Amniotic Membrane Transplantation in Strabismus Surgery

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ABSTRACT

Purpose: Adhesions between the extraocular muscles and surrounding tissues pose a main cause of failure of strabismus reoperations. Amniotic membrane (AM) transplantation during extraocular muscle surgery, to prevent the formation of adhesions, has been a subject of research during the past decade. This review aims to determine the value, indications, and tips on usage of AM transplantation during strabismus surgery.

Materials and methods: All references cited in PubMed in English were searched using the key words: amniotic membrane strabismus or amniotic membrane extraocular muscles, and a brief summary of these was described. In addition, certain articles were chosen to provide introductory information on wound healing and fibrosis, AM properties and how it works after transplantation, and AM processing and preservation.

Results: AM used for transplantation during extraocular muscle surgery may be cryopreserved, dried, or fresh. It may be oriented with its stroma or epithelium towards the muscle. It may or may not be fixed with sutures. What were the best choices? Various studies attempted to answer these questions. Many of the studies reviewed, however, were inconclusive or contradictory. Fresh AM seemed effective, but carried a risk of transmission of communicable diseases. Dried membrane was not of value in preventing adhesions. Histopathologically, cryopreserved membrane prevented the development of adhesions in the region of its presence, regardless of its orientation, and without the need for suture fixation. To accentuate this histopathological effect during clinical practice, it was recommended to utilize the largest segment possible of cryopreserved membrane and limit its usage to cases where adhesions are expected to be the main cause of failure of strabismus surgery.

Conclusion: Cryopreserved AM transplantation was safe and histopathologically effective in preventing adhesions. This effect was, however, less pronounced clinically. Its use during strabismus reoperations is merited if previous recommendations and precautions are considered.

Introduction

This is a review article of published data on amniotic membrane (AM) transplantation during extraocular muscle or strabismus surgery. All references cited in PubMed in English were searched using the key words: amniotic membrane strabismus or amniotic membrane extraocular muscles, and a brief summary of these was described. In addition, light was also shed on articles describing wound healing and the development of fibrosis and adhesions, as better understanding of the problem addressed helps understanding the solutions proposed. Moreover, certain articles were chosen to provide information on AM properties and how it works after transplantation, and AM processing and preservation.

Text of review

Wound healing and adhesions

Strabismus corrective surgery entails tenotomy of the extraocular muscles and their re-attachment to the sclera. Wound healing involves inflammation in the acute phase, granulation tissue in the intermediate phase, and scarring in the chronic phase. The initial peritendinous inflammatory reaction, on the superficial and deep surfaces of the tendon, consists of blood vessels, inflammatory cells, and fibroblasts. After the inflammatory reaction lessens, fibroblasts lay down collagen, which gradually matures and becomes well organized. The tendon eventually becomes attached to the sclera by firm fibrous tissue.

The initial inflammation involves cellular infiltration by polymorphonuclear neutrophils (PMNs), macrophages, and lymphocytes. PMNs will eventually undergo apoptosis and are removed by M2 macrophages via phagocytosis, which is essential to resolve inflammation. M2 macrophages also express a high level of anti-inflammatory cytokine (interleukin-10), which activates Treg lymphocytes that downregulate the proinflammatory responses enhanced by Th1 lymphocytes. On the contrary, under pathological states when there is a wider extent of injury, there is excessive PMN infiltration and delayed apoptosis, which exacerbate inflammation and activate M1 macrophages that are ineffective in phagocytic clearance of apoptotic neutrophils. M1 macrophages express high levels of proinflammatory cytokines and activate Th1 and Th17 lymphocytes, which enhance proinflammatory responses. The presence of activated T lymphocytes and a high amount of TGF-b during the
proliferative phase of wound healing are characteristic of hypertrophic scars.\textsuperscript{20}

The ideal goal following a strabismus operation is to limit the healing process to a thin neat line of fibrous tissue connecting the tendon to the sclera at the insertion site. This, however, is not always the case. Undesired adhesions, defined as fibrous bands (scar tissue) that connect tissues not normally connected, frequently develop between conjunctiva, Tenon’s capsule, extracellular muscle, and sclera, especially following strabismus reoperations, and result in ocular motility problems that adversely affect the surgical outcome.\textsuperscript{21}

Fibrosis results from the overgrowth, hardening, and/or scarring of various tissues and is attributed to excess deposition of extracellular matrix components including collagen. The key cellular mediator of fibrosis is the myofibroblast, which when activated serves as the primary collagen-producing cell.\textsuperscript{1} Transforming growth factor-beta (TGF-\(\beta\)) is the most potent cytokine promoting myofibroblast differentiation.\textsuperscript{12} TGF-\(\beta\) also upregulates the expression of such matrix components as collagens and proteoglycans, downregulates proteinase and matrix metalloproteinases, and upregulates their inhibitors. Collectively, these actions result in increased cell–matrix interactions and adhesiveness, as well as deposition and formation of scar tissue.\textsuperscript{13} The source of fibroblasts is the peritendinous loose connective tissue (peritendon). These fibroblasts are responsible for healing of the tendon and its attachment to the sclera, as well as healing of the wound in Tenon’s capsule. Fibrous tissue deposited in the tendon-to-sclera wound could intermingle with that deposited in the Tenon’s capsule wound, with resultant adhesions between these layers (Tenon’s capsule, tendon, and sclera).\textsuperscript{2}

**Prevention of adhesions**

Various techniques have been used with varying success to reduce the formation of postoperative adhesions.\textsuperscript{17,14–20} None, however, has been popularly accepted because of associated complications, unavailability, or inconsistent results.\textsuperscript{21}

The AM is a thin tissue that covers the placenta on the fetal side. The low immunogenicity of AM, with subsequent lack of rejection following transplantation, makes it a unique tissue that can be safely transplanted on the ocular surface for reconstruction (by promoting epithelialization)\textsuperscript{22} and around extraocular muscles.\textsuperscript{21} Following transplantation, the AM acts as a biological barrier between potentially adhesive surfaces, and its stromal matrix reduces inflammation, neovascularization, and scarring.\textsuperscript{23,24} These peculiar actions offer a rationale for its transplantation during strabismus reoperations to limit adhesions between muscle, sclera, Tenon’s capsule, and conjunctiva.\textsuperscript{21}

**AM properties**

**Anatomical properties**

Anatomically, the AM is the innermost layer of the placenta enwrapping the fetus in the amniotic cavity, which contains the amniotic fluid. The AM is transparent, avascular, and 0.02–0.05 mm thick. It consists of three layers from within outwards: (1) the epithelium; a monolayer of metabolically active cuboidal cells with microvilli; (2) the basement membrane, containing collagens IV and VII, fibronectin, laminins, and hyaluronic acid; and (3) the stroma, avascular and divided into three layers: (a) inner compact layer, consisting of a reticular network and contributes to the tensile strength of AM; (b) middle fibroblast layer with loose fibroblast network; and (c) outermost spongy layer, consisting of bundles of reticulum bathed in mucin. The stroma contains collagen types I, II, III, V, and VI.\textsuperscript{25}

**Physiological properties (in-utero functions)**

The fetal membrane (AM fused with the chorion) physically separates the fetus from the maternal environment (i.e., uterus).\textsuperscript{23} The AM’s barrier function is not only “physical” but also “biological”, where the AM exerts anti-inflammatory and anti-scarring effects to protect the fetus from cellular insults derived from the maternal environment.\textsuperscript{26} Heavy chain (HC) of inter-\(\alpha\)-trypsin inhibitor – hyaluronan (HA)/pentraxin 3 (PTX3) complex (HC-HA/PTX3) is mainly responsible for these actions.\textsuperscript{10,26}

**Anti-inflammatory actions.** HC-HA/PTX3 promotes apoptosis of activated neutrophils and activated M1 macrophages\textsuperscript{27} and enhances phagocytosis of apoptotic neutrophils by M2 macrophages.\textsuperscript{4} Furthermore, HC-HA/PTX3 promotes polarization of activated macrophages towards M2 phenotype, rather than M1 phenotype.\textsuperscript{27} Lastly, it suppresses Th1/Th17 lymphocytes and promotes Treg lymphocytes.\textsuperscript{10}

**Anti-scarring actions (scarless fetal wound healing).** The anti-scarring actions are effected indirectly through the anti-inflammatory effects and through a direct anti-scarring effect of the AM stroma.\textsuperscript{10,23} The latter is achieved through suppression of TGF-\(\beta\)1 activity by HC-HA/PTX3.\textsuperscript{28}

**Actions of transplanted AM**

Transplanted AM, in addition to yielding its anti-inflammatory and anti-scarring actions, also shows antiangiogenic and antimicrobial effects. HC-HA/PTX3 is responsible for the antiangiogenic effects, both directly and through its anti-inflammatory effect.\textsuperscript{29}

The antimicrobial impact of AM and amniotic fluid is attributed to the presence of ribonuclease, protease and other antimicrobial proteins and peptides.\textsuperscript{30} Furthermore, genes for antimicrobial peptides, including human beta-defensins, are expressed by AM and their expression levels positively correlate with the antimicrobial activity of AM.\textsuperscript{31}

**Immunology of AM**

A unique advantage of AM is the low immunogenicity of the tissue and lack of rejection.\textsuperscript{22} This is attributed to the limited expression of major histocompatibility antigens (human leukocyte antigens [HLA]) by the AM epithelial and mesenchymal cells.\textsuperscript{32}

Cryopreserved AM is, however, relatively immunogenic after xenotransplantation in an immunologically unprivileged site (with blood and lymphatic flow), due to the small amounts of HLA expressed by the human amniotic cells. These antigens may be indirectly presented to T cells by host antigen-presenting cells.\textsuperscript{22}
**AM procurement, preparation, and preservation**

Although the clinical application of AM is considered to be safe, complications have been recorded. In particular, the risk of transmitting communicable diseases (HIV, hepatitis, or syphilis) to recipients is possible if AM donors are not adequately screened for communicable diseases. Moreover, microbial infections (bacterial, viral, or fungal) may complicate AM transplantation if the membrane is not processed using aseptic technique in a sterile work environment or if storage is improper. Air-dried AM.

AM is used for transplantation only if all tests, on both occasions, are negative. AM may be fresh or preserved. Fresh AM, however, may pose a risk of transmission of communicable diseases (including HIV, hepatitis, and syphilis) as the time interval from procurement to transplantation is short, preventing repeat testing of the donor 6 months later. Preserved AM is, therefore, a safer alternative for transplantation.

Microbial infections after AM transplantation have been reported, although rarely. A study evaluated 326 patients, over a 7-year-period, who underwent AM transplantation for various ocular diseases. From the microbiological records of these patients, 11 positive cultures (3.4%) were identified. The time interval between AM transplantation and infection ranged between 6 days to 16 months. Gram-positive organisms were found in 7/11 (64%) positive cultures. The authors concluded that the infections rate after AM transplantation is low, especially when AM is prepared according to Good Tissue Banking Practice set forth by FDA. Moreover, it is sound to culture the storage medium immediately following AM transplantation and to monitor patients postoperatively for signs of infections.

Preserved AM is either cryopreserved or dried. The ideal method of preservation of AM would be one that facilitates transport and prolonged storage without affection of its tissue properties necessary to suppress inflammation and scarring. Cryopreservation is the best preservation method to guarantee maintenance of membrane properties. The biological properties of cryopreserved AM are similar to fresh one, except for the viability of epithelial cells, which are present but devitalized in the former.

**Cryopreserved AM**

The AM membrane is flattened onto nitrocellulose paper with the epithelium/basement membrane surface up and the stromal surface (the adhesive side) in contact with the paper. The membrane and the paper are cut and placed in a sterile vial containing Dulbecco’s Modified Eagle Medium (DMEM), ratio of 1:1, and frozen at −80°C, with the maximum storage times ranging between 1 and 2 years. The membrane is defrosted immediately before use by warming the container to room temperature for 10 min.

**Dried AM**

**Lyophilized (freeze-dried) AM.** Intact AM (with epithelial cells) or AM deprived of epithelial cells by incubation with EDTA may be used. The membrane is cut into pieces and rapidly frozen at −50°C to −80°C. Thereafter it is dried under high vacuum using a freeze-drier device. Tissue water is extracted through sublimation, reaching a final water content of 5–10%. This results in the inhibition of deleterious chemical reactions that lead to tissue alteration. At the end, packing and sterilization using gamma irradiation is performed. The product can be stored at room temperature. Lyophilization leads to a greater reduction in the amounts of growth factors compared to cryopreservation. Similarly, irradiation gives rise to morphological and structural changes in the AM, particularly in relation to the disintegration of the basal membrane, decomposition of fine collagen fibers and condensation of nuclear chromatin. Lyophilization, however, still maintains some of the most important characteristics of the AM and thus could be useful when the necessary infrastructure for the transport and storage of cryopreserved tissue is not available.

**Air-dried AM.** The AM is flattened and exposed to air to get dried. Packing and sterilization using gamma irradiation is then performed. Properties of the amnion are lost or altered remarkably due to dehydration.

**AM transplantation during extraocular muscle surgery**

Various clinical and histopathological studies evaluated the value of AM transplants during strabismus reoperations or complex strabismus cases. Among these, 12 were clinical studies involving humans, and 9 were experimental studies involving rabbits. Among the clinical studies, six were case reports, three were prospective, and three were retrospective studies.

First, Yamada and colleagues successfully treated diplopia, hypotropia, and limited elevation due to fat adherence syndrome, after retinal surgery, using AM. After removing the subconjunctival fibrosis, the authors recessed the inferior rectus muscle and applied a piece of AM on the exposed sclera and muscle with the epithelial side away from sclera and muscle. The AM was fixed with 9-0 silk sutures. The authors achieved stable improvement of binocular vision, hypotropia (from 45 to 6 PD) and elevation after more than 1-year follow-up.

Later, Kersey and Vivian described a technique which used both mitomycin C and AM patches to reduce fibrosis in two patients with complex strabismus: one with congenital fibrosis of extraocular muscles and the other with inferior oblique traction syndrome after six strabismus surgeries. The authors disinserted the muscle and allowed it to retract posteriorly, followed by dissection of fibrous tissue and application of mitomycin C. An 8 × 8-mm AM square patch was then sutured to the sclera using 7-0 Vicryl sutures, with the basement membrane towards the muscle. After a follow-up duration of 9 months for the first and 3 months for the second patient, the authors reported success, yet limited, with their
cases, which could be attributed to the marked complexity of the conditions treated in both cases and the presence of a tendon expander in the second case. The authors concluded that despite the limited success achieved, they believed the technique has allowed a better outcome than would have otherwise been achieved.45

These two previous studies did not specify whether the AM used was fresh or preserved.44,45 Later on various studies were performed to evaluate fresh and preserved AMs. These studies are summarized in Tables 1,46,47 248–52 and Supplementary Table 1.21,53–63

**Fresh AM transplantation (Table 1)**

*Experimental study.* Demirel et al. performed a controlled, histopathological study to evaluate the effect of fresh AM transplantation during superior rectus muscle surgery in rabbits. An AM sheet was sutured over a sclerectomy (to create adhesions) under the muscle. On histopathological examination, all AM eyes showed intact AM, with periamnion inflammation, and no adhesions between the muscle and surrounding tissues. Adhesions were detected in all control (C) eyes. Scleral inflammation was detected in 60% of AM and none of C eyes. FB inflammation was present in 60% of AM and 90% of C eyes. Muscle fibrosis was present in 40% of AM and 90% of C eyes ($p = .023$).46 The authors attributed the higher rate of scleral inflammation in the amnion group than the control group to the xenogenic nature of human AM that was used in rabbits.46,64 where fresh AM possessed viable amniotic epithelial cells with immunogenic potential.46,65 Minguini et al. suggested that the amount of resultant fibrosis would be proportional to the severity of previous inflammation, so inflammation would end up as fibrosis in the long term.66 Demirel et al. thought that although 2 months were adequate for complete healing, yet the AM group might have more fibrosis and adhesions after a long follow-up, due to the higher rate of inflammation. Demirel et al. concluded that although their data do not support the hypothesis that AM would be effective in reducing postoperative cell proliferation; however, it can prevent adhesion formation with possible barrier effect. Moreover, the authors expected less inflammation with the use of human AM in human subjects.46

*Clinical study.* Tugcu et al. prospectively evaluated the use of fresh human AM during strabismus reoperations in patients with restrictive strabismus. Strabismus surgery included the excision of adhesions and scar tissue, repositioning of extraocular muscles and placement of two AM sheets, one between muscle and tenon’s capsule (stroma facing tenon’s capsule) and the other between muscle and sclera (stroma facing the sclera). Conjunctival recession with covering of the bare sclera using AM (stroma towards the sclera) was also performed. The authors reported a success rate (<8 PD of ocular deviation with no duction deficit) of 86%. They concluded that AM placement around the extraocular muscle improved ductions (from 1.7 to 0.2) and decreased the residual deviation angle (from 34.3 to 4.6 PD) by inhibiting postoperative scar formation, with no complications.67 These findings supported those of the former histopathological study, where adhesions were absent and fibrosis was less with the use of AM transplants.46 The study of Tugcu
| Study                  | Type of study | Sample size (patients/rabbit eyes) | Type of AM | AM size | AM orientation | Suture fixation of AM | Condition treated | Follow-up duration/outcome |
|-----------------------|---------------|-----------------------------------|------------|---------|----------------|-----------------------|-------------------|--------------------------|
| Kassem et al. 48      | Experimental  | 20/16 (10/8 AM, 10/8 C)           | Human, dried (Matrix Health Care, Ameco, Egypt) | -       | Stroma could not be identified | Sutureless wrap | -                | 6w                       |
|                       |               |                                   |            |         |                |                       |                   | -                        |
| Chun et al. 49        | Experimental  | 12 (6 AM, 6 C)                    | Human, dried, intact epithelium (Ambiodry2; Okto Ophthalm, Costa Mesa, CA, USA) | 15 × 15 mm | Stroma | - | - | 2w |
|                       |               |                                   |            |         |                |                       |                   | -                        |
| Kennedy et al. 50     | Experimental  | 40 (10 dried AM, 10 Cryopreserved AM, 20 C) | Human dried (Ambiodry2, IOP Inc., Costa Mesa, CA); Human cryopreserved (AmnioGraft, Bio-Tissue, Miami, FL) | 2 sheets of 10 × 7.5 mm | Stroma | - | - | 1m |
|                       |               |                                   |            |         |                |                       |                   | 1.Tensile strength: No significant difference of tensile strength of muscle attachment or conjunctival adhesion in dried AM vs C & in cryopreserved AM vs C (p > .05). |
|                       |               |                                   |            |         |                |                       |                   | 2.Histopathology for adhesions, muscle fibrosis & inflammation: a. Dried AM: No significant difference in AM vs. C (p > .05). b. Cryopreserved AM: Scleral inflammation AM > C (p = .01); otherwise no significant in AM vs. C (p > .05). |
| Kassem et al. 51      | Clinical, case report | 1 | Human, dried (Matrix Health Care, Ameco, Egypt) | 5 × 25 mm | Stroma could not be identified | Sutureless wrap around MR muscles | Residual ET after 2 previous strabismus surgeries | 8 w |
|                       |               |                                   |            |         |                |                       |                   | -Consecutive XT, limited adduction. -On 4th reoperation: dense muscle/conjunctival adhesions & fibrotic inelastic MR muscles. |
| Mehendale and Dagi 52 | Clinical, case report | 1 | Human, dried (Ambiodry5; Okto Ophtho, Costa Mesa, CA, USA) | 4 × 4 mm | - | 6-0 & 8-0 Vicryl | Orbital trauma repaired with titanium plates | 3m |
|                       |               |                                   |            |         |                |                       |                   | Improvement of binocular single vision, hypotropia and limited elevation |

AM: amniotic membrane; m: month(s); w: week(s); a: The Table also includes one study evaluating both dried and cryopreserved AM grafts; b: AM: amniotic membrane group, where rectus muscle surgery was performed with application of an amniotic membrane graft; C = Controls where the same rectus muscle surgery was performed but without an amniotic membrane graft; d: 2 rabbits died before 6 weeks and were excluded, so documented results are for 16 eyes only; e: AM segment wrapped around the muscle, extending for 5 mm along the length of the muscle.
et al., however, included no control group, so it is not known whether the improvement was merely due to repositioning of extraocular muscles or the use of AM graft.

In the study of Tugcu et al., limitation of duction was stabilized in the first week, although complete healing occurs by 4–6 weeks. The authors stated that this might be attributed to the prevention of adhesions by the AM by forming a temporary biological barrier between the layers of perimuscular connective tissue. The assumption of the barrier effect agrees with that of Demirel and co-authors.

One of two patients with limited success in the study of Tugcu et al. had congenital fibrosis of extraocular muscles. This agrees with Kersey and Vivian who reported limited success with congenital fibrosis.

In conclusion, fresh AM transplantation seemed effective in preventing adhesions and improving the outcome of strabismus reoperations, based on the experimental and clinical study. Solid conclusions, however, cannot be based on merely two studies. Moreover, even if fresh AM proves effective, its use is discouraged due to the risk of transmission of communicable diseases.

### Dried AM transplantation (Table 2)

**Experimental studies.** Kassem et al. performed a controlled histopathological study on rabbits to evaluate the influence of dried human AM transplant on the development of postoperative adhesions and fibrosis after extraocular muscle surgery. In one eye of each rabbit, an AM sheet was placed on the sclera under the disinserted, resected muscle. After securing the muscle to the sclera, the AM was folded to wrap the muscle without sutures. The fellow eyes underwent superior rectus resection without an AM wrap and served as controls. The histopathological parameters scored are shown in Table 3. On histopathological evaluation, the AM was absent in all specimens. Adhesions were detected between the muscle and surrounding tissues in 100% of AM and control (C) eyes. Conjunctival inflammation level was significantly less in AM than C eyes \( (p = 0.034) \). Muscle fibrosis, FB inflammation, and conjunctival hyperemia were insignificantly less in AM than C eyes \( (p > 0.05) \). The authors concluded that dried AM insignificantly reduces postoperative adhesions and fibrosis, when used to wrap the operated upon extraocular muscles, limiting its benefit in strabismus surgery.

Kassem et al. attributed the contradictory outcomes of fresh and dried AM transplants to the deprivation of dried AM of epithelial cells during processing, while fresh membrane possesses its layer of epithelium. As epithelialized surfaces cannot adhere to other surfaces, no adhesions were found with fresh AM, due to its barrier effect. On the other hand, the absence of epithelium in dried AM was a cause of loss of its barrier effect, and its absorption and replacement by adhesions.

The partial degradation of stroma and basement membrane of AM during lyophilization was another suggested cause of failure of dried AM to prevent adhesions and fibrosis, as the stroma of AM is responsible for its anti-scarring functions. In a previous study by Thomasen et al., the authors incubated pieces of air-dried as well as cryopreserved AMs in vitro. On histochemical analysis, collagen IV, collagen VII, laminins, and fibronectin were detectable in the basement membrane of cryopreserved AM, but only collagen IV and fibronectin were present in air-dried AM. These findings suggest partial degradation of the basement membrane of the AM during lyophilization.

Kassem et al. also proposed the inability to identify the stromal surface of the dried AM, preventing proper orientation of the stroma towards the muscle, as a third factor leading to its failure. Their rationale was that the stroma of AM is mainly responsible for its anti-inflammatory, anti-adhesive, and anti-fibrotic effects. Accordingly, the membrane becomes more effective when the stromal side is in contact with the muscle.

The significantly lower rate of inflammation in AM than in control eyes in the study of Kassem et al. is another contradiction to the study of Demirel and co-authors. It has been demonstrated that the stromal matrix of AM, rather than the viable epithelial cells, is responsible for the AM anti-inflammatory effects. Accordingly, the absence of viable epithelial cells in dried membrane eliminated its xenogenic nature, while the presence of its matrix maintained its anti-inflammatory effects, resulting in less inflammation than in the study utilizing fresh membrane, as well as significantly less inflammation in AM than C eyes.

Later, Chun et al. conducted another controlled, histopathological study to evaluate the effect of dried human AM transplantation during extraocular muscle surgery in rabbits. AM was

| Parameters | Values |
|-----------|--------|
| Conjunctival membrane | 0 = absent |
| 1 = present |
| Conjunctival inflammation | 0 = no inflammation |
| 1 = a few lymphocytes and plasma cells beneath epithelium |
| 2 = mild inflammatory infiltrate composed of lymphocytes, plasma cells and polymorphonuclear leukocytes beneath epithelium and congestion |
| 3 = grade two plus neutrophils in the epithelium |
| 4 = high concentrations (collections) of lymphocytes, plasma cells, polymorphonuclear leukocytes and histiocytes (both intraepithelial and subepithelial) and ulceration |
| Scleral inflammation | 0 = absent |
| 1 = present |
| Foreign body inflammation | 0 = absent |
| 1 = present |
| Conjunctival vascularity | 0 = white avascular conjunctiva |
| 1 = some avascularity |
| 2 = normal vascularity |
| 3 = mildly increased vascularity suggestive of ongoing inflammation |
| 4 = moderately increased vascularity |
| 5 = severely increased vascularity |
| Adhesion between rectus muscle and sclera | zero = no fibrosis |
| 1 = mild perimucosal fibrotic reaction (stained collagen is detectable only in thin bands immediately adjacent to muscle) |
| 2 = easily detected thick bands |
| 3 = well-developed, dense bands of collagen |
| 4 = a severe fibrotic response replacing large areas |
| Rectus muscle fibrosis | 0 = absent |
| 1 = present |
applied between the resected SR muscle plane and Tenon's capsule. Based on histopathological examination, the authors concluded that the use of dried human AM was not effective in controlling the postoperative inflammation and scarring in rabbit eyes after extraocular muscle surgery and attributed this to the devitalized dry preparation of human AM, which may have lost the expected anti-inflammatory and anti-scarring properties.49

Kennedy et al. performed a controlled study to evaluate wound tensile strength and histopathologic changes after strabismus surgery with AM grafts in rabbits. Eyes were divided into three groups: control, those receiving processed dehydrated AM grafts, and those receiving cryopreserved AM grafts. An AM graft was placed between the sclera and inferior rectus muscle, extending anteriorly to the muscle stump. The inferior rectus was resected 4 mm using hang-back technique. A second AM graft was placed on top of the muscle, followed by conjunctival closure. Tensile strengths of the muscle-globe attachments and conjunctiva-globe attachments were measured, and histopathologic analysis of each eye was performed. Tensile strength was defined as the force required to disinsert the muscle from the underlying sclera as well as break through the overlying conjunctiva using a modified tensometer attach to a Green muscle hook. The authors concluded that there was no significant change in tensile strength of the muscle insertion using AM grafts as compared to control eyes, and that AM grafts did not reduce inflammation or improve scar formation.50

In the study of Kennedy et al.,50 the AM graft was placed under the muscle in a way that AM was present underneath the new muscle insertion site. As previously reported by Kassem et al.,48 dried AM was absent (probably absorbed) with adhesions present between muscle and sclera in all specimens. The muscle, in the study of Kennedy et al.,50 was, therefore, able to reattach to the sclera despite the application of an intervening AM graft, as the latter was probably absorbed. Accordingly, the two studies agree on failure of dried AM graft to prevent adhesions during extraocular muscles surgery.48,50

All three studies agreed on the inability of dried AM to limit fibrosis during extraocular muscles surgery in rabbits.48–50 The significantly diminished conjunctival inflammation in the study of Kassem et al.48 is a contradiction to the other two studies, where there were no significant differences in the inflammation level between eyes receiving dried AM grafts and control eyes.49,50 This could be attributed to the absence of epithelium in the AM used by Kassem et al., thus eliminating the xenogenic nature of human AM being used in rabbits,48 in contrast to the presence of intact epithelium (with potential immunogenic potential) in the AM used by Chun et al.49 and probably in that used by Kennedy et al. as both used Ambiodry.2

Clinical studies. Kassem et al. used dried AM as an adjunct in a strabismus reoperation in a 10-year-old girl. Forced duction testing of the medial and lateral rectus muscles bilaterally revealed no restriction. Dissection of subconjunctival tissue revealed filmy, easily separable adhesions. Both medial rectus muscles were re-recessed to 12.5 mm from the limbus and wrapped with AM. An unacceptable outcome necessitated a subsequent reoperation, on which dense adhesions and fibrosis were discovered affecting the muscles formerly wrapped with AM. The authors questioned if the fibrosis and adhesions were related to the use of AM, or due to excessive dissection to achieve a large recession. They concluded that although the cause of the fibrosis was not clear, still lyophilized AM was ineffective in protecting against its development.51

Later, Mehendale and Dagi used a dehydrated AM graft in a patient with diplopia, hypotropia, limited elevation and extensive adhesions following repair of traumatized medial and inferior orbital walls with titanium plates. After extensive dissection and recession of the inferior and medial rectus muscles, dehydrated AM graft was applied to the opposing surfaces along the inferior and medial surface of the globe, over the recessed muscles and adjacent sclera, and reflected to cover the palpebral conjunctiva inferiorly and medially. Improvement ensued in the form of enlarged field of binocular single vision, collapse of hypertropia from 30 to 5 PD in primary position and from 45 to 20 PD in upgaze, together with improvement of limited elevation from −5 to −3. Despite this favorable outcome, the authors could not be certain if their outcome would have been different without the graft.52

Mehendale and Dagi created a barrier with the membrane graft between the titanium plate and adjacent muscles and reported a favorable outcome,52 in contradiction to other reports on dried AM.48–51 First, in the case of Mehendale and Dagi,52 there was improvement and not cure. Second, this is the only report applying the AM graft between the muscles and a titanium plate,52 which biases a comparison to the other studies,48–51 as the titanium plate might have interfered with complete absorption of the membrane, maintaining in part its barrier effect. Lastly, if this form of membrane possessed intact epithelium (as previously mentioned by Chun et al.),49 it would be capable of providing a barrier effect.

In conclusion, all authors,48–51 except Mehendale and Dagi,52 reported lack of efficacy of dried AM during strabismus surgery.

Cryopreserved AM transplantation (Supplementary Table 1)

Experimental studies. Kassem et al. conducted a controlled histopathological study to evaluate the effect of wrapping the resected superior rectus muscle of rabbits with cryopreserved human AM.53 Histopathological evaluation was based on the parameters in Table 3.48 On histopathological examination, the AM was present in 8 of 10 eyes. In these eight eyes, no adhesions were detected between the muscle and the underlying sclera (Figure 1), or the overlying Tenon’s capsule and conjunctiva. Absence of adhesions was confined to the segment where the AM was present, but was detected elsewhere. All control eyes, undergoing superior rectus resection without AM wrap, showed adhesions. The frequency of muscle fibrosis was, however, equal in control and AM eyes (80%).53 The absence of adhesions despite frequent presence of muscle fibrosis after AM transplantation supports the barrier rather than the anti-fibrotic effect of AM as being the mechanism of its anti-adhesive action. Foreign body inflammation was significantly more in AM eyes (80%) than in control eyes (20%), suggesting an attack against the AM by the host tissue, although
devitalized (Figure 2). This is probably related to the xenogenic nature of the human AM that was used in rabbits.

Kirsch and colleagues performed a two-stage controlled, experimental study to evaluate the effects of wrapping the recessed superior rectus muscle of rabbits with cryopreserved human AM. Histopathological evaluation and dynamometry were performed 15 days (17 rabbits) and 30 days (5 rabbits) postoperatively. On both occasions, histopathological evaluation revealed significantly more inflammation and less fibrosis in eyes treated with AM than in control eyes. The authors recommended another study utilizing “rabbits” AM to test if the inflammatory reaction was only related to the use of “human” AM in rabbits. A dynamometer was used to measure the force required to displace the eyes. The authors considered the force measured on dynamometry as an indication of the restriction caused by adhesions that form during strabismus surgery. As no significant differences on dynamometry were documented between control and AM eyes at 30 days, the authors concluded that although AM decreased fibrosis, it did not prevent adhesion formation. They, however, considered a sample size of only five rabbits as a limitation to their results.

Chun and Rhiu performed a controlled histopathological study to evaluate the effect of cryopreserved “rabbit” AM allografts transplantation during experimental strabismus surgery in rabbits, to eliminate xenograft rejection phenomenon between humans and rabbits. Four weeks postoperatively, the degree of postoperative inflammatory infiltration and the extent of fibrosis were significantly less in AM versus control eyes. The authors collectively graded fibrosis and adhesions as a single parameter. A significant decrease in the expression of inflammatory cytokines [interleukin (IL)-12a, IL-12b, IL-17f, and tumor necrosis factor-alpha (TNF-α)], and a markedly increased expression of anti-inflammatory cytokines (transforming growth factor-beta-1 (TGFβ-1) and IL-10) were observed in the eyes treated with AM. The lower levels of inflammation in AM eyes (in contradiction to the previous two studies utilizing “human” AM), with resultant decrease in fibrosis levels, were attributed to the anti-inflammatory and anti-scarring actions of AM that could take effect after elimination of xenograft rejection by using “rabbit” AM allograft rather than human AM graft.

The study of Kassem et al. differed from the other two studies in terms of fibrosis and adhesions. Kassem et al. reported equal fibrosis levels in AM and control eyes, whereas the other two studies reported less fibrosis in AM than in control eyes. The anti-fibrotic effects of AM lie in its stroma and can be affected by the amount of stromal degradation during AM processing. This might explain the controversy in the results of the three studies. Moreover, Chun and Rhiu collectively graded fibrosis and adhesions as a single parameter.

Kassem et al. reported no adhesions in the region of AM presence, whereas Kirsch et al. suggested that AM did not lower adhesions. The absence of adhesions was depicted histopathologically (which is more accurate) and in a higher number of rabbits in the study of Kassem and co-authors. On the other hand, Kirsch et al. did not provide histopathological data on adhesions level, but only indirectly evaluated the latter using dynamometry and in a smaller number of rabbits. Moreover, as documented by Kassem et al., adhesions were absent only in the region of AM application, but present elsewhere. The results of the dynamometry test in the study of Kirsch et al. might have, therefore, been affected by the adhesions present outside the region of AM application, which biased their results.

Kennedy et al., as mentioned earlier, performed a controlled histopathological study to evaluate the effect of dried and cryopreserved AM transplantation during inferior rectus hang-back recession in rabbits. Their results agree with previous studies reporting higher levels of inflammation after human AM transplantation in rabbits. Their results, however, contradict previous studies reporting significantly lower levels of adhesions and fibrosis after cryopreserved AM transplantation, where Kennedy et al. reported...
insignificant differences between control and AM eyes in terms of adhesion and fibrosis detected histopathologically, as well as in terms of the tensile strength of muscle-globe and conjunctiva-globe attachments, which was an indirect indicator of the amount of fibrosis.\(^50\)

In the study of Kennedy et al., the AM graft was placed underneath the muscle in a way that AM was present underneath the new muscle insertion site.\(^50\) According to Kassem et al., no adhesions were found between the AM and surrounding tissues in the region of AM presence.\(^53\) Accordingly, failure of muscle reattachment to the new scleral insertion site is expected if the AM graft was in between. The muscle had, presumably, migrated to insert in a site where there was no AM between it and the sclera. Accordingly, the tensile strength measured was, probably, not related to the presence of AM graft, but simple muscle-sclera reattachment in absence of intervening AM graft. Moreover, in the study of Kassem et al., the absence of adhesions was confined to the segment of AM presence but was detected elsewhere.\(^53\) Kennedy et al. scored adhesions only once, not stating whether these were in the region of AM presence or elsewhere and did not comment on AM absence or presence as detected histopathologically. Moreover, their study lacked any figures showing gross or histopathologic appearance of the specimens for accurate evaluation.\(^50\)

Sierra et al. performed a study to determine the functional recovery of the superior rectus muscle after its partial resection in a rabbit model with and without equine cryopreserved AM. Muscle samples were extracted and electrically stimulated to register the force exerted by the samples, considered as its active behavior. They were, then, subjected to stretching test to obtain its resistance to deformation, known as passive behavior. The authors documented no significant differences in active behavior recovery between muscles wrapped or not wrapped with AM. Normal passive behavior, however, recovered at 30 days in muscles treated with AM versus 60 days in those not treated with AM.\(^50\) This study was the first to focus on functional rather than histopathological effects of AM transplantation. Their results highlight the lack of effect of AM on muscle contractility (active behavior). As stretch passive behavior) is an indicator of muscle elasticity, which is, in turn, an indicator of the amount of muscle fibrosis, their results support the anti-fibrotic value of AM transplantation during extraocular muscles surgery.

In conclusion, previous studies (with the exception of that of Kennedy et al.\(^50\)) all highlighted the anti-scarring effect of cryopreserved AM transplantation during experimental extraocular muscle surgery.\(^53\)–\(^56\) These proved that cryopreserved AM transplantation holds promise as a potential therapeutic agent to provide a safe biological barrier in strabismus surgery.

**Clinical studies.** Cryopreserved human AM transplantation during strabismus surgery was clinically evaluated by various authors with promising results.\(^21,57\)–\(^59\) These studies were, however, uncontrolled and evaluating single cases or small series of patients.

Kassem et al. performed the first prospective, controlled study, with the largest patient series, evaluating the role of cryopreserved AM wrapping of extraocular muscles during strabismus reoperations (Figure 3). The study comprised a control group undergoing strabismus reoperation without an AM wrap and an AM group undergoing strabismus reoperation with an AM wrap. Each group included 15 patients. Three patients of the AM group were excluded due to loss to follow-up. In the AM group, the muscles were wrapped with a \(5 \times 30\) mm AM sheet, extending for 5 mm along the muscle length, without sutures. Surgical success was defined as 0D-10PD of horizontal tropia and 0D-4PD of vertical tropia, with no limitation of ductions exceeding \(-1\). A cosmetically acceptable outcome was defined as a tropia of 0-15PD. After a follow-up of 3–12 months, a successful outcome was achieved in 58% and 47% in AM and control groups, respectively (\(p = .63\)). A cosmetically acceptable outcome was achieved in 83.3% and 80% AM and control groups, respectively (\(p = .48\)). The mean ocular deviation angles improved from \(38.67 \pm 15.9\) to \(8.67 \pm 12.03\) PD in AM group (\(p = .003\)) and from \(40.2 \pm 14.82\) to \(12.27 \pm 17.37\) PD control group (\(p = .002\), AM vs. C, \(p = .75\)). Limited ductions improved from a mean of \(-1.28 \pm 0.67\) to \(-1.24 \pm 0.58\) in AM group and from \(-1.36 \pm 0.5\) to \(-1.29 \pm 0.59\) in control group. Ductions improved in 66.7% and 36.4% of the muscles with limited motility in AM and control groups, respectively (\(p=0.019\)).\(^50\)

Comparing AM and control groups, it is evident that postoperative deviation did not differ significantly between both groups, but the rate of improvement of limited ductions was significantly higher in AM than control group. The authors concluded that wrapping the extraocular muscles with cryopreserved AM during strabismus reoperations was of limited clinical benefit and was not as prominent clinically as previously reported histopathologically. They attributed this to the presence of other causes of failure rather than adhesions, especially amblyopia, and to the small segment of AM used (extending for only 5 mm along the muscle length).\(^60\)

Kassem performed a third reoperation on one of AM group patients. Due to extensive adhesions found around the muscles previously wrapped with cryopreserved AM, the author discouraged the use of AM during strabismus reoperations.\(^61\) On later reviewing the video of the operation, the author, however, noticed that the hook could be easily passed under the muscles in the segment of presumed AM application, proving absence of adhesions in the region of AM application,\(^53\) although extensive in surrounding regions.\(^51\) This supports the assumption that a small AM segment produces a limited clinical effect.

Kassem et al. later reported the long-term follow-up of the AM group (15 patients) of the former short-term study,\(^60\) where the three formerly excluded patients returned for follow-up and were included.\(^65\) Surgical success criteria were the same as previously defined in the former study.\(^60\) A successful outcome was recorded in 46.7%. A cosmetically acceptable outcome was achieved in 66.7%. The mean ocular deviation angles improved from \(38.6 \pm 4.63\) to \(10.6 \pm 11.08\) PD (\(p < .001\)). Limited ductions improved from a mean of \(-1.24 \pm 0.63\) to \(-1.21 \pm 0.51\). Ductions improved in 57.1% of the muscles with limited motility. Duction limitation exceeding \(-1\) was noted in 4/180 muscles (2.2%). The authors concluded that the effect of cryopreserved AM transplantation...
on the success of strabismus reoperations was moderate in terms of ocular alignment, but was more pronounced in terms of ocular motility and that the latter better reflects the level of adhesions. No long-term complications were documented, denoting safety of cryopreserved AM usage during strabismus reoperations.

The development of postoperative adhesions is not the only cause of failure of achieving ocular alignment following strabismus corrective surgery. There are other surgeon and patient factors leading to surgical failure. The role of AM transplantation, however, is confined to ameliorating factors related to the development of adhesions, and has no role in ameliorating the remaining factors. Duction limitation reflects the presence of adhesions and is therefore, a more specific measure of AM success rather than ocular alignment. Kassem et al. stated, in their short-term study, that the rate of improvement of limited ductions (which is an indicator of less adhesions) was significantly higher in the AM group than in the controls, (Figure 4) despite insignificant difference of ocular alignment between the two groups (which is affected by all causes of surgical failure). They stated in their long-term study that if one considers motility limitation as the main outcome measure of AM success rather than ocular alignment, a high success rate would have been achieved, as motility limitation on the final follow-up visits exceeded −1 in only 4/180 muscles in AM group (2.2%).

It is noteworthy that although AM has the advantage of preventing adhesions, it also has disadvantages. Its application adds to the operative time and surgical manipulations. This in turn would presumably incite more inflammation, adhesions, and fibrosis, which, although would be ameliorated in the region of membrane presence, could be accentuated in the surrounding areas. It was, therefore, recommended to utilize a larger AM segment, adequate to cover the largest area possible, to accentuate its beneficial effect of prevention of postoperative adhesions.

In conclusion, a less favorable, yet good, outcome appeared to be achieved clinically, than histopathologically, after cryopreserved AM transplantation during strabismus surgery. The AM merely acts by preventing adhesions in the region of its presence. It is, therefore, recommended to limit the use of AM transplant to cases with extensive adhesions, as expected in those with markedly limited motility and positive forced duction test, and to use the largest AM segment possible, to benefit from its anti-adhesive effect. A new study with these criteria is warranted.

**AM orientation**

Does the orientation of the AM, whether AM stroma or epithelium towards the muscle, make a difference? The stromal side is the sticky one in contact with the paper. While the stromal side is rough, adhesive, and less reflective, the epithelial side is smooth and shiny. The stromal side can be identified by the presence of vitreous-like strands that can be raised with a sponge. Moreover, staining of AM with indocyanine green, rose Bengal, Trypan Blue, or lissamine green B aids in identification of stromal and epithelial surfaces. As it was suggested that the AM stroma is mainly responsible for its actions and that direct contact with the stroma is necessary for its actions to take effect, it was recommended to apply the AM with the stromal side towards the muscle to suppress peritendinous inflammation and
fibrosis. The AM orientation differed among the various studies. Some applied the AM with its stromal side towards the muscle, \(21,44,50,53-56,60,62\), while others applied its epithelial side towards the muscle. \(45,47,57,59\) Variable outcomes were reported by these studies, with no consistence with membrane orientation.

The AM orientation was verified histopathologically in the study of Kassem et al. to determine if the AM was truly oriented stroma towards the muscle, \(53\). The stroma was towards the muscle in three eyes, the AM was inadvertently folded on itself with the epithelium externally in two eyes, and orientation could not be determined in three eyes due to masking by inflammatory cells. Rectus muscle fibrosis was absent only in the two eyes where the AM was folded on itself and conjunctival hyperemia was significantly less. The authors assumed that this could be the best AM orientation. \(53\)

Kassem et al. later performed a histopathological study to compare three different AM orientations during extraocular muscle surgery in rabbits. The superior rectus muscle was resected without an AM wrap (control) or with an AM wrap (AM groups). The three AM groups were stroma towards the muscle (S), epithelium towards the muscle (E), and folded AM with epithelium externally being in contact with muscle and sclera (F). The authors concluded that AM orientation does not make a difference, and AM, at any orientation, was effective in preventing adhesions. \(63\)

The latter study agrees with the former one, \(53\) where adhesions were absent merely in the region of AM presence, but detected elsewhere. Two factors were postulated to explain this. First, the barrier effect offered by the AM acts only in the region of presence of the membrane. Accordingly, the benefit of AM does not extend beyond its area of presence. \(53\) Second, the stroma of the AM was considered mainly responsible for its anti-inflammatory, anti-fibrotic, and anti-adhesive effects; \(23,38\); therefore, these effects could not take place outside the region of AM presence.

It was postulated that direct contact with the stroma was necessary for its actions to take effect. \(23,38\) The absence of adhesions in the latter study in all specimens, regardless of AM orientation, \(63\) and in the former study even in the two eyes where the AM was inadvertently folded, \(53\) partly contradicts this postulation. Moreover, muscle fibrosis was absent in the former study only in the two specimens where the AM was inadvertently folded. \(53\) In addition, muscle fibrosis was least in group F, although insignificantly, in the latter study. \(63\) These again contradict the above postulation. Two suggestions could be presented in this setting. First, the anti-adhesive and anti-fibrotic actions of the AM stroma do not necessitate direct contact with the latter to take effect, but close contact is enough, where the effect is confined to the region of AM application, but does not necessitate an orientation of stroma towards the muscle. Accordingly, the stromal actions were effective even in the

Figure 4. Two patients showing improved versions following strabismus reoperation with cryopreserved AM wrapping of extraocular muscles. (a) Clinical photographs before (top) and after (bottom) left lateral rectus recession and left medial rectus resection with AM wrapping. Before strabismus reoperation there was a left consecutive exotropia with left limited adduction due to tight left medial rectus (forced duction test score of 2, and adhesions score of 3). Left adduction was normal postoperatively. (b) Clinical photographs before (top) and after (bottom) bilateral medial rectus recession and right lateral rectus advancement surgery with AM wrapping. Before strabismus reoperation there was a right residual esotropia with right limited abduction. Postoperatively right abduction was normal, and adduction of the right eye was limited due to overweakened right medial rectus muscle that was positioned 13 mm from the limbus. (Quoted from Kassem et al. with permission.)
specimens where the stroma was oriented away from the muscle. Second, it is noteworthy that the notion, that direct stromal contact is necessary for the stromal actions to take effect, was reported in cases of AM transplantation for ocular surface reconstruction, rather than during extraocular muscle surgery, so mechanisms of action could be different, and in turn direct stromal contact, although necessary during ocular surface reconstruction, might be unnecessary during extraocular muscle surgery. Third, the anti-adhesive and anti-fibrotic effects of AM during extraocular muscle surgery were a result of its barrier effect, rather than the stromal anti-fibrotic actions. In this case, muscle fibrosis was least where the AM was folded, because this provided a double layer of AM around the muscle, which better secluded the muscle from the perilimbal space which is the source of inflammation and fibrosis. Accordingly, although all orientations prevented adhesions, yet a folded AM had a further privilege of less muscle fibrosis, and could be the best orientation.

The mean conjunctival inflammation scores did not differ significantly between all groups (p > .05). Nevertheless, the mean conjunctival inflammation scores were higher in groups F and S (where AM epithelium was towards the Tenon’s capsule/conjunctiva) than in group E (where AM epithelium was away from the Tenon’s capsule/conjunctiva). The rate of development of F.B. inflammation was significantly lower in the control group than the AM groups (p < .05) and was present in 100% of group E eyes. An explanation is that inflammation is presumed to be related to the xenogenic nature of human AM used in rabbits, and it is the AM epithelial cells that possess immunogenic properties. In agreement, significantly less inflammation was previously reported when lyophilized AM (which is deprived of epithelium during processing) was used during extraocular muscle surgery in rabbits.

In conclusion, cryopreserved AM, when used to wrap the superior rectus muscle in rabbits, proved effective in preventing postoperative muscle-sclera adhesions in the region where the AM was present, regardless of membrane orientation. If the stroma cannot be identified with ease, the AM may be placed around the muscle at any orientation. This will omit the unnecessary extra time and manipulations employed to identify the stromal surface, and would, in turn, omit risks of inducing more inflammation and subsequent fibrosis.

**AM fixation**

Is it better to fix the membrane with sutures or not? Some authors fixed the AM with sutures, while others wrapped the membrane around the muscle without sutures. The AM is sticky. Accordingly, some authors believed no sutures are needed to fix the membrane, when it is wrapped all around the muscle, without fear of its migration. Moreover, absorbable sutures generate an inflammatory response during the absorption process, therefore may induce more foreign body inflammation, and subsequent adhesions and fibrosis. In addition, suture fixation adds to the operative time and manipulations, which could further induce more inflammation and subsequent fibrosis. On the other hand, sutureless AM application could carry a risk of postoperative loss or displacement of the membrane. In the study of Kirsch et al., however, the AM was exactly where it was placed during surgery after both 15 and 30 days, supporting the feasibility of the sutureless technique. Nevertheless, in our experience, sutureless AM application sometimes caused its plication during further surgical manipulations, such as during reattachment of the muscle to the sclera or during conjunctival repositioning. Careful and gentle manipulations are, therefore, necessary in case of sutureless application of AM is opted for. An alternative technique is to first reattach the muscle to the sclera, then insert the membrane under the muscle, spread it properly and wrap it.

**Conclusions**

AM transplantation during strabismus reoperations and complex strabismus procedures could be a promising option to limit adhesions and improve surgical success rates, provided certain precautions are considered. Fresh AM transplantation poses a risk of transmission of communicable diseases, therefore better avoided. Dried AM was ineffective in preventing adhesions, therefore is not recommended. Cryopreserved AM is the best option, being safe and effective in preventing the development of adhesions in the region of its presence. A less favorable outcome was, however, achieved clinically, than histopathologically, after cryopreserved AM transplantation during strabismus surgery. The AM has the advantage of preventing adhesions in the region of its presence, yet it has some disadvantages. Its application adds to the operative time and surgical manipulations. This, in turn, would presumably incite more inflammation, adhesions, and fibrosis, which although would be ameliorated in the region of membrane presence, could be accentuated in the surrounding areas. Four recommendations are, therefore, proposed. First is to limit the use of AM transplant to cases with extensive adhesions, as expected in those with markedly limited motility and positive forced duction test, to benefit from its anti-adhesive effect. Second is to utilize the largest AM segment possible to limit adhesions over large areas. Third, if the stroma cannot be identified with ease, the AM may be placed around the muscle at any orientation. This will omit the unnecessary extra time and manipulations employed to identify the stromal surface, and would, in turn, omit risks of inducing more inflammation and subsequent fibrosis. Lastly, sutureless AM application could be better, as this incites less inflammatory reaction and fibrosis and shortens the surgical time, provided care is taken during subsequent surgical steps to avoid membrane displacement or plication.

A number of further studies may be proposed. One is a controlled, prospective study on the value of cryopreserved AM transplantation during strabismus reoperations, including only patients with extensive adhesions and utilizing the largest possible segment of AM to cover as much areas as possible, around the extraocular muscle and subconjunctivally. In addition, all reports, describing the findings obtained on reoperating on extraocular muscles previously wrapped with AM, are encouraged.

**Disclosure statement**

No potential conflict of interest was reported by the authors.
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