# Analysis of intraocular amniotic membrane in vitreoretinal disease with complicated retinal detachment - case reports and in-vitro safety assessment.

**Anna Hillenmayer**
University of Ulm
**Christian M. Wertheimer**
University of Ulm
**Maximilian-Joachim Gerhard**
Ludwig-Maximilians-University Munich
**Siegfried G. Priglinger**
Ludwig-Maximilians-University Munich
**Andreas Ohlmann**
Ludwig-Maximilians-University Munich
**Armin Wolf** ( armin.wolf@uniklinik-ulm.de )
University of Ulm

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## Abstract

# Purpose

Amniotic membrane (AM) is a popular treatment for ocular surface diseases due to its wound healing effects. First intraocular implantations showed good anatomical and functional results. Here, three cases of intravitreal AM transplantation and the morphological and functional outcome are described. The reaction to AM was examined by immunostaining and cellular reactions were investigated on retinal cells in-vitro.

# Methods:

Three patients with epiretinal implanted AM during PPV for complicated retinal detachment are presented retrospectively. We investigated the AM-influence in-vitro on RPE cells (ARPE-19), Müller cells (Mio-M1), and retinoblasts (661W). An anti-histone DNA ELISA and live/dead assay for apoptosis, a BrdU ELISA for proliferation and a WST-1 assay for viability were performed. Following AM-removal, tissue-specific cellular responses were evaluated by light microscopy and immunohistochemical staining.

# Results:

Despite severe vitreoretinal disease, good anatomical and functional outcomes were achieved in all three cases. Immunostaining of AM showed no immunological rejection. In-vitro, AM mediates no toxic or proliferating effect on RPE and Müller cells but a neuroprotective effect on differentiated retinoblasts.

# Conclusion:

AM offers possible advantages treating severe vitreoretinal disease. The pathobiological mode of action was elucidated and we could not determine issues regarding rejection reactions and biocompatibility. Further studies are needed to assess clinical potential.

## Introduction

Severe vitreoretinal disease with complicated retinal detachment as observed in trauma or endophthalmitis can result in loss of vision.[1] Depending on the extent of the initial damage, disruption of integrity of the vitreoretinal barrier and blood-retinal barrier as well as direct damage to the retina occurs. Further complications arise from the development of fibrotic membranes in proliferative vitreoretinopathy (PVR).[2]

Amniotic membrane (AM) transplantation is widely used to treat external ocular diseases due to its anti-inflammatory, anti-apoptotic, anti-infective and anti-fibrotic effects as well as its good biocompatibility.[3]

Bearing this potential, AM was recently used to treat vitreoretinal disease by direct implantation during pars plana vitrectomy (PPV). Several indications have been treated with either epiretinal or subretinal AM. Examples are large macular tears,[4] high myopic retinal detachment associated with macular holes,[5] retinal tears,[6] optic pits,[7] and advanced age-related macular degeneration.[8]

A further interesting use of AM in vitreoretinal surgery is complicated retinal detachment. Subretinal AM was demonstrated in six patients in a case series with good anatomical and functional outcomes.[9] There is also evidence from in-vitro and animal studies that pathobiological processes leading to worse outcomes can be modified by AM. In retinal pigment epithelial cells (RPE), AM was phenotype-stabilizing, indicating an action against PVR formation.[10, 11]

Based on these previously mentioned positive properties, we aimed at using AM in severe vitreoretinal disease with complicated retinal detachment. Three cases of vitreoretinal AM in two penetrating choroidal wounds as described elsewhere in a case report[12] and one in endophthalmitis are presented. In all patients, the AM was removed during silicon oil removal and tissue specific reactions were evaluated by light microscopy and immunohistochemical staining to assess safety. In additional in-vitro experiments, we investigated the mode of action and biocompatibility on retinal cell types such as RPE, Müller glial cells, and differentiated retinal neurons.

## Results

# Case presentation

In the first case, a 67-year-old male patient suffered from symptoms of protracted endophthalmitis due to blebitis six days before the examination. Fundoscopy was not possible because of purulent vitreous material and BCVA was light perception. A PPV with peeling and intravitreal antibiotics was performed. Intraoperatively, the retina was covered with pus, multiple membranes, and subretinal infiltrates. Four weeks later, a second PPV was required because a retinal detachment had developed due to a large full thickness macular hole. Closure of the macular hole was not possible because of the size and fragile edematous retina. The internal limiting membrane could not be mobilized, and a posterior capsulotomy had already been performed. The macular hole was therefore patched with AM, which was fixed with a silicone oil tamponade. In the postoperative course, the intraocular irritation decreased, and the retinal findings stabilized. Corrected visual acuity increased to 1.3 logMAR measured eight months after surgery. Silicone oil and the amniotic membrane patch were removed another two months later. The anatomic result was confirmed 22 months after initial presentation. (Fig. 1a, b)

Secondly, a 48-year-old male patient suffered a car battery explosion. The trauma caused several minor injuries in the craniofacial area and a second-degree burn on the left eye. The right eye, however, exhibited complete hyphema and only light perception. On the sixth day, a PPV was performed. During surgery, a perforating injury with incarcerated retina was observed at the posterior pole. After removal of the lens and loading the eye with AM via the anterior chamber, the exit wound was covered, and the wound gap

was filled with AM. At the end of surgery, a gas tamponade was instilled. Due to bleeding next to the AM, we decided to remove the AM 8 weeks later. A wide-angle angiography performed preoperatively showed no evidence of inflammatory or vascular changes in the surrounding retina and vessels. Six weeks after the second surgery, the best-corrected visual acuity was 0 logMAR with irritation-free intraocular findings. A few weeks later, secondary implantation of an intraocular lens into the sulcus was performed. Six months after the initial trauma, good anatomic and functional results were observed, and the patient reported subjective freedom from symptoms.

The third case is a 28-year-old male patient, who presented with a traumatic ruptured globe during a fist fight. Visual acuity was light perception, and the eye was hypotonic. In due course, exploratory surgery with primary wound closure was performed. A 16-mm scleral rupture at the superior insertion of the lateral rectus muscle, extending posteriorly to well behind the equator, and a triangular rupture extending to the posterior insertion of the superior oblique muscle, with prolapsed choroid, vitreous, and retina, could partly be sutured, however the posterior part of the wound needed to be left unsutured. Due to persisting hypotony eleven days after primary wound closure, PPV was performed. Intraoperatively, incarcerated retina and choroidal hemorrhage were noted. After cataract extraction, the incarcerated retina was carefully mobilized from the tear. An AM was used to completely cover the tear and associated minor retinal defects. Silicone oil was used as a tamponade. Within 4 weeks after PPV, corrected visual acuity increased to 0.3 logMAR (Fig. 1c, d).

# Histopathology of explanted amniotic membranes

To investigate potential immunological reactions against the implanted amniotic membrane as well as its role on PVR reaction, light microscopy and immunohistochemical staining against various lymphoid markers were performed.

In the ocular trauma cases, light microscopy revealed an almost acellular and avascular stroma of the explanted amniotic membranes, which were lined on one side with devitalized epithelial ghost cells without nucleus (Fig. 2a). Furthermore, the epithelial origin of the epithelial ghost cells could be confirmed by staining against cytokeratin (Fig. 2b). However, only a few lymphocytes showed an immunoreactivity for CD45 in both cases suffering from an ocular trauma (Fig. 2c). No signal was obtained for CD3 and CD20, as markers for mature T-cells and B-cells, respectively.

After the explantation of the amniotic membrane that was used for macular hole closure, spindle shaped cells with surrounding connected tissue were detected in the center of the specimen (Fig. 3a, b), suggesting that potential PVR cells grow onto the amniotic membrane. Furthermore, thin acellular membranes at the surface of the amniotic membrane were observed, which had a fibrillar structure after staining against glial fibrillary acidic proteins (GFAP), indicating partial adhesions between the amniotic and the inner limiting membrane (Fig. 3c, d). In addition, several CD68 positive cells, which is a marker for macrophages and microglia cells, were seen adjacent to the amniotic membrane and within their fibrotic tissue (Fig. 3e, f). Again, no immunohistochemical staining for CD3 or CD20 was detected.

# Amniotic membranes mediate no toxic or proliferative effects on immortalized RPE cells

Since the RPE has critical functions in maintaining retinal homeostasis and is involved in the formation of PVR membranes, the potential toxic and proliferative effects of amniotic membranes on immortalized RPE cells were investigated in-vitro. After three days of incubation of ARPE-19 cells with amniotic membrane patches, no difference in the relative amount of histone-DNA-fragments was detected compared with the untreated controls (Fig. 4a). These results are consistent with the live-dead assay in which only sporadic apoptotic cells were seen (Fig. 4d, e). Furthermore, in the WST-1 assay, an increase of metabolic activity of more than 17% ($p = 0.004$) was observed in treated cells when compared to controls (Fig. 4b). However, no increase in cell proliferation was detected by BrdU ELISA in ARPE-19 cells following incubation with amniotic membrane patches (Fig. 4c).

# Amniotic membranes mediate no toxic or proliferative effects on immortalized Müller cells

Like RPE cells, Müller cells play a crucial role in maintenance of neuroretinal homeostasis and are involved in PVR formation. To this end, possible toxic and proliferative effects of amniotic membranes on immortalized Müller cells (Mio-M1) were investigated by anti-histone DNA ELISA, BrdU ELISA, and WST-1 assays in-vitro. After incubation of Mio-M1 cells with amniotic membrane patches, no difference was observed between the amount of histone DNA complexes (Fig. 5a), WST-1 metabolism (Fig. 5b) and BrdU incorporation (Fig. 5c) compared with untreated controls. Our data strongly suggest that amniotic membranes have no toxic effects and a negligible effect on the metabolic or proliferative activity of immortalized Müller cells.

# Amniotic membranes mediate protective effects on differentiated retinal neuroblasts

Photoreceptor-derived retinal neuroblasts (661W) were differentiated with staurosporine. To induce apoptosis, the differentiated 661W cells were incubated in serum-depleted cell culture medium. In the control group without amniotic membrane exposure, a substantial number of cells were detached or showed cell rounding, shrinkage or bleb formation after 24 hours signs, which strongly indicate cellular apoptosis. In contrast, the incubation of differentiated 661W cells with AM reduced the number of cells with apoptotic signs (Fig. 6c, d). To quantify these morphological observations, apoptosis and viability were analyzed by anti-histone DNA ELISA and WST-1 assay, respectively. In amniotic membrane-treated cells, the amount of histone DNA complexes was reduced by more than 20% ($p = 0.004$) compared with untreated controls. The viability of differentiated 661W cells was increased by approximately 18% ($p < 0.001$) after incubation with AM compared with untreated controls (Fig. 6a, b).

# Discussion

In summary, despite severe vitreoretinal disease, an unexpected good anatomical and functional outcome was achieved in all three cases when using intravitreal amniotic membranes. None of the explanted amniotic membranes showed evidence of immunological rejection reactions or cell proliferation in the sense of PVR. Further on, by clinical observation and in-vitro experiments, we could almost rule out that intravitreal amniotic membranes mediate toxic effects on retinal neurons. Our conclusions rest upon the observation that

1) clinically, no intravitreal inflammation and no lymphocytes adjacent to the explanted amniotic membranes were observed, 2) clinically, no PVR reaction, no proliferating RPE cells adjacent to the explanted amniotic membrane and no proliferative effect of amniotic membranes onto immortalized RPE or Müller cells were detected, and finally, 3) the absence of retinal degenerations or excessive scare formation as well as the non-attendance of toxic effects of amniotic membranes onto retinal cells in-vitro was noted.

Proliferative effects in the sense of a PVR reaction were not detected in explanted AM. In the explanted amniotic membranes of all three patients, no T- or B-cells could be detected by immunohistochemistry, strongly suggesting that a rejection reaction against the AM does not take place in a classic “host vs graft” manner after implantation into the vitreous cavity. During the AM-retinal exposure, we did not observe any sign of unspecific inflammation upon imaging during follow-up.

Our cell culture experiments also suggest that an amniotic membrane itself has no toxic or proliferative effect on immortalized RPE cells as well as immortalized Müller cells but rather a neuroprotective effect on differentiated retinoblasts. In addition, amniotic membranes did neither mediate toxic nor proliferative effects on all tested cell types.

Sensitive neuroretinal tissue reactions to already small mechanical as well as biochemical disruptions can lead to extensive functional damage, secondary degeneration and remodeling. [2] [13] [14] The following neuretinal degeneration is one the main causes of severe vision loss and blindness worldwide. Hence, when operating in the posterior segment of the eye and implanting allogeneic tissue, biocompatibility and immunocompatibility is a major concern to halter and prevent further immunological response. [15] We therefore evaluated the biocompatibility in cell culture on three classical retinal cell lines known for driving retinal degeneration and stress response. [16] The explanted AM were stained for immunohistochemistry and we clinically assessed function, reaction and morphological integrity of the retina in all three cases via fundus examination and OCT of the macula as well as UWF imaging (autofluorescence, color-photo). Other studies including human and animal studies confirm these effects of AM and even postulate a regenerative rather than degenerative effect on the outer retinal layers. [8] Small clinical case series with AM do not show toxic or graft rejection effects and AM seems to be well tolerated in the posterior segment.[4–8]

AM was used successfully as an intraocular healing method for severe intraocular trauma cases with complicated, hard to treat wounds and secondary tissue reactions. It needs to be pointed out, that PVR as a major complication after open globe injury did not occur.

Study limitations that should be discussed are the retrospective character, no control group and a small sample size which was connected to the relatively low incidence of the disease. The heterogenicity of our group must also be noted. Further prospective studies, with a larger number of participants need therefore to be conducted in this rare retinal entity. Other limitations of the study include inherent problems that arise from the use of cell culture as a model for retinal disease. The cells used are a very simplified model and do not fully represent the pathologic basis of the retina as a complex tissue with glial cells, neurons, microglia cells, and blood vessels. As this study was meant to serve as an evaluation of the biocompatibility of intraocular amniotic membrane implantation and possible cellular responses as well as possible clinical advantages, we argue that first conclusions can be made following the found results.

Amniotic membranes predominately consist of extracellular matrix with various cytokines and growth factors in high amounts.[17, 18] Depending on their concentrations and interactions, cytokines and growth factors can induce, beside other effects, apoptosis or proliferation in different cell types. Since both events could have severe side effects in the retina, such as neuroretinal degeneration or development of PVR membranes after surgery as discussed above, both phenomena were analyzed in our current study in Müller cells, RPE cells and differentiated retinoblasts.[19] In all cell lines, neither toxic nor proliferative effects of amniotic membranes were observed. On one hand, an enhanced proliferation of fibroblasts or skin keratinocytes during wound healing was observed following treatment with amniotic membranes.[20] On the other hand, several reports suggest that amniotic membranes have the distinct potential to induce growth arrest in hepatocarcinoma cells or epithelial ovarian cancer cells.[21, 22] In RPE cells cultured on human amniotic membranes, a more differentiated phenotype of the cells was induces when compared to control cells.[23] Since a higher differentiation of cells is often associated with reduced proliferation, it is tempting to speculate that amniotic membranes could mediated their antiproliferative effects on PVR cells via growth arrest induction and differentiation. In summary, at the current state of investigation, we suspect that intravitreal AM will not increase the risk for the formation of PVR.

Furthermore, the number of surviving differentiated retinoblasts was significantly increased after treatment with amniotic membranes, suggesting that amniotic membranes could mediate protective effects on retinal neurons, which has also been indicated in other studies trying to regenerate severed neurological tissue.[24] In line with our observations, the intranasal application of the secretome of amnion-derived multipotent progenitor cells attenuated retinal ganglion cell degeneration following optic nerve crush in mice.[25] In addition, a subretinal amniotic membrane patch in dry age related macular degeneration in six patients was able to promote some partial retinal function restoration 6 months after surgery with visual acuity improvement as argued by the authors.[8]

A potential rejection reaction against allogeneic implanted amniotic membranes, which could induce a chronic inflammation in the vitreous body and the adjacent retina and subsequently lead to reduced retinal function, is a major concern against the intraocular use of amniotic membranes. To address potential rejection reactions in our patients, immunohistochemical staining against markers for macrophages and microglial cells as well as for T- and B-cells were performed. A few macrophages or

microglial cells were detected on the amniotic membrane in one patient. Since there is evidence that in this patient, the amniotic membrane was attached to the inner retina, it is tempting to speculate that the appearance of macrophages or microglial cells on the graft could be due to normal migration of these cells after injury.[26] However, classical rejection reactions against allogeneic transplants can be classified in hyperacute, acute and chronic, which all need specific subtypes of T-cells to induce their specific immunological processes.[27] By immunohistochemistry, no T-cells were detected in all three explanted grafts, strongly suggesting that a rejection reaction did not occur in our patients. In line with our observations, in previous studies implanting amniotic membranes into the subretinal space of humans, no clinical signs of rejection reactions were reported.[8] Intriguingly, in-vitro experiments on T-cells from mice demonstrate that amniotic membranes mediate distinct immunosuppressive properties on immune cells.[27] In summary, our data strongly indicates that amniotic membranes at the vitreoretinal interface do not induce relevant rejection reaction or inflammation.

In conclusion, intravitreal transplantation of amniotic membranes is a feasible procedure in treating severe vitreoretinal injury. Since we could almost rule out severe rejection reactions as well as toxic effects, it appears reasonable to investigate the intravitreal use of amniotic membranes in prospective studies.

## Methods

# Clinical cases

In addition to the laboratory investigation, case reports of 3 eyes of 3 patients suffering from severe vitreoretinal disease that were treated at the Department of Ophthalmology of the Ludwig-Maximilians-University of Munich are presented. All three eyes required PPV (pars plana Vitrectomy) to treat the underlying disease. In addition, an AM was placed in different anatomical locations of the retina. Research approval from the institutional review board of the department of ophthalmology of the Ludwig-Maximilians-University Munich was obtained as well as informed written consent from all subjects. All research was performed in accordance with relevant guidelines and regulations and adhered to the Declaration of Helsinki.

# Amniotic membrane patch

The cryopreserved amniotic membrane patches were produced by a certified supplier (Gewebebank Mecklenburg-Vorpommern, Rostock, Germany). The procedures adhered to the currently valid German guidelines for the preparation of amniotic membranes. Tissue that was left over after surgery and would have been discarded was transferred to the laboratory for further investigation.

# Surgical procedure

All surgical procedures were performed by one experienced vitreoretinal surgeon (AW). For PPV, three 23-gauge trocars were placed under sterile conditions, each 3.5 mm from the limbus. Lens surgery was conducted as required. Central parts of the vitreous body were cut free. The posterior vitreous was

detached followed by an indentation with the squint hook and excision of peripheral vitreous parts. The AM was inserted into the eye through an additional sclerostomy or in case of combined phakovitrectomy through the anterior chamber before implanting the lens. A fluid air exchange was performed and the AM was unfolded on the epiretinal surface. The AM was then mobilized to position in the area of interest. In the majority of cases, silicone oil was used as a tamponade. Endolaser coagulation was performed if necessary. Suturing over the sclerostomy and removal of the trocars ended the procedure.

After 2 to 12 months, silicone-oil was removed in an additional procedure. During this procedure the large AM was carefully lifted with endgripping forceps and extracted via a sclerotomy.

# Immunohistochemistry and staining of explanted AM

Explanted Amniotic membranes were fixed in 4% formaldehyde, embedded in paraffin, and stained with haematoxylin and eosin according to standard protocols. Additional sections were deparaffinized and pretreated with Ultra CC1 (Ventana Medical Systems, Tucson, AZ, USA) for antigen retrieval for anti-CD45, anti-CD20, anti-CD68 and anti-CD3 antibody staining and with target retrieval solution pH 9 (Dako, Heverlee, Belgium) for anti-pan-cytokeratin and anti-GFAP antibody staining. After endogenous peroxidase blocking, sections were incubated with 1:25 anti-CD45 (Dako, Heverlee, Belgium, clone 2B11 + PD7/26), 1:200 anti-CD20 (Dako, clone L-26), 1:1000 anti-CD68 (Merck, Darmstadt, Germany), 1:100 anti-pan-cytokeratin (Dako, clone 6F2), 1:200 anit-GFAP (Dako, clone AE1/AE3) and 1:20 anti-CD3 (Monosan, Uden, Netherlands, clone PS-1) antibodies. After incubation with peroxidase-labelled secondary antibodies, sections were visualized with a chromogen DAB (3,30diaminobenzidine) solution.

# Cell culture of 661W, Mio-M1 and ARPE-19 cells

ARPE-19 (ATCC, Manasas, VA, USA), immortalized retinal precursor cells (661W, ATCC) as well as immortalized Müller cells (Mio-M1, kindly provided by Dr. Wolfram Eichler, Leipzig, Germany) were incubated at 37°C in humidified 5% $CO_2$. Mio-M1 and 661W were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco by Life Technologies, Paisley, UK) and ARPE 19 in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (Gibco by Life Technologies). Both cell culture media contained 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin.

For experimental procedures, 4,000 or 10,000 cells / well of ARPE19 or Mio M1 were seeded into a 96-well tissue culture plate and allowed to attach for 4 h. For differentiation into a neuroretinal phenotype, 20,000 661W cells/ well were treated with staurosporine (Merck, Burlington, MA, USA) as described previously.[28] In brief, after their attachment, cells were washed twice with 1x phosphate buffered saline (1xPBS) and incubated for 1 h with 1 µM staurosporine in unsupplemented medium. For recovery, treated 661W cells were incubated in DMEM with 10% FBS for additional 24 h.

# Cell viability

Cell viability was assessed by a WST-1-assay (MilliporeSigma) as described previously.[29] In brief, 10,000 cells / well were incubated in serum-free cell culture medium with or without an amniotic membrane patch (2mm x 2mm length). After 3 d, the medium was removed and serum-free cell culture

medium containing 10% WST-1 was added. After incubation for 30 min, extinction was measured at 450 nm using an ELISA plate reader (Molecular Devices, San Jose, CA, USA).

# Cell proliferation

Cell proliferation was analyzed by BrdU labeling of dividing cells (Merck) as described previously.[30] In brief, 4000 cells / well were plated and cultured in serum-free cell culture medium with or without an amniotic membrane patch and 10 µM BrdU. After 72 h, cells were fixed and incorporated BrdU was detected by ELISA using an ELISA plate reader (Molecular Devices) at 450 nm.

# Apoptosis detection ELISA

To exclude a toxic effect, cellular apoptosis was investigated after incubation with amniotic membranes by measuring histone-DNA complexes in accordance with the manufacturer's instructions (cell death detection ELISA, Merck). In brief, 20,000 differentiated 661W cells / well as well as 10,000 ARPE-19 or Mio-M1 cells / well in were incubated in serum-free cell culture medium with or without amniotic patch for 24 h or 72 h, respectively. After treatment, the cell culture medium was centrifuged, and histone-DNA fragments were detected by ELISA using an ELISA plate reader (Molecular Devices) at 405 nm.

# Live / dead staining

For histological analysis of dead cells, glass coverslips with ARPE-19 cell were washed 3 x with 1 x PBS and incubated with 5 µg/ml propidium iodide (Thermo Scientific, Waltham, MA, USA) in 1 x PBS (red, dead cells) and 5 µg/ml Hoechst 33342 (Thermo Scientific) in 1 x PBS (all cells) for 15 min. After additional 3 washings, the cells were fixed with 4% paraformaldehyde for 15 min and mounted on a glass slide upside down.

# Statistics

All values are expressed as mean ± standard error of the mean. For statistical analyses a one-way ANOVA was performed, followed by an LSD post-hoc test for data that met the criteria of the assumption of homogeneity of variances and a Games-Howell post-hoc test for data that did not. P-values less than 0.05 were considered statistically significant.

# Declarations


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### Author contributions statement

A.H. made a substantial contribution to the analysis and interpretation of the data and drafted the work substantively. C.W. and M.G. made a substantial contribution to the analysis and interpretation of the data and revised it. S.P. substantively revised it. A.O. and A.W. made a substantial contribution to

conception and drafted the work substantively. They made a substantial contribution to acquisition and interpretation of the data, to the design of the work and the analysis. All authors reviewed the manuscript.

### Data availability statement

All data generated or analyzed during this study are included in this published article.

### Additional information

### Competing Interests Statement

All authors declare that no funds, grants, or other support was received for this work.

All authors certify that they have no affiliations with -or involvement in- any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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## References

1. A. D. Negrel and B. Thylefors, "The global impact of eye injuries," *Ophthalmic Epidemiol*, vol. 5, no. 3, pp. 143 – 69, Sep 1998, doi: 10.1076/opep.5.3.143.8364.
2. J. A. Cardillo *et al.*, "Post-traumatic proliferative vitreoretinopathy. The epidemiologic profile, onset, risk factors, and visual outcome," (in eng), *Ophthalmology*, vol. 104, no. 7, pp. 1166-73, Jul 1997, doi: 10.1016/s0161-6420(97)30167-5.
3. S. C. Tseng, "Amniotic membrane transplantation for ocular surface reconstruction," Biosci Rep, vol. 21, no. 4, pp. 481–9, Aug 2001, doi: 10.1023/a:1017995810755.
4. T. Caporossi, R. Tartaro, L. De Angelis, B. Pacini, and S. Rizzo, "A human amniotic membrane plug to repair retinal detachment associated with large macular tear," Acta Ophthalmol, vol. 97, no. 8, pp. 821–823, Dec 2019, doi: 10.1111/aos.14109.
5. T. Caporossi *et al.*, "A human Amniotic Membrane plug to manage high myopic macular hole associated with retinal detachment," (in eng), *Acta Ophthalmol*, vol. 98, no. 2, pp. e252-e256, Mar 2020, doi: 10.1111/aos.14174.
6. T. Caporossi, L. De Angelis, B. Pacini, and S. Rizzo, "Amniotic membrane for retinal detachment due to paravascular retinal breaks over patchy chorioretinal atrophy in pathologic myopia," Eur J Ophthalmol, vol. 30, no. 2, pp. 392–395, Mar 2020, doi: 10.1177/1120672119891415.

7. S. Rizzo, T. Caporossi, B. Pacini, L. De Angelis, M. L. De Vitto, and F. Gainsanti, "Management of Optic Disk Pit-associated Macular Detachment with Human Amniotic Membrane Patch," (in eng), *Retina*, Jan 10 2020, doi: 10.1097/iae.0000000000002753.
8. S. Rizzo *et al.*, "Human Amniotic Membrane Plug to Restore Age-Related Macular Degeneration Photoreceptor Damage," (in eng), *Ophthalmol Retina*, vol. 4, no. 10, pp. 996–1007, Oct 2020, doi: 10.1016/j.oret.2020.04.017.
9. S. Rizzo *et al.*, "A Human Amniotic Membrane Plug to Promote Retinal Breaks Repair and Recurrent Macular Hole Closure," *Retina*, vol. 39 Suppl 1, pp. S95-S103, Oct 2019, doi: 10.1097/IAE.0000000000002320.
10. C. Capeans *et al.*, "Amniotic membrane as support for human retinal pigment epithelium (RPE) cell growth," Acta Ophthalmol Scand, vol. 81, no. 3, pp. 271–7, Jun 2003, doi: 10.1034/j.1600-0420.2003.00076.x.
11. K. Ohno-Matsui *et al.*, "The effects of amniotic membrane on retinal pigment epithelial cell differentiation," (in eng), Mol Vis, vol. 11, pp. 1–10, Jan 6 2005.
12. D. Zhu, X. Jin, and J. Zhou, "Transplantation of amniotic membrane for choroidal hole to treat suprachoroidal silicone oil migration," Acta Ophthalmol, vol. 95, no. 6, pp. e522-e523, Sep 2017, doi: 10.1111/aos.13516.
13. E. I. Paschalis *et al.*, "Microglia Regulate Neuroglia Remodeling in Various Ocular and Retinal Injuries," (in eng), J Immunol, vol. 202, no. 2, pp. 539–549, Jan 15 2019, doi: 10.4049/jimmunol.1800982.
14. K. Eastlake, W. D. B. Lamb, J. Luis, P. T. Khaw, H. Jayaram, and G. A. Limb, "Prospects for the application of Müller glia and their derivatives in retinal regenerative therapies," (in eng), *Prog Retin Eye Res*, vol. 85, p. 100970, Nov 2021, doi: 10.1016/j.preteyeres.2021.100970.
15. M. A. Stepp and A. S. Menko, "Immune responses to injury and their links to eye disease," (in eng), *Transl Res*, vol. 236, pp. 52–71, Oct 2021, doi: 10.1016/j.trsl.2021.05.005.
16. G. Kaur and N. K. Singh, "The Role of Inflammation in Retinal Neurodegeneration and Degenerative Diseases," (in eng), Int J Mol Sci, vol. 23, no. 1, Dec 30 2021, doi: 10.3390/ijms23010386.
17. M. S. Murri, M. Moshirfar, O. C. Birdsong, Y. C. Ronquillo, Y. Ding, and P. C. Hoopes, "Amniotic membrane extract and eye drops: a review of literature and clinical application," Clin Ophthalmol, vol. 12, pp. 1105–1112, 2018, doi: 10.2147/OPTH.S165553.
18. T. J. Koob *et al.*, "Biological properties of dehydrated human amnion/chorion composite graft: implications for chronic wound healing," Int Wound J, vol. 10, no. 5, pp. 493–500, Oct 2013, doi: 10.1111/iwj.12140.
19. S. Idrees, J. Sridhar, and A. E. Kuriyan, "Proliferative Vitreoretinopathy: A Review," (in eng), *Int Ophthalmol Clin*, vol. 59, no. 1, pp. 221–240, Winter 2019, doi: 10.1097/iio.0000000000000258.
20. J. P. McQuilling, M. Kammer, K. A. Kimmerling, and K. C. Mowry, "Characterisation of dehydrated amnion chorion membranes and evaluation of fibroblast and keratinocyte responses in vitro," Int Wound J, vol. 16, no. 3, pp. 827–840, Jun 2019, doi: 10.1111/iwj.13103.

21. S. Bu, Q. Zhang, Q. Wang, and D. Lai, "Human amniotic epithelial cells inhibit growth of epithelial ovarian cancer cells via TGFbeta1-mediated cell cycle arrest," Int J Oncol, vol. 51, no. 5, pp. 1405–1414, Nov 2017, doi: 10.3892/ijo.2017.4123.
22. R. Riedel *et al.*, "Human amniotic membrane conditioned medium inhibits proliferation and modulates related microRNAs expression in hepatocarcinoma cells," Sci Rep, vol. 9, no. 1, p. 14193, Oct 2 2019, doi: 10.1038/s41598-019-50648-5.
23. H. Akrami *et al.*, "Evaluation of RPE65, CRALBP, VEGF, CD68, and tyrosinase gene expression in human retinal pigment epithelial cells cultured on amniotic membrane," *Biochem Genet*, vol. 49, no. 5–6, pp. 313 – 22, Jun 2011, doi: 10.1007/s10528-010-9409-1.
24. M. Bourgeois, F. Loisel, L. Obert, I. Pluvy, and F. Gindraux, "Can the amniotic membrane be used to treat peripheral nerve defects? A review of literature," Hand Surg Rehabil, vol. 38, no. 4, pp. 223–232, Sep 2019, doi: 10.1016/j.hansur.2019.05.006.
25. G. A. Grinblat, R. S. Khan, K. Dine, H. Wessel, L. Brown, and K. S. Shindler, "RGC Neuroprotection Following Optic Nerve Trauma Mediated By Intranasal Delivery of Amnion Cell Secretome," *Invest Ophthalmol Vis Sci*, vol. 59, no. 6, pp. 2470–2477, May 1 2018, doi: 10.1167/iovs.18-24096.
26. Y. Huang *et al.*, "Dual extra-retinal origins of microglia in the model of retinal microglia repopulation," Cell Discov, vol. 4, p. 9, 2018, doi: 10.1038/s41421-018-0011-8.
27. A. Sanchez-Fueyo and T. B. Strom, "Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs," *Gastroenterology*, vol. 140, no. 1, pp. 51–64, Jan 2011, doi: 10.1053/j.gastro.2010.10.059.
28. L. J. Frassetto *et al.*, "Kinase-dependent differentiation of a retinal ganglion cell precursor," *Invest Ophthalmol Vis Sci*, vol. 47, no. 1, pp. 427 – 38, Jan 2006, doi: 10.1167/iovs.05-0340.
29. R. Seitz, S. Hackl, T. Seibuchner, E. R. Tamm, and A. Ohlmann, "Norrin mediates neuroprotective effects on retinal ganglion cells via activation of the Wnt/beta-catenin signaling pathway and the induction of neuroprotective growth factors in Muller cells," J Neurosci, vol. 30, no. 17, pp. 5998–6010, Apr 28 2010, doi: 10.1523/JNEUROSCI.0730-10.2010.
30. A. Ohlmann, R. Seitz, B. Braunger, D. Seitz, M. R. Bosl, and E. R. Tamm, "Norrin promotes vascular regrowth after oxygen-induced retinal vessel loss and suppresses retinopathy in mice," *J Neurosci*, vol. 30, no. 1, pp. 183 – 93, Jan 6 2010, doi: 10.1523/JNEUROSCI.3210-09.2010.

# Figures

### Figure 1

Clinical examples of case one that suffered from large macular hole after severe endophthalmitis **(a)** after surgery and **(b)** after explanation of the AM with closed macular hole. The last two images are taken

from case three, which suffered from ocular trauma with large scleral rupture extending far beyond the equator **(c)** after surgery and **(d)** after explanation of the AM with a quiet scar formation.

## Figure 2

Amniotic membranes induce no rejection reaction following double perforating eye injury. H&E staining as well as immunohistochemistry for pan-cytokeratin, a marker for epithelial cells, and CD45, a marker for lymphocytes, is shown. By CD45 immunostaining only a few lymphocytes could be detected, whereas an intense staining for cytokeratin was observed in amniotic epithelial cells. Magnification bars: 100 µm.

## Figure 3

Amniotic membrane traps PVR cells following macular hole closure (Histopatho 11250). H&E staining (**a, b**) and immunohistochemistry for GFAP (**c,d**), a marker for Müller cells and astrocytes, and CD68 (**e, f**), a marker for macrophages and microglia cells, is shown. On surface of the amniotic membrane focal accumulation of spindle or oval shaped cells with surrounding connecting tissue was observed (asterisk in **b**). By immunohistochemistry, fibrillar GFAP positive membranes on the surface of the amniotic membrane (arrowhead in **d**) and plenty of CD68 positive cells adjacent to the amniotic membrane and within the fibrotic tissue were detected (open arrowhead in **f**). Magnification bars: **b**, D100 µm; **a, f,** 200 µm; **c, e,** 400 µm.

## Figure 4

Incubation of ARPE-19 cells with amniotic membranes has no toxic or proliferative effects. **a.** Quantification of histone-DNA fragments, **b.** quantification of cell viability, and **c.** quantification of incorporated BrdU after incubation of ARPE-19 cells in serum free cell culture medium with or without amniotic membrane patches for 3 d (mean ± SEM of 3 independent experiments; **p < 0.01). **d, e.** ARPE-19 cells were stained with Hoechst 33342 (blue) and propidium iodide (red), which is a marker for apoptosis. Following incubation only rare sporadic apoptotic cells were detected in both amniotic membrane exposed and control cells. Magnification bars, 50 µm.

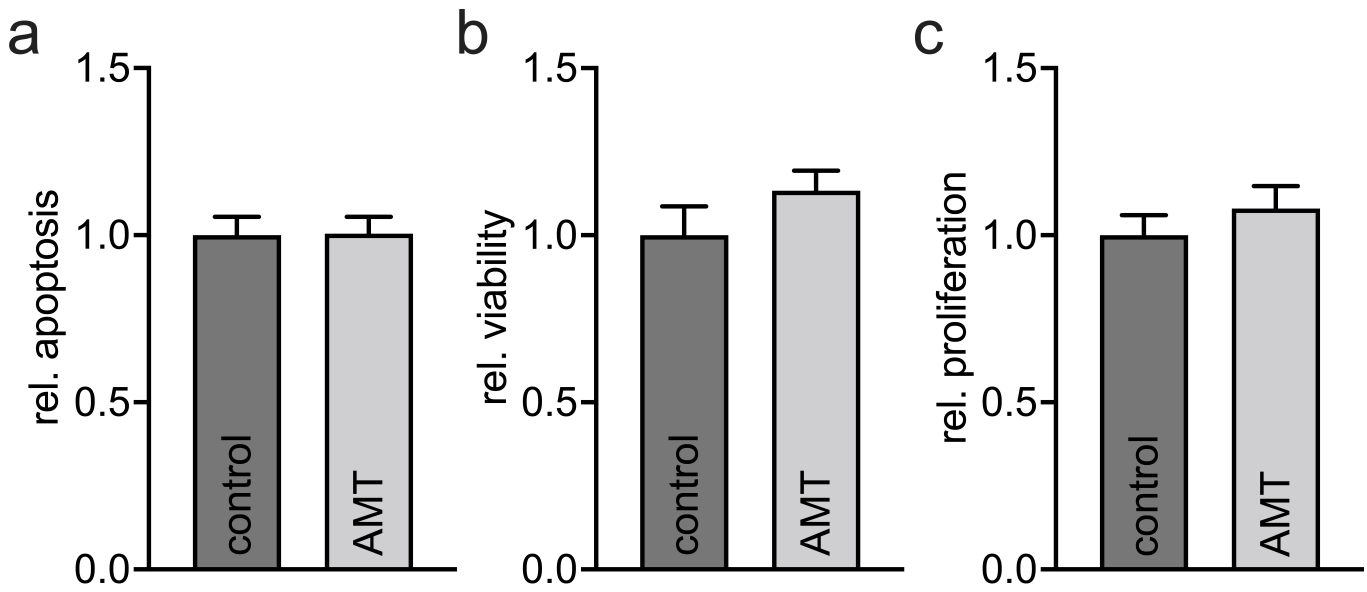


**Figure 5**

Incubation of Mio-M1 cells with amniotic membranes has no toxic or proliferative effects. **a.** Quantification of histone-DNA complexes, **b.** cell viability and **c.** BrdU incorporation after incubation of Mio-M1 cells with or without amniotic membrane patches in serum free cell culture medium for 3 d (mean ± SEM of 3 independent experiments).

**Figure 6**

Amniotic membranes mediate protective effects on differentiated retinal neuroblasts. **a.** Quantification of histone DNA fragments, **b.** cell viability after incubation of differentiated W661 cells in serum-free cell culture medium with or without amniotic membrane patch for 24 h (mean ± SEM of 3 independent experiments; **p < 0.01, ***p < 0.001). **c, d.** 661W cells were differentiated with staurosporine [1 µM] and incubated for 24 h in non-supplemented cell culture medium with or without amniotic membrane patch. After 24 hours of treatment with amniotic membrane patch, the number of differentiated W661 cells was significantly increased **(d)** compared with control cells **(c).** Magnification bar, 100 µm.