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REVIEW

Insights on the Human Amniotic Membrane in Clinical Practice with a Focus on the New Applications in Retinal Surgery

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Lay Summary

Recently, the use of the human amniotic membrane (hAM) has been extended to treat retinal disorders such as refractory macular holes, retinal breaks and dry and wet age-related macular degeneration. Not only the hAM has proved to be an excellent tool for repairing retinal tissue, but it has also shown a promising regeneration potential. This review aims to highlight the novel use of the hAM in treating retinal diseases. Although the hAM has been used in the ocular anterior segment reconstruction for more than 60 years, in the last 2 years, we have found in literature articles showing the use of the hAM in the retinal surgery field with interesting results in terms of tissue healing and photoreceptor regeneration.

Keywords Human amniotic membrane · Retinal regeneration · Nerve regeneration · Post-stroke recovery · Corneal regeneration

Introduction

Retinal diseases such as refractory macular holes, age-related macular degeneration and retinal detachment are characterized by a permanent loss of a large part of the photoreceptors. In recent years, vitreoretinal surgeons have proposed numerous procedures and substances to induce a regeneration of the retinal layers and, especially, the photoreceptor layers.

Recently, Prof Rizzo, Dr. Caporossi, et al. published a group of articles on the use of the human amniotic membrane (hAM) to repair refractory macular holes or peripheral retinal tears ([1]). Not only have they proved that the human amniotic membrane (hAM) is an excellent tool for repairing retinal tissue, but they also have shown that it can induce a regeneration of the retinal layers. The authors have shown optical coherence tomography (OCT) images indicating a regeneration of the retinal layers (Fig. 1) with a cellular migration that occurred above the amniotic membrane (Fig. 2). The authors demonstrated a visual acuity improvement in almost all patients.

Moreover, the authors extended the use of hAM in other retinal pathologies such as myopic macular holes associated with retinal detachment [2] and dry and wet age-related macular degeneration [3]. Prof Rizzo and Dr. Caporossi's group have proposed, for the first time, the use of the hAM to treat these pathologies. The purpose of this review is to report all the scientific evidence that has induced Prof Rizzo and Dr. Caporossi to apply the hAM graft in retinal pathologies.

The hAM is non-immunogenic [4], contains high levels of regenerative growth factors [5] and has anti-inflammatory and antiangiogenic properties [6].

Placental transplants have been used in surgical practice due to their biochemical properties and bio-architectural structure.

The hAM is the innermost layer of the placenta, and it is also called amnion. The hAM is a semipermeable avascular layer characterized by nerves, lymphatic tissue and matrix containing cytokines, enzymes and other active molecules. The hAM is translucid, 0.02–0.05-mm thick and made up of an epithelium: cuboidal cells with abundant microvilli and active metabolism, a basement membrane composed of IV and VII collagen, laminin,

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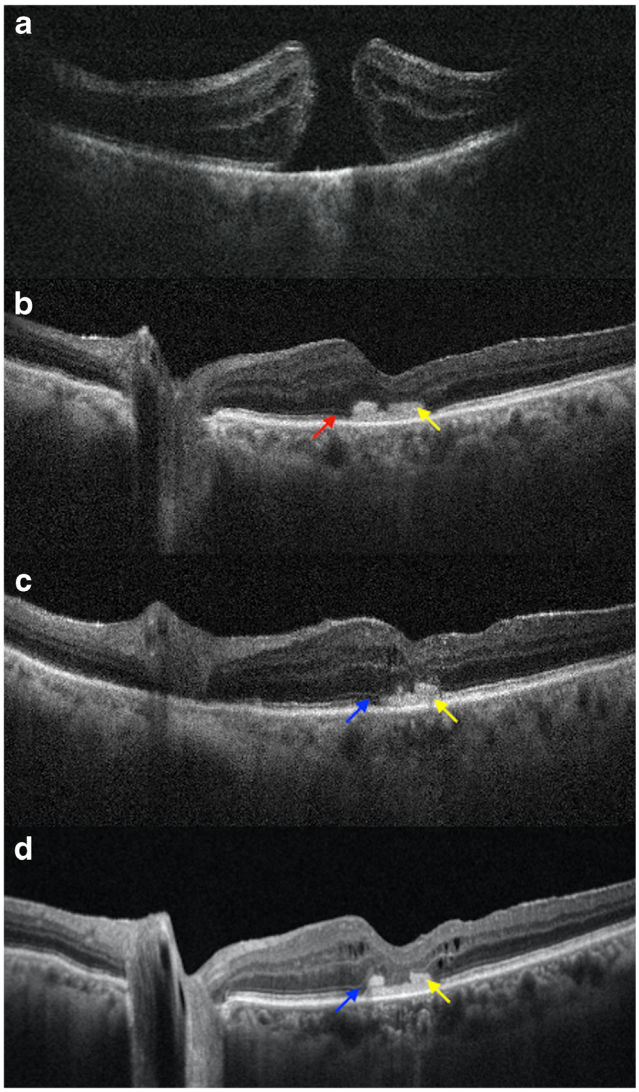

Fig. 1 Preoperative OCT showing a macular hole that failed to close, with an already peeled ILM (A). Three months postoperative OCT showing the macular hole closure. The regenerative process of the external retinal layers is starting (red arrow) (1B). Six months OCT (1C) and 12-month OCT (1D), the regeneration of the external retinal layers progressed (red arrows) and we cannot observe any sign of rejection. The amniotic membrane partially dissolved after 1 year (yellow arrow)

fibronectin, hyaluronic acid and an avascular stroma [7–9]. The hAM can produce a wide variety of growth factors such as transforming growth factor (TGFα, TGFβ-1, β-2 and β-3), basic fibroblast growth factor, the epithelial growth factor, hepatocyte growth factor and its receptor and keratinocyte growth factor and its receptor [10–15]. Surgically implanted hAM wraps, furthermore, are a reservoir of neurotrophic factors as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), glial cell–derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF) [16, 17].

Human Amniotic Membrane Cells in Nervous Tissue and Neuronal Regeneration

It has been demonstrated that hAM cells can differentiate in neural cells in vivo and in vitro [18]. Moreover, the expression of glial cell–derived neurotrophic factor (AF-GDNF), secreted by the hAM cells, can determine a neuroprotective effect [19].

Studies, conducted in the animal model, have shown that the hAM can regenerate small, surgically sectioned, nerves [20, 21].

Riccio et al. applied a hAM graft wrapping in five human patients with a median nerve injury and showed promising results [22, 23].

Amnion epithelium produces brain natriuretic peptides and hormones that release corticotropin, a cell proliferation promoter [24].

Human amniotic epithelial cells (hAECs) can be obtained from the epithelial sheet of the amnion, which are immediately available and a relatively economical reservoir of cells for clinical application.

hAECs are foetal in principle and therefore exhibit a high level of pluripotency. Contrarily to other stem cell lineages, hAECs are readily available and do not require invasive harvesting procedures. hAECs are immunologically inert due to the low surface expression of human leukocyte antigens (HLA)-A, HLA-B, HLA-C and HLA-DR, which are critically implicated in the post-transplant rejection [25, 26]. Conversely, hAECs express and deliver the non-polymorphic, non-classical antigen, HLA-G, which is capable of repressing immune responses. Furthermore, hAECs do not form neoplasia in vivo and do not differentiate into fibroblasts [25, 26]. The non-immunogenicity characteristics of the hAM suggest that they are safer than many other stem cells. In the animal model, hAECs have shown regenerative and protective potentials after an experimentally induced stroke [27].

A group of researchers [28] have enrolled a Phase I clinical trial to determine the maximum tolerable dose of hAECs in human ischemic stroke patients (Australian New Zealand Clinical Trials Registry: ACTRN12618000076279p; [26]). Gong et al. [29] showed that hAMSCs can differentiate into Schwann-like cells, with the proper chemical mediators' administration in vitro, and a considerable quantity of promoting nerve growth factors are released during the differentiation process.

HAECs have been used in the animal model with good results concerning the physical recovery in traumatic cerebral injuries [30, 31], hypoxic-ischemic injury [32] and ballistic-like brain injury [33].

HAECs can modulate the local inflammation favouring a protective/pro-regenerative environment in the damaged brain. This may produce an improvement in the neurological function.

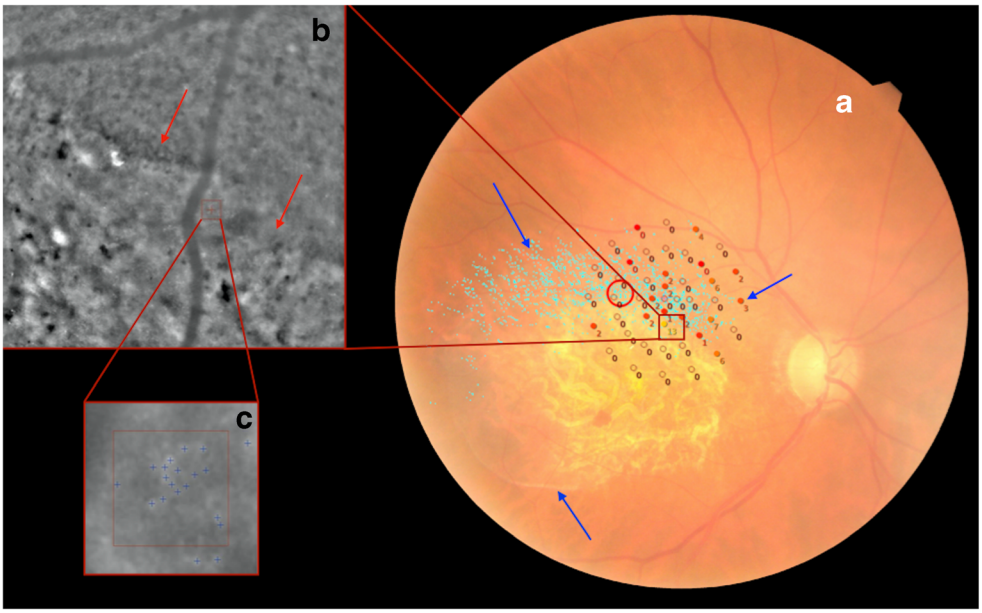

Fig. 2 Microperimetry (A) of a patient treated using choroidal neovascularisation removal and subretinal amniotic membrane implant. We can observe the amniotic membrane disc edges (blue arrows), a stable fixation at the superior edge of the amniotic membrane disc (blue dots) and the microperimetric numeric values (red dots with numbers). The adaptive optic scan was conducted in the area represented by the red square (B); we can observe the amniotic membrane edges (red arrows) and a magnified image showing the photoreceptors cells, calculated using the in-built software (Imagine eyes Rx, France) (C)

We can observe that both hAM wraps, sheets and HAECs can induce a regeneration in the neuronal tissue. It is clear that, when the target tissue is more accessible, such as a nerve, it is easier to use sheets or wraps. On the other hand, when a brain suffers from a stroke, we need to use HAECs, because this tissue is not accessible for a hAM implantation.

Human Amniotic Membrane in Other Surgical Specialities

Amnion epithelial and mesenchymal cells are pluripotent stem cells which have the ability to differentiate into all three germ layers. Amniotic cells also secrete growth factors, anti-inflammatory and anti-bacterial agents which create a synergic environment for tissue healing.

The hAM controls the scarring formation through the reduction of fibrosis [34]. This effect is achieved by the downregulation of TGF-b and its receptor expression [35]. The hAM has been used in different surgical subspecialties; it can be used as a dressing or as a transplanted graft.

One of the most modern advances in wound therapy is the use of hAM transplantation for the treatment of acute and chronic skin injuries. An artificial dehydrated human amnion/chorion membrane allograft (dHACM, EpiFix; MiMedx Group, Marietta, GA) was used to treat a refractory ulcer in a 10-month-old girl, affected by a facial hemangioma [36].

EpiFix® is a cellular amniotic sheath allograft that carries many growth factors, cytokines and extracellular matrix proteins already in amniotic tissue. Moreover, it contains platelet-derived growth factor (PDGF) and more than 50 growth factors, cytokines and chemokines [37]. Epifix® was also used for cutaneous ulcers such as scalp ulcers [38], burns [39], gastric ulcers [40], venous leg ulcers [41] and diabetic foot ulcers [42, 43].

The amniotic membrane is histologically similar to cutaneous and nervous tissue; in fact, it arises from the embryonic ectoderm. Moreover, its transparency, which offers the possibility to surveil scar formation, is very useful in the tissue regeneration field. In addition, the amniotic membrane has other interesting properties such as the capacity of decreasing exudation and wound infection, and it also accelerates epithelial regeneration, has analgesic properties, decreases heat loss and reduces scar tissue formation [44].

Swim et al. [45] created an amnion-derived scaffold to repair arteries. They obtained multi-layered compositions characterized by in vitro biocompatibility with smooth muscle cells, endothelial cells, cardiac myocytes and thymus and cord-blood-derived mesenchymal stem cells. When applied in a piglet model of left pulmonary artery graft, the multi-layered sheet showed its in vivo biocompatibility and its usefulness for vascular reconstruction, as demonstrated by the generation of a newly developed endothelium in the intima,

a smooth muscle cell-rich intermediate layer and an adventitia including new vasa vasorum [45].

Moreover, it has been demonstrated that the hAM acts as a biological dressing, accelerating re-epithelialization and preventing invasive bacterial infection in burn injuries [46].

The hAM was used in dogs as a pericardial coverage to reduce post-surgical adhesion [47], and it elicited insignificant host to graft response due to its low immunogenicity. Preliminary results, in the animal model, have advised that the AM grafts may be a safe and useful method for restricting continued air leakage from the pleura after thoracic surgery [48].

Amniotic Membrane in Ocular Anterior Segment Treatments

The AM is an excellent substrate and acts as a scaffold for tissue reconstruction, supporting the germination of corneal epithelial progenitor cells by increasing their lifespan, supporting their clonogenicity and limiting epithelial cell apoptosis [49]. The hAM has an anti-inflammatory effect which is achieved through the secretion inhibition of a wide range of mediators, such as the interferon (IFN), bFGF, interleukin (IL)-1,TNF-b and platelet-derived growth factor (PDGF) on the damaged corneal and conjunctival surface [50, 51]. Its stromal matrix can induce apoptosis in inflammatory cells and contains several forms of protease inhibitors [52–54]. These properties of the AM may reduce scars and inflammation after its transplantation.

The structural integrity, transparency and elasticity of the amniotic basement membrane make it a widely accepted tissue replacement for ocular surface reconstruction.

The use of amniotic membrane transplantation to treat ocular surface abnormalities was first reported in 1940 as a graft for conjunctival surface reconstruction [55]. Later, it has been used for treating acute ocular caustic burns [56]. After these experiences, the use of amniotic membrane disappeared from the literature until 1995, when Kim and Tseng reported a study which described a rabbit cornea reconstruction [57]. The interest in this procedure has grown after the improvements in the methods of processing and preserving the hAM, and so the possibility of maintaining its inherent properties [53].

In most recent years, it has been demonstrated that the human amniotic membrane can be successfully used in the treatment of persistent corneal epithelial lesions [58–60], corneal ulcerations [59, 61, 62], symptomatic bullous keratopathy [63], band keratopathy [64], chemical and thermal burns [65, 66], recurrent pterygium [67, 68], ocular cicatricial pemphigoid and Stevens-Johnson syndrome [69]. In these cases, the hAM worked as an optimal biological support for cell growth. Moreover, clinical outcomes of repeated autologous cultivated limbal epithelial cells' transplantation for ocular surface burns showed that the hAM is a useful promoter for stem cells' growth and differentiation [70, 71]. In treating the ocular surface diseases, the hAM has shown remarkable reconstructive and regenerative capacities; moreover, it has demonstrated anti-inflammatory, antiangiogenic, and antimicrobial effects [53, 54, 72–77].

Many authors think that the hAM triggers the repression of conjunctival fibrosis, the reduction of inflammatory cytokines, the epithelialization promotion and the suppression of protease activity [78], although the specific regulatory mechanisms are not entirely understood. Transplanted hAM can remain on the corneal surface for a long time without being deteriorated or assimilated, but its presence does not cause an inflammatory reaction, rejection or opacity [79].

The use of hAM in anterior segment ophthalmic surgery has gained enormous popularity, as shown by the over 700 reports by 2009 [80].

Amniotic Membrane in Retinal Pathologies

The hAM seems to be a good candidate in the treatment of retinal degenerative diseases, including age-related macular degeneration, thanks to its anti-inflammatory and antiangiogenic properties and the ability to create an excellent growth support for the host's retinal pigment epithelium (RPE) cells. Recently human RPE cells have been cultured in vitro over a hAM sheet, which has demonstrated to be a feasible substrate for growth and proliferation of the RPE. The RPE cells maintained epithelial features and constituted a highly organized monolayer [81]. Moreover, Ohno-Matsui et al. [82] have demonstrated that RPE cells cultured on a hAM sheet acquire an epithelial phenotype and secrete several growth factors necessary for maintaining the retinal homeostasis. Later, hAM was implanted into pig's subretinal space, which underwent the RPE surgical removal and a mechanical damage to the Bruch's membrane. The hAM was well tolerated, caused only limited inflammation, reduced significantly the choroidal neovascularization and was covered by a monolayer of pigmented cells in contact to the host RPE [83].

Finally, the hAM plug was used in the treatment of suprachoroidal silicone oil migration related to a choroidal hole in a traumatic globe rupture that had caused hypotony. The hAM acted as a mechanical scaffold and promoted collagen formation and choroid vascular growth, achieving the tissue regeneration 3 months postoperatively [84].

Prof Rizzo, Dr. Caporossi and colleagues [1] implanted, for the first time, in the human eyes a hAM plug under the retina to promote tissue healing and regeneration. They used a cryopreserved hAM which was contained in a solution composed of a cryoprotectant (glycerol or dimethyl sulfoxide–DMSO) and a Dulbecco's modified Eagle's medium. This type of preparation allows a 20 to 50% amniotic cell survival rate [85] and represents the current format in clinical practice and clinical trials [86]. Inside the vitreous cavity, the hAM was handled under

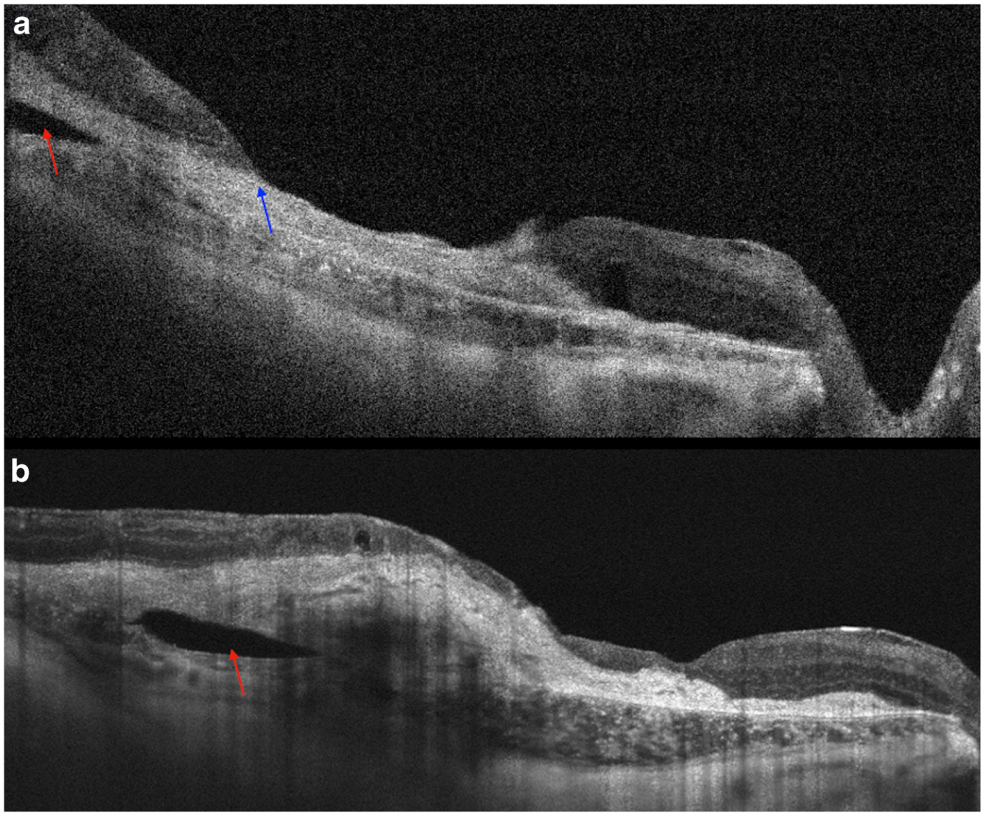

Fig. 3 Postoperative OCT showing an extremely large iatrogenic macular tear (4.78 mm) with an amniotic membrane disc underneath. The blue arrow indicates the macular hole edges (A). The 6-month OCT showed an inward migration of the hole margins that covered the large hole. (B) The same choroidal lacuna is evidenced in both figures using a red arrow

fluid and grafted through a retinal break or a macular hole into the subretinal space; it was then spread to cover the entire portion of the retinal defect. A silicone oil injection was performed at the end of the surgery. The OCT analysis revealed a retinal adhesion over the amniotic membrane plug in the macular holes or the retinal break sites during the first week after surgery. During the first 2 to 3 months, a retinal ingrowth at the retinal break's margin over the hAM plug was observed in all the cases. After the silicone oil extraction, the OCT scan revealed the retinal regeneration of the external layers over the hAM plug (Fig. 1). All the cases improved their visual acuity, except

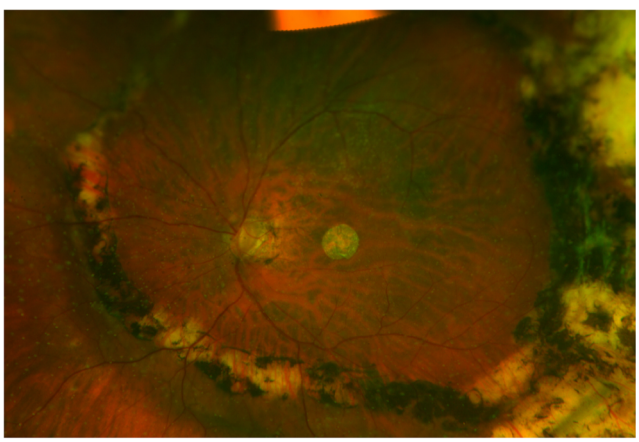

Fig. 4 A hAM successful transplant in an age-related macular degeneration case

a severe macula-off rhegmatogenous retinal detachment which maintained the same low visual function. They did not observe any proliferative vitreoretinopathy or abnormal scarring. After these results, Prof Rizzo and Dr. Caporossi's group published numerous articles, extending the hAM's indications for other retinal pathologies such as myopic macular holes associated with retinal detachment [2] and chronic post traumatic macular holes [87]. Moreover, they published interesting surgical techniques, inspired by corneal surgery, for the hAM insertion [88]. The first macular holes were tamponed using silicone oil and gas because the authors were concerned about hAM's postoperative dislocation, whereas the most recent cases were tamponed with air only because they understood that the hAM plug was stable inside the macular hole in the postoperative time [89]. Prof Rizzo's group obtained interesting results, using the hAM, also in the end-stage dry and wet age-related macular degeneration treatment [3]. Not only the hAM, positioned under the retina, demonstrated its safety and non-immunogenicity, but it showed a good microperimetry function and photoreceptor cell repopulation (observed using adaptive optics technology) (Fig. 2). Age-related macular degeneration is the leading cause of blindness in the industrialized world and Prof Rizzo's group for the first time introduced the hAM in the treatment of this chronic degenerative disease. In recent times, interesting new surgical treatments have been suggested for AMD. Autologous retinal and chorioretinal transplants have been proposed by Dr. Parolini [90], and Prof. Da Cruz created

Table 1 Dr. Caporossi's and Prof Rizzo's outcomes on the use of the hAM in retinal pathologies

Paper	Pathology treated	Operation performed	Anatomical results	Functional results
Caporossi et al. Acta o phthalmologica 2019	Case report describing a large irregular iatrogenic macular tear (around 5 mm max diameter) revealed after choroidal neovascularisation membrane removal	The patient underwent a PPV and the cryopreserved hAM was placed under the macular defect	The retina grew over the hAM patch and the macular large tear closed	The visual function improved from the only light perception to 20/400
Rizzo et al. Retina 2018	8 eyes with a refractory macular holes and 6 eyes with a retinal detachment	The cryopreserved hAM was placed into the macular hole and inside the retinal tears	The refractory macular holes closed with a regeneration of the external retinal layers in almost all cases. The retinal detachment cases resolved with the retinal tears well sealed by the amniotic membrane	In the macular hole group, the BCVA improved from 20/800 preoperatively to 20/100 3 months after the operation, and to 20/50 6 months after the operation In the retinal detachment group, BCVA improved from 20/2000 preoperatively to 20/400 after 3 months, and to 20/125 2 months after silicone oil removal
Caporossi et al. Acta Opthalmologica 2019	10 eyes of 10 patients with recurrent high myopic macular hole and retinal detachment	PPV with a cryopreserved hAM plug implanted into the macular hole	The retina reattached in all the patients and the macular hole closed with a partial regeneration of the retinal layers	The BCVA improved from 1.73 logMAR to 0.94 logMAR after 6 months
Rizzo et al. Ophthalmology Retina 2020	6 eyes with age-related macular degeneration complicated by choroidal neovascularization and 5 eyes complicated with geographic atrophy	The cryopreserved hAM was placed under the macular area	No major adverse events were reported; and the hAM remained in place in all the patients. The adaptive optics imaging showed a possible photoreceptor presence over the hAM membrane	The mean preoperative BCVA was 20/2000 The mean final BCVA improved to 20/400
Rizzo et al. Retina 2020	3 eyes of 3 patients affected by macular detachment associated with optic nerve head pit	The cryopreserved hAM was placed into the optic pit	The subretinal fluid resolved in all cases during the 6-months of follow-up	The mean BCVA improved from 20/200 to 20/25 at the sixth month after surgery
Caporossi et al. OSLI 2019	Case report describing a case of large posttraumatic macular hole that was opened 25 years before. The macular hole basal diameter was 971 μm	The patient underwent a pars plana vitrectomy with an hAM plug implanted into the macular hole	The macular hole closed 10 days after the operation	The preoperative BCVA was 20/400. The postoperative BCVA improved to 20/100

an embryonic stem cell–derived retinal pigment epithelium patch [91]. However, the autologous choroidal and retinal transplant are very challenging procedures with a high risk of complications such as retinal detachment with proliferative vitreoretinopathy, and the embryonic stem cell–derived retinal pigment epithelium patch was associated with systemic corticosteroid therapy because of the risk of immunogenic graft rejection. Prof Rizzo and Dr. Caporossi think that the combination of these new treatments with the hAM may be a step forward in the treatment of the AMD also because it has been demonstrated that the hAM can actively reduce abnormal neovascularization [75, 92] which complicates many inflammatory/degenerative diseases such as AMD. Finally, I would like to mention a case report involving a large iatrogenic macular hole that was repaired using a hAM sheet [93]. The patient had a near 4.78-mm-diameter iatrogenic macular hole after a choroidal neovascularization membrane removal. The amniotic membrane, positioned under the retina, triggered an inward migration of the hole margins that led to the hole closure. It is the first time that such a large macular hole closure was reported (Fig. 3). Regarding the anatomical and functional outcomes between dehydrated and cryopreserved hAM, a group of authors reported no significant differences in 17 consecutive cases of recurrent macular holes [94]. It may be interesting in the future to investigate micronized hAMs for retinal surgical cases. Micronization is a novel process to obtain amnion sheets, recently taken into consideration by several authors. Micronized amniotic membranes, although they have a reduced diameter, are still a reservoir of regenerative molecules and still represent a valid scaffold for tissue regeneration [95]. It may be interesting to publish the results of micronized hAMs in complicated retinal cases because these types of engineered hAMs have shown optimal properties with also a reduced diameter, which may be more suitable for micrometric retinal nervous tissue (Fig. 4).

Conclusion

To conclude, the hAM has several advantages: it is non-immunogenic, contains high levels of regenerative growth factors and has anti-inflammatory and antiangiogenic properties.

The hAM can be a tool for the creation of valid biomaterials to regenerate human nervous structure such as the retina and the RPE.

The use of hAM in surgery is increasingly proposed in the era of regenerative medicine. Prof Rizzo's and Dr. Caporossi's articles, in the field of macular holes, optic pit, retinal detachment and age-related macular degeneration, are cutting edge and promising. Specialists in the field of tissue regeneration should continue investing in this easily accessible technology.

Table 1 shows Dr Caporossi's and Prof Rizzo's outcomes on the use of the hAM in retinal pathologies.

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