods include lyophilisation and air-drying. In ophthalmology, amniotic membrane is increasingly used for ocular surface reconstruction, including the treatment of persistent epithelial defects and non-healing corneal ulcers, corneal perforations and descemetoceles, bullous keratopathy, as well as corneal disorders with associated limbal stem cell deficiency, pterygium, conjunctival reconstruction, corneoscleral melts and perforations, and glaucoma surgeries.

Keywords Amniotic membrane · Properties · Procurement · Preparation · Storage · Graft indications

Method of Literature Search: PubMed/Medline database searches were undertaken in an iterative manner from July 2012 onwards using the key words of placenta and amniotic membrane, together with other specific keywords for each described section (i.e. amniotic membrane plus procurement).

K. Jirsova (✉)
Laboratory of the Biology and Pathology of the Eye, Institute of Inherited Metabolic Disorders, General Teaching Hospital and 1st Faculty of Medicine, Charles University, Czech Republic, Ke Karlovu 2, 128 08 Prague 2, Czech Republic
e-mail: katerina.jirsova@lf1.cuni.cz

G. L. A. Jones
The Veneto Eye Bank Foundation, Padiglione Rama - Via Paccagnella n. 11, 30174 Zelarino, Venice, Italy

Introduction

Amniotic membrane (AM) was first used for therapeutic purposes by Davis in 1910 for skin transplantation (Davis 1910), whilst the first application of human fetal

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membranes in ophthalmology was described by de Rotth in an attempt to reconstruct the ocular surface in patients with symblepharon (de Rotth 1940). Subsequently, numerous reports appeared regarding the use of AM as a biological bandage for dressing burns, for the treatment of non-healing ulcers or as an aid to physiological wound-healing (for references see the review by Dua et al. 2004).

However, the modern ophthalmic usage of AM really blossomed as a result of the work of Tseng and his team (Kim and Tseng 1995a, b). Due to its transparent structure, lack of immunogenicity and excellent potential as a substrate for the growth, migration and adhesion of epithelial corneal and conjunctival cells, AM is increasingly used for ocular surface reconstruction in a variety of ocular pathologies including corneal disorders associated with limbal stem cell deficiency, surgeries for conjunctival reconstruction, as a carrier for the ex vivo expansion of limbal epithelial cells, glaucoma surgeries and scleral melts and perforations (Baradaran-Rafii et al. 2007; Malhotra and Jain 2014; Rahman et al. 2009). Further significant advantages of AM are the relative availability of placenta, easy preparation and its immediate availability for grafting.

Structure and function

AM is the innermost layer of the placenta, located next to the fetus. It is semi-transparent and 0.02–0.05 mm thick. AM has neither blood vessels nor a direct blood supply. It consists of three different layers: the epithelium, a monolayer of metabolically active cuboidal cells with numerous microvilli, a basement membrane containing in particular collagens IV and VII, fibronectin, laminins and hyaluronic acid, and an avascular stroma which itself is divided into three layers: an inner compact layer, a middle fibroblast layer with a loose fibroblast network, and an outermost, almost acellular spongy layer. The stroma contains mostly collagens I, II, III, V and VI (Bourne 1960; Cunningham et al. 2001; Fukuda et al. 1999; Champliaud et al. 1996) (Fig. 1).

Amniotic epithelial cells contribute to the homeostasis of the amniotic fluid, while the presence of intracellular vacuoles and bleb formations suggests some secretory activity (Dua et al. 2004). In addition, AM is important due to its ability to allow for the transport of water and soluble compounds, the production of growth factors such as epithelial growth factor (EGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and its receptor, keratinocyte growth factor (KGF) and its receptor, and tumour growth factors (TGF α, TGF β-1, β-2, and β-3 isoforms) (Koizumi et al. 2000b). Moreover, cytokines (predominantly interleukins 6 and 8 and amniotic IFN-γ) and vasoactive peptides are secreted by AM (Cunningham et al. 2001; Keelan et al. 1997).

Properties, mechanisms of action

AM promotes epithelialization and exhibits anti-fibrotic, anti-inflammatory, anti-angiogenic and anti-microbial features (for references see Baradaran-Rafii et al. 2007). The lack of immunogenicity is an important feature of AM in relation to its utility for grafting. On account of its transparent structure it can be favourably applied to the ocular surface, where AM acts as an excellent substrate for the growth, migration and adhesion of epithelial cells. In addition, AM is able to maintain a physiological moist microenvironment and as such reduce water loss and promote wound healing.

Several mechanisms have been put forward to explain the beneficial effects of AM, which are inferred from both its structure and composition.

Mechanical properties

The basement membrane of the AM closely resembles that of the conjunctiva and cornea especially in relation to its collagen composition (Fukuda et al. 1999). It thus serves as a substrate on which epithelial cells of the ocular surface can easily grow.

Epithelialization

AM stimulates the epithelialization and differentiation of the epithelium (Guo and Grinnell 1989; Meller et al. 2002), reinforces the adhesion of basal epithelial cells (Keene et al. 1987), and prevents epithelial cell apoptosis (Boudreau et al. 1995). Epithelialization is facilitated by the production of EGF, HGF and KGF. The expression of these growth factors, as well as TGF, are preserved even after storage of AM at −80 °C (Koizumi et al. 2000b). Moreover, amniotic

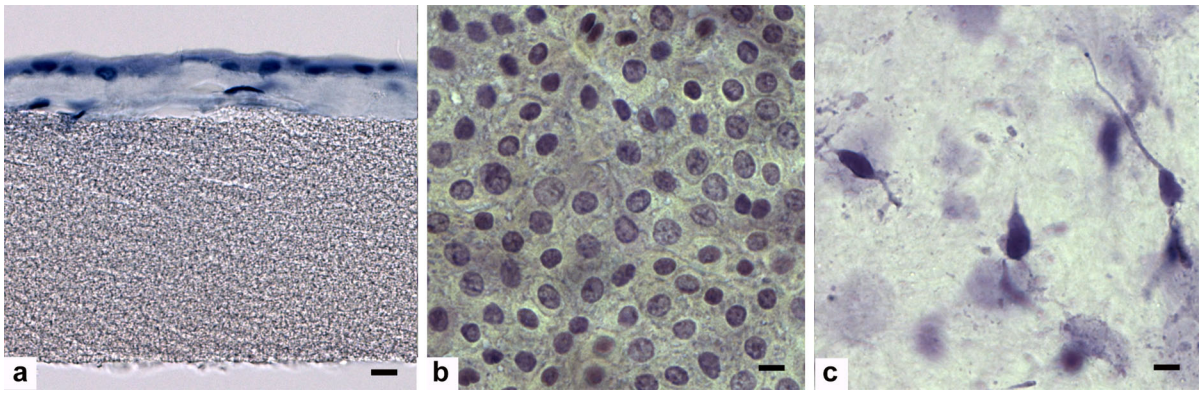

Fig. 1 Human amniotic membrane. **a** Cryopreserved tissue oriented with the stromal side in contact with the nitrocellulose filter paper and the epithelial side facing up, **b** fresh amniotic membrane—epithelial cells, **c** fresh amniotic membrane—stroma with mesenchymal cells. Haematoxylin staining, *scale bar* = 10 μm

epithelium produces brain natriuretic peptides and corticotrophin-releasing hormones that support cell proliferation and calcium metabolism (Cunningham et al. 2001).

Anti-fibrotic and anti-inflammatory properties

The anti-fibrotic effect of AM is exerted by the suppression of TGF β signalling along with the reduced expression of TGF β-1, β-2, and β-3 isoforms and TGF-beta receptor II. This inhibits the proliferation of corneal, limbal and conjunctival fibroblasts as well as the differentiation of fibroblasts into myofibroblasts (Lee et al. 2000; Tseng et al. 1999).

The anti-inflammatory impact of AM is driven by the inhibition of the expression of proinflammatory cytokines such as interleukin (IL)-1a, IL-2, IL-8, IL-10, IFN-γ, bFGF, tumour necrosis factor β and platelet derived growth factors from the damaged ocular surface (Solomon et al. 2001). Moreover, in patients with persistent epithelial defects, it has been demonstrated that inflammatory cells may be trapped by the AM stroma where they undergo apoptosis (Shimmura et al. 2001). Some anti-inflammatory and anti-fibrotic effects of AM are believed to be due to the mediation of apoptosis, particularly through the Fas receptor expressed on epithelial and stromal cells (Kubo et al. 2001; Runic et al. 1998).

Anti-angiogenic properties

Specific anti-angiogenic effects have also been ascribed to AM as a result of the production of several potent anti-angiogenic compounds including thrombospondin-1, endostatin and tissue inhibitors of metalloproteases (TIMP-1, 2, 3 and 4) (Hao et al. 2000). Nonetheless, the anti-angiogenic effect (promoting ischemia) of AM must be balanced against its other potential benefits in the treatment of limbal stem cell deficiency (Dua et al. 2004).

Anti-microbial properties

The anti-microbial impact of AM and amniotic fluid is attributable to the presence of bactricidin, beta-lysin, lysozyme, transferrin and 7-S immunoglobulins in the amniotic fluid (Galask and Snyder 1970; Gusdon 1962). Furthermore, AM works as an effective physical barrier against the potential entry of infection to the inner parts of the eye due to its close adherence to the wound surface (Kjaergaard et al. 1999; Talmi et al. 1991).

Immunomodulatory effect

Initially it was believed that the surface of AM cells was free of the major histocompatibility antigens HLA-A, B, or DR (Adinolfi et al. 1982), but subsequent studies have demonstrated a limited expression of HLA class Ia (HLA-A, B, C, DR) and class Ib (HLA-E, G) antigens by AM epithelial and mesenchymal cells (Houlihan et al. 1995). Nevertheless, the grafting of cryopreserved AM does not lead to immunological rejection (Akle et al. 1981). This feature of AM obviates the need for any immunosuppressive treatment in AM transplantation.

Donor screening

By virtue of legal requirements in different countries, various protocols exist for donor screening, AM preparation and storage. However, a choice of basic recommendations concerning AM procurement and screening can be found in the 2nd Edition of the "Guide to the quality and safety of tissues and cells for human application", implemented by the Council of Europe through the European Directorate for the Quality of Medicines & HealthCare. Naturally, a healthy donor medical and social history are prerequisites for donation (Dua et al. 2004; Keitel 2015). An exhaustive pre-natal screening of the donor's medical history for malignancies, genetic or transmissible diseases is undertaken. In addition, a careful evaluation of the donor's social history (taking careful note of a history of high-risk sexual behaviour, intravenous drug or alcohol abuse, blood transfusion etc.) is performed and a thorough physical examination is carried out,with particular attention paid to tattoos and needle marks (Rama et al. 2001; Baradaran-Rafii et al. 2007; Dua et al. 2004).

Although the clinical application of AM is considered to be safe, complications have been recorded. In particular, the risk of transmitting bacterial, viral or fungal infections to recipients is possible if AM donors are not adequately screened for communicable diseases, if the membrane is not procured and processed using aseptic techniques in a sterile working environment or if storage conditions are improper (Malhotra and Jain 2014). Maternal blood sampling should be obtain at the time of donation or, shortly after childbirth, but <7 days after donation at maximum (Keitel 2015). In the absence of advanced diagnostic testing techniques, such as PCR testing, serological screening of the donor should be repeated after 6 months, as the potential transmission of communicable diseases is real due to the window period between infection and sero-conversion (Simonds et al. 1992). Screening for HIV-1/2, hepatitis B and C, and syphilis is mandatory. Other serology tests, e.g., for HTLV-I/II, toxoplasmosis, CMV etc., are performed in accordance with national laws or guidelines (Kim et al. 2001; Meller et al. 2011). Processed AM tissue must be retained in quarantine until all test results have been received and only released for surgery when all tests are negative/non-reactive (Dua et al. 2004). In the case of positive test results, all of the tissue prepared from the respective donor must be withdrawn from distribution for surgical use. Besides maternal blood, blood from the umbilical cord may be used for screening.

Procurement and preparation

Procurement

Placenta should be collected by suitably trained, designated and authorised medical staff in appropriate gynaecological facilities from pre-selected living donors after caesarean section or vaginal delivery after a full-term pregnancy. As AM could be contaminated by normal vaginal flora during vaginal delivery (Adds et al. 2001; Dua and Azuara-Blanco 1999), procurement under strict aseptic conditions after elective caesarean section is preferred (Keitel 2015). Informed consent to retrieve AM for therapeutic purposes has to be obtained from all donors. Retrieved placenta is placed in a sterile container and transported to the tissue bank for processing as soon as possible after collection. The transport time of procured tissue should be kept as short as possible (the recommended maximum time is 24 h) and a temperature of 2–8 °C should not be exceeded. If transport time exceeds 2 h a sterile solution/medium should be placed in the container to protect tissue against drying and to maintain semi-physiological conditions (Keitel 2015).

Preparation and sterilisation

The utilization of sterile conditions and aseptic techniques should be applied at all stages in the preparation and processing of AM (Lee and Tseng 1997). First, the placenta must be decontaminated to remove potential pathogens that may by present on the surface of the placenta. Then the placenta should be repeatedly rinsed with sterile saline or antibiotic or antibiotic/antimycotic solutions. Antibiotics that are effective against both Gram-positive and Gram-negative bacteria as well as fungi should be used. Most often an antibiotic–antimycotic cocktail consisting of 50 µg/mL penicillin, 50 µg/mL streptomycin, 100 µg/mL neomycin, and 2.5 µg/mL amphotericin B is used, based on previously described methods (Kim et al. 2001; Malhotra and Jain

2014). Certified commercially available decontamination and rinsing solutions are also now available (Gatto et al. 2013).

Alternatively, the sterilisation can be performed via gamma radiation, most often by 25 kGy, which dose was selected as it is 40% above the minimum dose required to kill the resistant microorganisms. Very often, but not in the each case is used for terminal sterilisation of AM after freeze- or air-drying (Tallentire 1980; Singh et al. 2003; Gajiwala and Gajiwala 2004). The irradiation ≥20 kGy gives rise to morphological and structural changes in the AM, particularly in relation to the disintegration of the basal membrane, the decomposition of fine collagen fibres and the condensation of nuclear chromatin (Mrazova et al. 2015; Paolin et al. 2016b). Irradiation also led to a significant decrease of growth factors (e.g. EGF or bFGF) and TIMP-1, 2 and 4 (Paolin et al. 2016b). Sterilisation using peracetic acid/ethanol mixture was also used for freeze- or air-dried AM (von Versen-Höynck et al. 2004).

AM preparation is started by a cut performed close to the umbilical cord, then blunt dissection is used to separate the amnion from the chorion (Fig. 2a). Large pieces of AM are then removed, rinsed from any remnants of blood or blood clots, and stretched (Fig. 2b). Prepared segments of AM should be transparent and of regular thickness. The AM is then transferred onto a carrier, usually with the epithelial side up (Kim et al. 2001), and cut into multiple pieces of different dimensions (e.g., 2 × 2, 3 × 3, 2 × 3, 5 × 5, 10 × 10 cm) (Fig. 2c) (Baradaran-Rafii et al. 2007). During preparation, careful macroscopic examination of placenta should be undertaken to exclude visible pathological abnormalities and ensure AM integrity, and AM should continuously be kept wet.

Carriers

The most commonly used carrier is nitrocellulose paper (Kim et al. 2001). In some cases AM does not need to be attached to a carrier (dry state AM) (Malhotra and Jain 2014). On the other hand, carriers help to recognize side-orientation (as indicated on graft leaflet, mostly epithelial side up).

Preservation and storage

Although fresh and preserved AM have been found to be equally effective when transplanted onto the ocular surface (Adds et al. 2001), not all of the above-described characteristics are applicable to both types. Preserved AM is considered to be an inert tissue, generally with no viable cells. On the other hand, some cell viability and proliferative capacity have been observed after short-term cryopreservation (Kubo et al. 2001) As a consequence, the ability of preserved AM to support healing by regulating changes in the local milieu of growth factors or cytokines is very limited (Dua et al. 2004).

There are several basic preservation methods for storing human AM: cryopreservation, lyophilization and storage in a dry form. Additionally, a suspension of homogenized AM can be used therapeutically (Bonci et al. 2005).

Cryopreservation techniques

Pieces of AM resting on a carrier are placed in a vial containing storage solution. Based on a methodology introduced by Tseng's laboratory, glycerol (86%) in Dulbecco's Modified Eagle Medium (DMEM), at a

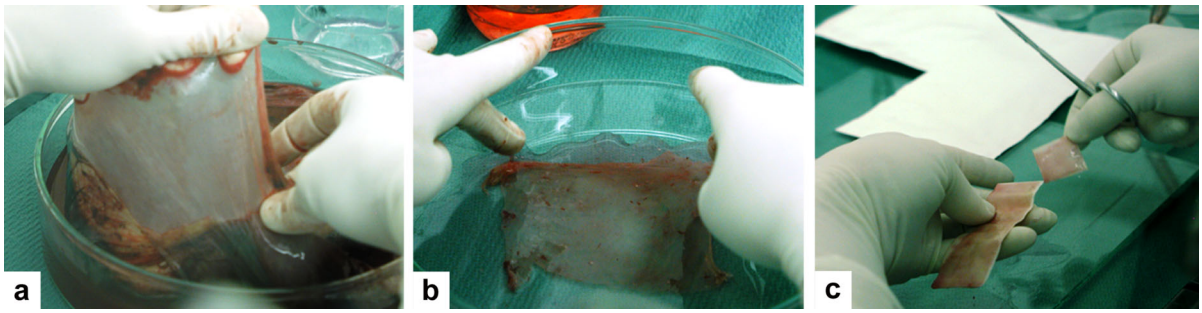

Fig. 2 Preparation of AM under aseptic conditions. **a** Blunt preparation; **b** removal of blood clots; **c** transfer of AM onto nitrocellulose paper

ratio of 1:1, is the most often used solution (Kim and Tseng 1995a, b; Lee and Tseng 1997; Malhotra and Jain 2014), which is also the technique recommended by the U.S. Food and Drug Administration (Fernandes et al. 2005). Storage temperatures of −75 to −85 °C are utilised, with the maximum storage times ranging between 1 and 2 years (Dua et al. 2004; Meller et al. 2011; Thomasen et al. 2011). Prolonged storage up to 5 years with excellent clinical outcomes has been shown after storage at −140 °C as with air-dried AM (Singh and Chacharkar 2011; Paolin et al. 2016a). Instead of DMEM, other commonly available tissue culture media can be successfully used (Dekaris and Gabric 2009).

Other known storage solutions include undiluted glycerol and pure or diluted dimethyl sulfoxide (DMSO) (EEBA Directory 2017; Shimazaki et al. 1998). AM preserved in glycerol may be safely and effectively used for over a year with the added advantage of having an antiviral and antibacterial effect (Maral et al. 1999). Indeed, it has been shown that the preservation of skin in 85% glycerol at 4 °C for 5 days leads to the complete inactivation of HIV-1 in the tissue (Cameron et al. 2000). More recently it has been shown that AM stored in 98% glycerol is clinically effective as a biological dressing (Zidan et al. 2015), and CE-certified storage glycerol is now commercially available at this purity (Glyo·ON, Alchimia, Italy).

Generally, solutions containing DMSO are less often used for AM cryoconservation as opposed to glycerol; a limiting factor is its high toxicity and DMSO as cryoprotectant is not suggested for AM storage in the Council of Europe "Guide to the quality and safety of tissues and cells for human application" and other EU recommendations (Keitel 2015; EEBA Directory 2017). However AM storage solutions containing DMSO have been studied, mostly at experimental conditions, with the aim to increase cell viability in AM (Hettiarachchi et al. 2016).

As the cells of cryopreserved AM are devitalized after thawing, no enzyme activity appears and no intact RNA can be extracted; these substances are released from the devitalized cells. Histological examination has shown no significant morphological alterations after AM cryopreservation (Kruse et al. 2000).

Lyophilisation (freeze-drying)

In this method, pieces of AM are rapidly frozen at −50 to −80 °C and then dried under high vacuum using a freeze drier device. Water present in the tissue is extracted by sublimation to diminish water concentration to a maximum of 5–10%. The tissue is usually sterilized using gamma-irradiation (Nakamura et al. 2004; for review see Baradaran-Rafii et al. 2007; Burgos and Sergeant 1983; Allen et al. 2013). The pretreatment of AM with saccharide lyoprotectans reduces cellular damage and better protects its biochemical stability (Allen et al. 2013).

Air-drying

Pieces of AM are kept at room temperature under a biohazard hood and exposed to air for different time-periods (overnight to 24 h) (Singh et al. 2003; von Versen-Höynck et al. 2004; Singh and Chacharkar 2011; Ab Hamid et al. 2014). Sterilisation using gamma-irradiation usually follows (Singh et al. 2003; Singh and Chacharkar 2011; Ab Hamid et al. 2014).

Storage method influence on AM attributes

The methods described above for the preparation and storage of AM bring specific benefits. It has been shown that lyophilisation leads to a greater reduction in the amounts of growth factors compared to cryopreservation (Rodriguez-Ares et al. 2009; Russo et al. 2012). On the other hand, no significant differences in the concentration of various growth factors and TIMPs have been found between fresh-frozen and lyophilized AM (Paolin et al. 2016b). The thickness of air-dried and lyophilized AM varying from 20 to 30 µm, AM preserved in glycerol varied from 45 to 50 µm (von Versen-Höynck et al. 2004). It has been shown that cryopreserved AM has better preserved basement membrane components and as a result are deemed more suitable for cell cultivation in that they release more soluble wound-healing modulating factors when compared to air-dried AM (Thomasen et al. 2009). Lyophilized and air-dried AM have the advantage of allowing storage and shipment at room temperature, and longer expiration time (von Versen-Höynck et al. 2004; Singh and Chacharkar 2011). It has to be

emphasised that only preparation and storage according to standardized operating procedures may allow for a comparison of long-term clinical outputs (Paolin et al. 2016a).

Microbiology

AM can be contaminated by normal vaginal flora if placenta is obtained during normal vaginal delivery however, the risk of contamination from tissue obtained in this manner can be minimized by sterilising the AM using gamma irradiation (Keitel 2015; Mrazova et al. 2015).

Microbiological screening of the placenta (before decontamination) and from prepared AM specimens (before and after decontamination) should be undertaken in order to check for the presence of fungi and aerobic and anaerobic bacteria. Where samples taken before antibiotic decontamination yield microorganisms that are considered pathogenic and highly virulent, the tissue should not be released for therapeutic use. Additionally, after decontamination (sterilisation), AM should is not released for clinical use if the samples taken for microbiological testing show signs of any microbial growth (Keitel 2015; Mrazova et al. 2015). Notwithstanding microbiological screening and the processing and grafting of AM under sterile conditions, post-operative contamination rates of 1.6–8.0% have been described with Gram-positive isolates most frequently being reported (Khokhar et al. 2001; Marangon et al. 2004; Messmer 2001).

Preparation before grafting

Prior to surgery, cryopreserved AM should be warmed to room temperature, then the storage solution (particularly cryoprotectants such as glycerol or DMSO) should be thoroughly removed by rinsing with a sterile solution (buffered normal saline, balanced salt solution) (Dua et al. 2004). Dried amniotic membrane can be rehydrated for improved manipulation during surgery. Just prior to use, the AM is peeled from the carrier (Fig. 3).

Grafting

Ophthalmosurgery

There are several main objectives in the ophthalmic use of AM: to promote epithelialisation, to reduce pain and to minimize inflammation of the ocular surface and prevent surgical adhesions (Dua et al. 2004; Meller et al. 2011). Moreover, an important feature of AM is its ability to integrate into the host tissue (e.g., the corneal stroma), which helps improve the structural quality of the tissue. This integration, associated with the formation of hemidesmosomes and desmosomes, provides anchorage and stability for the regenerating epithelium (Resch et al. 2006). AM is predominantly used for grafting in ophthalmology for the following indications: persistent epithelial defects and non-healing corneal ulcers, corneal perforations and descemetoceles, symptomatic bullous keratopathy, corneal disorders with associated limbal stem cell deficiency, pterygium, conjunctival reconstruction (including conjunctival tumours and ocular surface squamous neoplasia), and corneoscleral melts and perforations, as well as in glaucoma treatment to reduce scarring in filtering surgery (for references see the reviews by Malhotra and Jain 2014; Rahman et al. 2009). For the most part AM is used as a substrate facilitating corneal or conjunctival epithelial cells growth. In these situations AM placed epithelial side up. In a minority of instances, particularly when AM is utilised to inhibit acute inflammation, AM is placed epithelial side down) (Dua et al. 2004).

Fig. 3 Amniotic membrane is peeled from the nitrocellulose filter paper

Depending on the particular pathology, there are three main surgical techniques by which AM can be utilised.

Graft or inlay technique

AM is intended to act as a substrate or scaffold for epithelial cells to grow on, thus the AM is placed epithelial side up. Besides a single layer graft inlay, a multilayer graft inlay may also be used (e.g., multiple layers of AM are placed into the ground of an ulcer).

Patch or overlay technique

AM is sutured to the ocular surface using a patch larger than the underlying defect so that the host epithelium is present below the membrane. The AM may be used epithelial side up or stromal side up as the host epithelium is expected to grow under the AM, which acts as a biological bandage or contact lens. For reasons of applicability, the AM is normally supplied on the carrier epithelial side up, with the stromal side in direct contact with the paper. The stromal surface can be identified by the presence of vitreous-like strands that can be raised with a sponge or fine forceps.

Combined (inlay and overlay) technique (sandwich technique)

Two or more layers of AM are used, with the inner smaller layer/layers acting as a graft and the outer larger AM acting as a patch. The epithelium is expected to grow between the uppermost inlay and the patch (Dua et al. 2004; Baradaran-Rafii et al. 2007; Meller et al. 2011; reviewed by Malhotra and Jain 2014).

Non-ophthalmic usages

Besides ophthalmology, AM has been used widely in dermatology, serving as an alternative treatment for venous leg ulcers, skin ulcers or used for epidermal sheets of skin substitutes, including temporary skin coverage after burns (Koller and Orsag 2006; Lo and Pope 2009; Serena et al. 2014; Tauzin et al. 2014). Additionally, AM has been used in vaginal reconstruction, the prevention of surgical adhesions, in oral and maxillofacial surgery, or in head and neck surgeries (for references see the review by Dua et al. 2004; Kesting et al. 2014).

AM as a scaffold or substrate for cell cultivation

In vitro, AM is regularly used to culture limbal epithelial cells for grafting. Despite the lack of a limbus-specific environment, AM has been found to be an ideal substrate in promoting the formation of a well-differentiated stratified corneal epithelial equivalent for tissue engineering strategies (Dietrich-Ntoukas et al. 2012). It has also been shown that significantly higher proliferation occurs on denuded AM (Koizumi et al. 2000a), and several methods for the de-epithelialisation of AM have been suggested (Koizumi et al. 2007; Saghizadeh et al. 2013; Shortt et al. 2009). In addition, it has been demonstrated that AM cryopreserved in glycerol negatively influences the morphology and the expansion of limbal epithelial cells (Shortt et al. 2009). The successful use of AM as a carrier for expanding cells, proliferation and the clinical application of cells on the ocular surface has been repeatedly shown (Tseng et al. 2010). Besides ophthalmology, AM may be used as a biodegradable scaffold for nerve or cartilage regeneration or as a carrier for chondrocytes, keratinocytes, endothelial or epithelial cells (for references see the reviews by Mamede et al. 2012; Huang et al. 2013).

The use of epithelial and mesenchymal cells in modern medicine

By virtue of the fact that AM contains two types of pluripotent cells, both epithelial and mesenchymal ones, it is considered to be a potentially attractive source of such cells for grafting. AM cells express various stem cells markers, including octamer-binding transcription factor (OCT-4), nestin, nanog, and hepatocyte nuclear factor-3β (HNF-3 β) [for references see the review by Toda et al. (2007)]. Recently, AM was used as a source of stem cells for potential clinical application (Insausti et al. 2014). AM-derived cells have the potential to differentiate into cardiomyogenic, chondrogenic, adipogenic, hepatic, and neural insulin-producing cells (Ilancheran et al. 2007; Miki et al. 2005; Parolini et al. 2008). Furthermore, the utilization of AM cells seems very promising, particularly epithelial cells, for the treatment of cancer, the prevention of tumor metastasis and the restoration of

tissue damaged by chemotherapy. The anti-cancer effect of AM derives from its anti-angiogenic, immunoregulatory and anti-apoptotic properties (Niknejad et al. 2014; Wang et al. 2013). Besides AM cells, AM fluid or extracts can be used clinically in regenerative therapy (Kim et al. 2014; Kordic et al. 2013).

Conclusion

The selection of a suitable placenta and the subsequent meticulous preparation and storage of the amniotic membrane in a certified tissue establishments are crucial to the maintenance of the particular healing properties for which AM has become a recognized therapeutic option, not only in ophthalmology but also in other medical specialities such as regenerative medicine. Its anti-inflammatory, anti-microbial, anti-angiogenic and low immunogenicity characteristics, together with its relatively easy availability, have led to the rapid expansion of the use of AM itself, or its cells, in tissue engineering and in the development of new therapeutic strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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