Novel Method of Plugging the Hole: Anatomical and Functional Outcomes of Human Amniotic Membrane-Assisted Macular Hole Surgery

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Abstract

Purpose: To describe the surgical outcomes of macular holes (MHs) by inserting a human amniotic membrane (hAM) plug.

Methods: In this retrospective, interventional, comparative case series, 10 patients who had undergone hAM plugging for a MH were included in the study. Seven patients had idiopathic full-thickness MHs, 1 patient had traumatic MH, and 1 patient each had a MH-induced retinal detachment and combined retinal detachment. The control group included 10 cases with similar configuration and duration of MHs treated with the inverted peeling of the internal limiting membrane technique. All patients underwent a standard 3-port, 25-gauge transconjunctival pars plana vitrectomy and hAM plug transplantation in the subretinal space under the MH. The anatomic and functional outcomes were assessed at 4 weeks postsurgery.

Results: At the 4-week follow-up visit, all the MHs in the hAM plug group achieved hole closure, whereas 80% of the eyes in the control group were able to achieve hole closure. Improvement of 0.1 logMAR vision was noted in 8 of the 10 patients. At the 4-week follow-up visit, the external limiting membrane and ellipsoid zone layer continuity over the hAM was defined only in one case. No significant difference was found between the hAM plug group and controls in visual and anatomical responses. No complications following hAM transplantation such as rejection, endophthalmitis, or hypotony were noted following surgery.

Conclusion: Subretinal hAM graft transplantation can be a useful option in the repair of primary or refractory MHs not only for achieving anatomic closure but also for the accompanying visual improvement.

Keywords: Amniotic membrane transplantation, Macular hole, Outcomes, Surgery

INTRODUCTION

The human amniotic membrane (hAM) is the innermost layer of the fetal membranes consisting of a stromal matrix, a thick collagen layer, and an overlying basement membrane with a single layer of epithelium.[1] Literature has shown that hAM constitutes an adequate substrate for *in vitro* growth and expansion of conjunctival epithelial progenitor cells,[2,3] thereby making amniotic membrane graft (AMG) transplantation a successful and useful technique in patients with persistent epithelial lesions, corneal ulceration, symptomatic bullous keratopathy, band keratopathy, chemical and thermal burns, conjunctival surface reconstruction, ocular cicatricial pemphigoid, and Stevens–Johnson syndrome.[4-13]

In vitro studies have demonstrated hAM to be a useful matrix substrate to retain the differentiated and epithelial phenotype

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of the retinal pigment epithelial (RPE) cells for subretinal transplantation and thus could prove to be useful in the future for the treatment of macular diseases such as age-related macular degeneration.[14,15] Kiilgaard *et al*. studied the effects of AMG transplantation on subretinal wound healing in pigs. They found that AMG modified choroidal neovascularization after basement membrane damage and concluded that hAM may serve as a basement membrane substitute for the RPE.[16] In rabbits, Rosenfeld demonstrated that subretinal implantation of hAM was tolerated without any evidence of inflammation. Capeáns *et al*. demonstrated that human RPE cells seeded over hAM in the first 24 h. They were able to maintain epithelial features and could proliferate over epithelium-free hAM, constituting a tight monolayer with well-defined intercellular and cell-substrate interactions.[17]

A macular hole (MH) is a full-thickness retinal defect that opens up in the center of the retina due to traction primarily by the vitreous and/or internal limiting membrane (ILM). Following surgery, MHs close by a "bridging" mechanism along the inner retinal surface with the migration of proliferative RPE cells assisted by the capillary forces generated by fluid–air interface deformation at the edges of the MH.[18] The hAM could provide a useful substrate matrix for the proliferation of RPE cells, thereby helping in the MH closure. There are very few papers in the literature studying the *in vivo* utilization of hAM transplantation in subretinal space for the treatment of retinal disorders. Rizzo *et al*. studied the effects of hAM plugging in the subretinal space for the closure of retinal breaks and recurrent MHs. They found a significant improvement in the retinal hole closure and visual acuity (VA) following this surgical technique.[19] Caporossi *et al*. demonstrated successful retinal reattachment in complex cases of retinal detachment having large macular tears using hAM plug. They concluded that hAM transplantation can be a valid option not only to help the retinal reattachment but also for a partial regenerative effect which can lead to a VA recovery.[20] Recently, *in vivo* hAM plug transplantation was used for choroidal hole repair in a case of globe rupture to treat suprachoroidal silicone oil migration.[21]

With this background, in this case series, we report the clinical data and outcomes of 10 patients with MHs who underwent subretinal space hAM plugging.

METHODS

This retrospective, interventional, comparative study was carried out in the retina department of a tertiary super specialty eye hospital in South India. The institutional review board approval (C/2019/10/02) for the study was obtained, which was by following per under the tenets of the Declaration of Helsinki, and all the patients signed a written informed consent form.

Patients with full-thickness MHs operated between the period from June 2018 and December 2019 and who had undergone hAM plugging into the subretinal space having a minimum follow-up of 4 weeks were selected. A total of 10 eyes of 10 patients were enrolled in the study.

All patients underwent a standard 3-port, 25-gauge transconjunctival pars plana vitrectomy (Alcon Laboratories, Fort Worth, TX) under peribulbar anesthesia, ILM peeling, and hAM plug transplantation in the subretinal space under the MH. All the surgical procedures were performed by the same experienced surgeon (N.K.Y.). The 25-gauge system was chosen to facilitate the insertion of the hAM through the non-valved cannula. A complete vitrectomy was performed with an accurate vitreous base shaving (Constellation; Alcon Surgical). A chandelier endoilluminator was inserted to facilitate bimanual maneuvers. A patch of fresh hAM was obtained from the tissue bank on the morning of the surgery. A portion of the hAM was taken from the hAM patch and was manually fashioned with the vitreoretinal scissors before entering into the vitreous cavity. The hAM tissue was flipped and rolled inside the vitreous cavity with vitreoretinal forceps and inserted through the cannula into the vitreous chamber. Inside the vitreous cavity, the hAM was manipulated under air, fluid, or perfluorocarbon liquid (Auro Octane, Aurolab, India) and transplanted through the MH into the subretinal space. It was stuffed to plug the entire portion of the hole. Fluid–air or perfluorocarbon–air exchange and an endotamponade injection were performed at the end of surgery [Figure 1].

The control group included 10 cases of MHs having a similar hole configuration and duration to the study group. In this group, all the patients underwent a standard 3-port, 25-gauge transconjunctival pars plana vitrectomy (Alcon Laboratories, Fort Worth, TX) under peribulbar anesthesia with inverted ILM peeling technique and ILM stuffing into the MH followed by an endotamponade injection at the end of the surgery. The surgical technique was performed by the same experienced surgeon (N.K.Y.) for this group.

Preoperative evaluation included an ophthalmic history and a complete ophthalmic examination including refraction with an
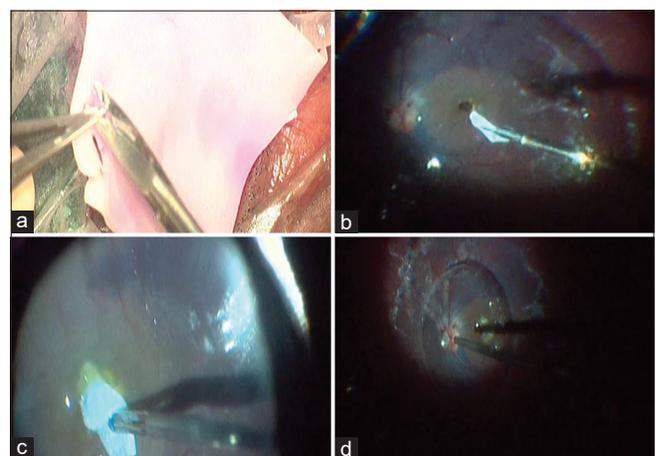
Figure 1: Intraoperative snapshots describing the surgical technique for insertion of human amniotic membrane (hAM) plug inside the macular hole (MH): (a) Fashioning the freshly acquired amniotic membrane graft according to the MH size. (b and c) Holding the hAM graft with the help of forceps and using the blunt end of the forceps to insert the hAM under the edges of the MH. (d) Performing perfluorocarbon liquid–air exchange away from the hAM graft to avoid the displacement of hAM from the MH

assessment of best corrected visual acuity (BCVA, Snellen, and logMAR), Goldmann applanation tonometry, standard fundus dilated ophthalmic examination, spectral-domain optical coherence tomography (OCT, Heidelberg Spectralis), analysis of the MH with an accurate measurement of the height and outer diameter of MH, and fundus photography using the multicolor imaging modality of Spectralis Heidelberg machine. Patients in both the groups were examined on the first postoperative day and subsequently on days 7, 14, and 28 following surgery. OCT scans were performed for all patients on days 7, 14, and 28 and at final follow-up visit as well. Postoperative assessments done at week 2 and 4 follow-up visits were analyzed. The following parameters were noted and analyzed: BCVA, presence of hAM *in situ*, MH status (closed or open) and integrity of the external limiting membrane (ELM), and ellipsoid zone layers. For a better understanding of the clinical outcomes, we further divided the patients in the hAM plug group into two subgroups: Group A: primary hAM plug group which included cases of MHs where hAM plug insertion was done during the primary surgery itself and Group B: secondary hAM plug group which included MHs where hAM plug insertion was done during the second vitreous surgery.

All the data were analyzed with the GraphPad Prism software (version 8.4.2 [679] for Windows, San Diego, CA). Only non-parametric statistical tests were used in this study. VA was converted to logMAR for statistical analysis. Quantitative variables between the two groups (hAM plug group and no-hAM plug group) were analyzed using the Mann–Whitney U test. Chi-square test was used to compare the categorical data between the two groups. $P < 0.05$ was considered statistically significant.

RESULTS

Ten eyes of 10 patients (4 males and 6 females) in the hAM plug group and 10 control eyes (no-hAM plug group) of 10 patients (5 males and 5 females) were included in the study. In the hAM group, 7 patients suffered from idiopathic full-thickness MHs, 1 patient had full-thickness MH secondary to blunt trauma, and 1 patient each had a MH-induced retinal detachment and combined retinal detachment. Of the 10 eyes, hAM transplantation was done during the primary MH surgery in 4 eyes, whereas in the remaining 6 cases, MHs either remained open or reopened after primary macular surgery.

The mean age in the hAM plug group was 62 ± 15.9 years, and in the control group, it was 67.6 ± 4.6 years, respectively ($P = 0.403$). Table 1 describes the comparisons about the clinical features and outcomes between the hAM plug and the control groups. At the 4-week follow-up visit, 100% of the cases in the hAM plus group achieved hole closure, whereas 80% of the eyes in the control group were able to achieve hole closure. The mean postoperative VA improved in both the groups from the baseline; however, it was not statistically significant ($P = 0.24$). Discontinuous ELM and ellipsoid zone layers were noted in 9 eyes each in both the groups.

Four eyes were included in Group A, and the remaining 6 eyes were included in Group B [Table 2]. The decision to use hAM for the primary MH cases was based on the size of the MH. In the primary hAM plug group, the insertion of hAM into the subretinal space was done in the initial surgery itself due to the larger hole size (>800 µm) [Figures 2 and 3]. In the secondary hAM plug group, all the patients underwent re-vitrectomy and additional ILM peeling, hAM plug into the subretinal space, and endotamponade. The mean preoperative BCVA was 1.05 ± 0.33 logMAR (20/225) ranging from 1.5 to 0.7 logMAR (20/630–20/100) for the primary hAM plug group and 0.93 ± 0.32 logMAR (20/170) ranging from 1.5 to 0.6 logMAR (20/630–20/80) in the secondary hAM plug group. Five of the 10 patients in the case group were phakic, of which only one patient underwent combined phaco-vitrectomy surgery. The remaining 5 patients were pseudophakic. BCVA improved from 1.05 ± 0.33 logMAR (20/225) preoperatively to 0.73 ± 0.01 logMAR (20/107) 1 month after the operation in Group A. In Group B, the BCVA improved from 0.93 ± 0.32 logMAR (20/170) preoperatively to 0.63 ± 0.19 logMAR (20/85)
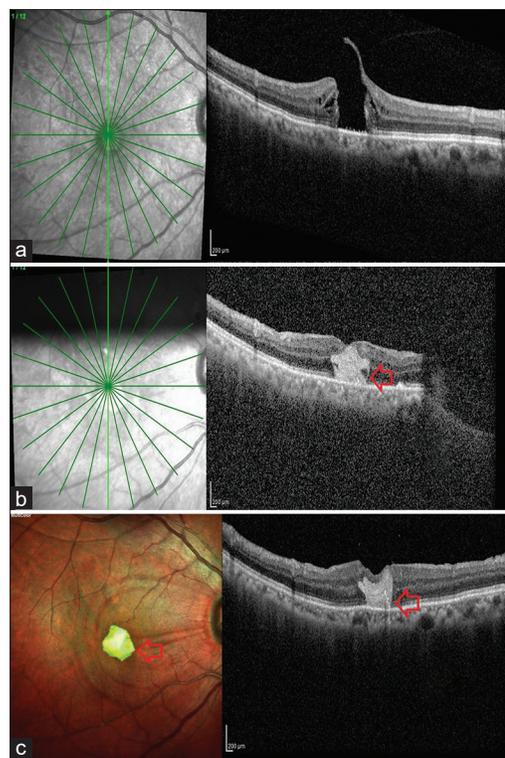
Figure 2: Pre and postoperative outcomes of a patient with idiopathic full-thickness macular hole (MH) having undergone primary human amniotic membrane (hAM) plug surgery (case 2, Group A). (a) This patient developed a large full-thickness primary MH (outer diameter = 897 µ). The patient underwent pars plana vitrectomy with internal limiting membrane peeling with hAM plugging into the MH in the primary surgery itself. (b and c) Optical coherence tomography image at 2-week and 4-week post-surgery shows the MH closed and hAM plug *in situ* (arrow with red border). The external limiting membrane and ellipsoid layer have not yet differentiated. Multicolor image shows the hAM plug to be integrated under the macula (arrow with red border)

Table 1: Comparisons in the clinical features and outcomes between the human amniotic membrane (hAM) plug and the no hAM plug groups

	hAM plug group (*n*=10)	No-hAM plug group (*n*=10)	*P*
Age	62±15.9	67.6±4.6	0.403
Sex (male:female)	4:6	5:5	>0.999
Study eye (RE/LE)	7/3	4/6	0.369
Preoperative visual acuity (logMAR)	0.98±0.312, 20/190	0.958±0.224, 20/181	0.999
Lens status	Phakic - 5	Phakic - 4	
	Pseudophakia - 5	Pseudophakia - 6	
MH duration (months)	8.68±3.56	9.11±3.07	0.316
MH outer diameter (μm)	1804±1596	1791±1420	0.807
MH height (μm)	506±230	484±276	0.912
Number of eyes with hAM plug during primary surgery	4 (40)	-	
Number of eyes with hAM plug during secondary surgery	6 (60)	-	
Tamponade used	SF6-3	SF6-2	>0.999
	C3F8-5	C3F8-6	>0.999
	SiO - 2	SiO - 2	>0.999
2-week postoperative visual acuity (logMAR)	0.810±0.296, 20/129	0.916±0.311; 20/164	0.467
Hole closure status at 4-week postoperative	10 (100)	8 (80)	0.136
Presence of discontinuity in ELM and EZ layers	9 (90)	9 (90)	>0.999
4-week postoperative visual acuity (logMAR)	0.67±0.157, 20/93	0.758±0.225, 20/115	0.24
Total follow-up duration	5.6±1.71	5.2±2.7	0.814

hAM: Human amniotic membrane, RE: Right eye, LE: Left eye, MH: Macular hole, ELM: External limiting membrane, EZ: Ellipsoid zone, SF6: Sulfur hexafluoride, C3F8: Perfluoropropane, SiO: Silicone oil

Table 2: Clinical features and outcomes of macular holes in the primary human amniotic membrane (hAM) plug and secondary hAM plug groups

n	Age/sex/eye	Pathology	Preoperative lens status	Preoperative logMAR	Tamponade	2-week postoperative logMAR	4-week postoperative logMAR	Hole status at 4-week postoperative	ELM and IS-OS integrity	Final follow-up duration
1	61/male/RE	PDR + CRD	Phakic	1.5	SiO	0.8	0.8	Closed	Discontinuous	7
2	66/female/RE	FTMH	PCIOL	1	C3F8	0.7	0.7	Closed	Discontinuous	6
3	74/female/LE	MHRD	PCIOL	1	C3F8	0.8	0.8	Closed	Discontinuous	6
4	18/male/RE	FTMH	Phakic	0.7	SF6	0.6	0.6	Closed	Discontinuous	3
5	67/female/RE	FTMH	PCIOL	1	SF6	1	1	Closed	Discontinuous	8
6	64/male/RE	FTMH	Phakic	0.8	C3F8	1	0.6	Closed	Discontinuous	8
7	65/female/LE	FTMH	PCIOL	1.5	C3F8	1.5	0.5	Closed	Discontinuous	5
8	66/female/RE	FTMH	PCIOL	0.7	SiO	0.5	0.5	Closed	Discontinuous	5
9	70/female/LE	FTMH	Phakic	1	C3F8	0.6	0.6	Closed	Discontinuous	4
10	69/male/RE	FTMH	Phakic	0.6	SF6	0.6	0.6	Closed	Continuous	4

ELM: External limiting membrane, IS-OS: Inner segment-outer segment, RE: Right eye, LE: Left eye, PDR: Proliferative diabetic retinopathy, CRD: Combined retinal detachment, FTMH: Full-thickness macular hole, MHRD: Macular hole-induced retinal detachment, PCIOL: Posterior chamber intraocular lens, SiO: Silicone oil, C3F8: Perfluoropropane, SF6: Sulfur hexafluoride

1 month after the operation [Figure 4]. The difference between these two groups was not statistically significant (*P* = 0.24).

OCT analysis showed complete hole closure with retinal adhesion over the amniotic membrane plug in all cases as early as 2 weeks post-surgery. However, at the 4th-week follow-up visit, the continuity of the ELM and ellipsoid zone layer over the hAM patch was seen only in one case (Group 2, case 6) [Figure 5], and in the remaining cases, the continuity of the ELM and ellipsoid zone layer over the hAM patch was absent. None of the cases showed any adverse events such as raised intraocular pressure, intraocular inflammation, endophthalmitis, hypotony, or retinal or choroidal reactions. In only one patient (case 2 of Group

B), the hAM was not found in the subretinal space and had got dislodged into the vitreous cavity on clinical examination at the 2nd-month follow-up visit; however, the MH remained closed. During the silicone oil removal, the adhesion over the amniotic membrane plug was checked intraoperatively and no additional laser photocoagulation was done.

DISCUSSION

The findings of this study suggest that using the hAM plug for the closure of the large-sized MH can achieve comparable anatomic and functional outcomes in comparison to the ILM flap technique.
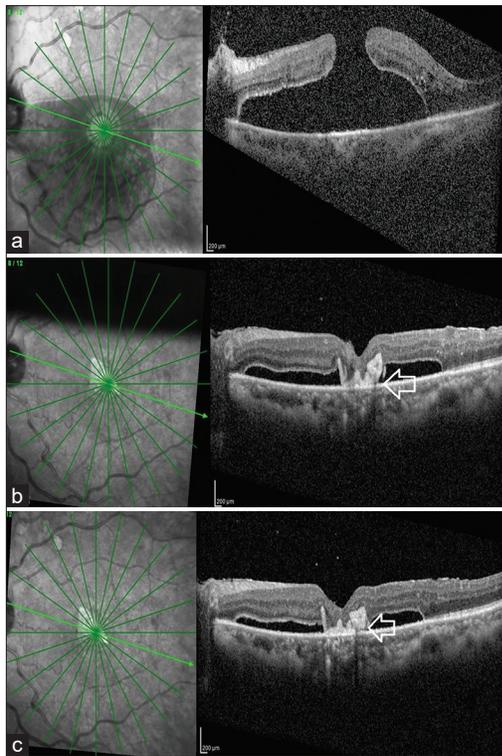
Figure 3: Pre and postoperative outcomes of a patient with full-thickness macular hole (MH) with retinal detachment having undergone primary human amniotic membrane (hAM) plug surgery (case 3, Group A). (a) This patient developed a posterior pole retinal detachment secondary to a large full-thickness MH. The patient underwent pars plana vitrectomy with internal limiting membrane peeling with hAM plugging into the MH in the primary surgery itself. (b and c) Optical coherence tomography image at 2-week and 4-week post-surgery shows the MH closed with hAM plug *in situ* and gradually resolving subretinal fluid (arrow with white border). The external limiting membrane and ellipsoid layer have not yet differentiated

Figure 4: Graph showing best corrected visual acuity changes of eyes undergoing primary and secondary human amniotic membrane plug transplantation

The principles of primary MH repair surgery include relieving the retinal traction by vitrectomy and ILM peeling, allowing the retinal cells to proliferate, thereby pulling the MH edges centripetally, and achieving a successful closure. This could be enhanced further if a useful matrix substrate is inserted inside the MH, thus allowing for the proliferation of the cells over the substrate and resulting in complete hole closure.[18] Plugging the hole with an additional substance and achieving retinal adhesion with the plug helps in obtaining a successful anatomic hole closure, especially in patients with failed primary surgeries. These include the use of ILM peeled from the periphery of the posterior pole and inserted into the MH, a neurosensory retinal free flap, or capsular lens fragments.[22-27] Previous *in vitro* studies have shown the hAM to be a useful substrate matrix for the human RPE cells to proliferate.[14,17] Hence, we proceeded to use hAM to plug both primary and secondary MHs in our series of cases.

In this series, the hAM plug was inserted inside the MH in 2 cases of MH with retinal detachments (cases 1 and 3 of Group 1) in the primary vitreous surgery itself [Figure 3]. In

eyes with retinal detachment associated with MH, peeling of the ILM may be difficult over the detached retina, even with the help of perfluorocarbon liquid. Thus, the hAM proves to be a good alternative option to plug the MH and help in the retinal attachment. Rizzo *et al*. performed a similar surgical technique which involved plugging of a retinal break with hAM in cases with recurrent retinal detachment and recurrent MHs.[19] In this study, the authors described the technique of inserting the hAM following vitrectomy and also noted the accurate placement of the hAM with intraoperative OCT visualization. Successful anatomic closure was obtained, and VA improved significantly in their group of cases. In comparison, in our series, we used the hAM to plug the large-sized (>800 μm) MHs during the primary surgery itself. Our series also had 1 case each of MH-induced retinal detachment and MH with combined retinal detachment in a patient with proliferative diabetic retinopathy (cases 1 and 3 of Group A).

In our series, anatomic closure of MH was achieved in all the 10 patients within 2 weeks of the surgery. An improvement of 0.1 logMAR vision was noted in 8 of the 10 patients. VA remained the same in two eyes at 4-week follow-up: One eye with optic disc pit maculopathy secondary to the extensive underlying RPE atrophy and another patient with a reopened full-thickness macular hole. In eyes with VA improvement, the OCT scans demonstrated closed MH and the presence of subretinal hAM patch with a fully intact retinal tissue over the hAM. However, the outer layers such as the ELM and inner segment-outer segment (IS-OS) junction layers were not well defined on the OCT scans at the 4-week visit in most cases except in case 6 of Group 2. This patient did not show
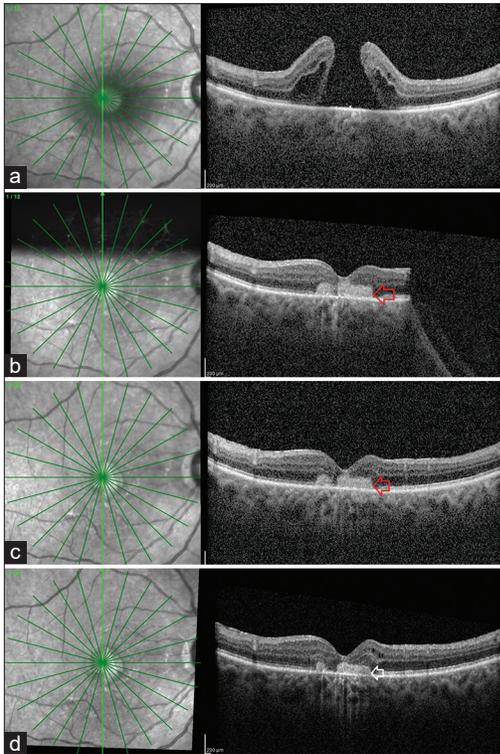
Figure 5: Pre and postoperative outcomes of a patient with re-opened full-thickness macular hole (MH) having undergone secondary human amniotic membrane (hAM) plug surgery (case 6, Group B). (a) This patient redeveloped a large full-thickness primary MH (outer diameter = 2185 μ) following initial MH repair surgery. The patient underwent hAM plugging into the MH 4 weeks after the primary surgery. (b and c) Optical coherence tomography image at 2-week and 4-week post-surgery shows the MH closed and hAM plug *in situ* (arrow with red border). The external limiting membrane and ellipsoid layer have differentiated over the hAM graft. (d) At 12-week final follow-up visit, the macula still remains closed with shrinking hAM plug *in situ* and integrated with the retina (arrow with white border). The patient had undergone cataract surgery with intraocular lens implantation, and final visual acuity improved to 0.3 logMAR

VA improvement at 4-week follow-up due to the presence of nuclear sclerosis cataract. At the final follow-up visit, after undergoing cataract surgery, the patient had a VA of 0.3 logMAR. Retinal fibrosis and pigment epithelial atrophy have been reported by some authors following autologous ILM transplantation for refractory MH.[28] A rolled and crumpled hAM was noted with the absence of ELM and IS-OS junction layers over it in the patient showing no visual improvement. Our analysis suggested that spreading the hAM patch as a uniform layer underneath the MH edges and not just by inserting the hAM inside the MH would allow the RPE cells to proliferate more uniformly with better stratification of the retinal layers and improvement in VA. However, unrolling the hAM inside the vitreous cavity is not simple. Using additional assistance of perfluorocarbon liquid or air may ease the unrolling of the hAM inside the vitreous cavity. Furthermore, the preoperative hole characteristics such as tall MH edges would allow easier insertion of hAM underneath the edges of the MH as seen in case 6 of Group 2 of our series. Some authors proposed the

possibility of amniotic membrane rejection when implanted in the subretinal space, but Rizzo *et al*. did not report any visible signs of rejection or retinal disorganization in their series of cases.[17,19] Similarly, this study did not show any hAM rejection, retinal detachment, or reopening of MH after the surgery.

The hAM can secrete growth factors and adhesion molecules, thus providing adequate support for RPE growth and facilitation of RPE integration into the subretinal space.[2,3,14,16,17] We believe that the hAM characteristics can play an important role even in primary retinal repair. hAM patches are easier to obtain from the tissue banks compared to other substrates used to close MHs, such as autologous ILM plugs or capsular lens fragments. Procuring an adequate size ILM or capsular fragment may involve additional effort and can be associated with an extra set of intraocular complications.

The small number of cases, short follow-up time, and varied set of indications are the major drawbacks of our study. Nonetheless, this study highlights the utility of hAM plug in MH closure. It further describes anatomic and functional outcomes and also discusses possible refinements in surgical technique for better visual outcomes. This study of 10 new cases adds to a very small number of published cases of hAM plugged MHs. Further studies are necessary to determine the efficacy of this new technique, and more intraoperative and postoperative analyses would be useful to understand the interaction between the amniotic membrane and the retina.

To conclude, hAM transplantation in the subretinal space is a useful and valid option for the repair of primary or refractory MHs, not only for anatomical success but also for the accompanying visual recovery. Further studies in this regard are required to improvise and simplify the surgical technique.

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Conflicts of interest
There are no conflicts of interest.

References

1. van Herendael BJ, Oberti C, Brosens I. Microanatomy of the human amniotic membranes. A light microscopic, transmission, and scanning electron microscopic study. Am J Obstet Gynecol 1978;131:872-80.
2. Grueterich M, Tseng SC. Human limbal progenitor cells expanded on intact amniotic membrane *ex vivo*. Arch Ophthalmol Chic Ill 1960 2002;120:783-90.
3. Meller D, Dabul V, Tseng SC. Expansion of conjunctival epithelial progenitor cells on amniotic membrane. Exp Eye Res 2002;74:537-45.
4. Anderson DF, Prabhasawat P, Alfonso E, Tseng SC. Amniotic membrane transplantation after the primary surgical management of band keratopathy. Cornea 2001;20:354-61.
5. Ashraf NN, Adhi MI. Outcome of application of amniotic membrane graft in ocular surface disorders. JPMA J Pak Med Assoc 2017;67:1045-9.
6. Bandeira F, Yam GH-F, Fuest M, Shing Ong H, Liu YC, Shen SY, *et al*. Urea-de-epithelialized human amniotic membrane for ocular surface reconstruction. Stem Cells Transl Med 2019;8:620-6.
7. Kim JS, Kim JC, Hahn TW, Park WC. Amniotic membrane transplantation in infectious corneal ulcer. Cornea 2001;20:720-6.
8. Le Q, Deng SX. The application of human amniotic membrane in

the surgical management of limbal stem cell deficiency. Ocul Surf 2019;17:221-9.

9. Lee SH, Tseng SC. Amniotic membrane transplantation for persistent epithelial defects with ulceration. Am J Ophthalmol 1997;123:303-12.

10. Letko E, Stechschulte SU, Kenyon KR, Sadeq N, Romero TR, Samson CM, *et al*. Amniotic membrane inlay and overlay grafting for corneal epithelial defects and stromal ulcers. Arch Ophthalmol Chic Ill 1960 2001;119:659-63.

11. Siu GD, Kam KW, Young AL. Amniotic membrane transplant for bullous keratopathy: Confocal microscopy & anterior segment optical coherence tomography. Semin Ophthalmol 2019;34:163-7.

12. Sahay P, Goel S, Maharana PK, Sharma N. Amniotic membrane transplantation in acute severe ocular chemical injury: A randomized clinical trial. Am J Ophthalmol 2019;205:202-3.

13. Rosenthal P, Cotter JM. Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. Am J Ophthalmol 1996;122:914-5.

14. Ohno-Matsui K, Ichinose S, Nakahama K, Yoshida T, Kojima A, Mochizuki M, *et al*. The effects of amniotic membrane on retinal pigment epithelial cell differentiation. Mol Vis 2005;11:1-10.

15. Stanzel BV, Espana EM, Grueterich M, Kawakita T, Parel JM, Tseng SC, *et al*. Amniotic membrane maintains the phenotype of rabbit retinal pigment epithelial cells in culture. Exp Eye Res 2005;80:103-12.

16. Kiilgaard JF, Scherfig E, Prause JU, la Cour M. Transplantation of amniotic membrane to the subretinal space in pigs. Stem Cells Int 2012;2012:716968.

17. Capeáns C, Piñeiro A, Pardo M, Sueiro-López C, Blanco MJ, Domínguez F, *et al*. Amniotic membrane as support for human retinal pigment epithelium (RPE) cell growth. Acta Ophthalmol Scand 2003;81:271-7.

18. Pak KY, Park KH, Kim KH, Park SW, Byon IS, Kim HW, *et al*. Topographic changes of the macula after closure of idiopathic macular hole. Retina Phila Pa 2017;37:667-72.

19. Rizzo S, Caporossi T, Tartaro R, Finocchio L, Franco F, Barca F, *et al*. A human amniotic membrane plug to promote retinal breaks repair and recurrent macular hole closure. Retina 2019;39:S95-103.

20. Caporossi T, Tartaro R, De Angelis L, Pacini B, Rizzo S. A human amniotic membrane plug to repair retinal detachment associated with large macular tear. Acta Ophthalmol 2019;97:821-3.

21. Zhu D, Jin X, Zhou J. Transplantation of amniotic membrane for choroidal hole to treat suprachoroidal silicone oil migration. Acta Ophthalmol (Copenh) 2017;95:e522-3.

22. Morizane Y, Shiraga F, Kimura S, Hosokawa M, Shiode Y, Kawata T, *et al*. Autologous transplantation of the internal limiting membrane for refractory macular holes. Am J Ophthalmol 2014;157:861-9.e1.

23. De Novelli FJ, Preti RC, Ribeiro Monteiro ML, Pelayes DE, Junqueira Nóbrega M, Takahashi WY. Autologous internal limiting membrane fragment transplantation for large, chronic, and refractory macular holes. Ophthalmic Res 2015;55:45-52.

24. Grewal DS, Mahmoud TH. Autologous neurosensory retinal free flap for closure of refractory myopic macular holes. JAMA Ophthalmol 2016;134:229-30.

25. Thomas AS, Mahmoud TH. Subretinal transplantation of an autologous retinal free flap for chronic retinal detachment with proliferative vitreoretinopathy with and without macular hole. Retina Phila Pa 2018;38:S121-4.

26. Chen SN, Yang CM. Lens capsular flap transplantation in the management of refractory macular hole from multiple etiologies. Retina Phila Pa 2016;36:163-70.

27. Peng J, Chen C, Jin H, Zhang H, Zhao P. Autologous lens capsular flap transplantation combined with autologous blood application in the management of refractory macular hole. Retina Phila Pa 2018;38:2177-83.

28. Lee SM, Kwon HJ, Park SW, Lee JE, Byon IS. Microstructural changes in the fovea following autologous internal limiting membrane transplantation surgery for large macular holes. Acta Ophthalmol (Copenh) 2018;96:e406-8.