

Comparison of three techniques of harvesting full-thickness retinal tissue for large or persistent macular holes

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Purpose: To evaluate the success rate of autologous retinal graft (ARG) for the closure of full-thickness macular holes (MHs) and compare the outcomes of three different techniques of harvesting the graft. **Methods:** Clinic files of all patients who had undergone ARG for MH using intraocular scissors, membrane loop, or retinal punch to harvest retinal tissue were retrospectively reviewed. All patients were evaluated for MH closure, retinal reattachment, and visual improvement. **Results:** Twenty-two eyes of 22 patients were included. ARG was done for 16 eyes (72.7%) with failed, large persistent MH, and six eyes (27.3%) also underwent simultaneous repair of retinal detachment. The basal diameter of MH was 1103.67 ± 310.09 (range 650–1529) μm . Intraocular scissors were used in 10 eyes (45.5%), a membrane loop in five eyes (22.7%), and a retinal punch in seven eyes (31.8%). Silicone oil tamponade was used in seven (31.8%) eyes and gas in 15 (68.1%) eyes. The follow-up ranged from 6 to 18 months. The hole closure rate was 72.7% (16/22). Visual improvement was noted in 18 eyes (81.8%). Retinal reattachment was seen in all eyes. Good graft integration with the surrounding area was seen in 17 eyes (77.3%). Graft retraction was seen in four eyes (18.18%) and graft loss in one eye (4.55%). No significant differences were noted among the three groups. **Conclusion:** ARG is successful in closing large, failed MH with and without retinal detachment. A membrane loop and retinal punch are equally useful in harvesting the graft, but scissors are preferable in case the retina is detached. With all three techniques, integration of the graft with the surrounding tissue can be achieved.

Key words: Autologous Retinal Graft, macular hole, retinal detachment, retinal punch, vitrectomy

Following its introduction by Kelly and Wendel, vitrectomy along with peeling of the internal limiting membrane (ILM) with gas tamponade has become the treatment of choice for managing full-thickness macular holes (MHs).^[1] Various modifications and refinements in this technique have led to anatomic success rate of nearly 90% for idiopathic MHs.^[2] Certain factors such as large size, concurrent retinal detachment (RD), high myopia, and chronicity may be responsible for non-closure of MHs.^[3] Surgical options for failed MHs, where vitrectomy and ILM peeling have been done, are limited. Various adjuvant materials to plug the retinal defect during vitrectomy have been reported, which include a free ILM flap,^[4] use of amniotic membrane (AMG),^[5,6] anterior lens capsule,^[7] and autologous retinal graft (ARG).^[8,9]

ARG was first described by Grewal and Mahmoud in 2016 in a patient with high myopia with an open MH following vitrectomy.^[10] Thereafter, several other reports including one from a large global consortium have shown the feasibility of using ARG for closure of the MHs.^[7,11-17] Results with

ARG showed it to be comparable to the other techniques for refractory MHs, such as AMG, autologous blood, and re-tamponade with gas alone.^[18] Tabandeh showed good uptake of the full-thickness retinal graft by demonstrating vascularization and reperfusion of the graft.^[19] Good functional recovery with improvement in retinal sensitivity was shown on multifocal electroretinography (mfERG) and microperimetry.^[15,20,21] The surgical technique involves harvesting of full-thickness neurosensory retinal tissue from mid-peripheral retina and transferring the tissue to plug the MH under air or perfluorocarbon liquid (PFCL). Various techniques are used by different surgeons to harvest the graft in the least traumatic manner and to prevent graft dislodgement. The purpose of this analysis was to see whether the method of harvesting the graft has any effect on the surgical success or functional outcome in patients who had undergone ARG.

Methods

This was a multicentric retrospective cases series of patients who had undergone vitrectomy with ARG for refractory full-thickness MH or large MH with RD. Surgeries were

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performed by six surgeons at five centers. The institutional review board approval was taken at each center, and the study adhered to tenets of the Declaration of Helsinki. All patients had signed a written informed consent before surgery.

All patients with MH size $>500\ \mu\text{m}$ associated with RD, open MHs following vitrectomy with adequate ILM peeling, or large MH ($>500\ \mu\text{m}$) associated with high myopia were included. Patients were excluded if they had a traumatic MH, uveitis, uncontrolled glaucoma, diabetic retinopathy, retinal degeneration, or retinal vascular occlusions. Patients with uncontrolled diabetes, severe systemic comorbidities, and those unable to maintain prone position were also excluded from the study. Patients were evaluated with pre- and postoperative spectral domain optical coherence tomography (SDOCT). The primary outcomes were closure of MH and reattachment of the retina. Secondary outcomes were change in best-corrected visual acuity (BCVA), restoration of foveal contour, and integration of the graft with restoration of outer and inner retinal layers on SDOCT.

Surgical technique

A standard three-port vitrectomy was performed with 23G or 25G system. Patients with visually significant cataract underwent simultaneous phacoemulsification with intraocular lens implantation before vitrectomy. A peripheral circumferential belt buckle was used based on the surgeon's discretion in cases with total RD. Following core vitrectomy, posterior hyaloid was detached using suction or forceps and total vitrectomy including base excision was performed, especially in eyes with RD. Any remnant epiretinal fibrous tissue was dissected. In cases with retinal detachment, all preretinal membranes were removed with spatula and end-gripping forceps. A donor site was chosen either in superotemporal, superonasal, or inferotemporal quadrant. The size was determined based on the size of the MH. Three different methods were used to harvest the ARG. In the first method, an intraocular curved scissors was used to cut the desired sized retinal graft after doing endodiathermy around it. A bubble of PFCL was placed over the MH, especially if the retina was detached. In a bimanual technique, the graft was held with two intraocular end-gripping forceps and taken to the MH site while ensuring correct orientation at all times. The graft tended to get crumpled in the PFCL bubble, hence the graft was laid on the retinal surface under the PFCL with

the photoreceptor side facing inferiorly and dragged slowly toward the MH. The graft was placed covering the MH, and no attempt was made to tuck or stuff the edges into it. Fluid-air exchange was done, PFCL bubble was removed, and all peripheral breaks including the donor site were lasered. Air was replaced with silicone oil or non-expansile gas, as per the surgeon's discretion.

In the second method, a membrane loop (Finesse Flex loop; Alcon, Fort Worth, TX, USA) was used to gently lift the retinal flap from its bed following endodiathermy and localized laser barrage. The area of diathermy makes the retina friable and easier to lift up using the loop. Full thickness of the graft was ensured by visualizing the retinal pigment epithelium (RPE)-choroidal bed underneath. An intraocular forceps to hold the edge of the ARG assisted in completing the graft removal. The graft was then transferred to the MH under PFCL or air. The rest of the procedure was the same. This method was used to harvest graft from the attached retina and could not be used in cases with total RD.

In the third method, a specially designed retinal punch (Epsilon India, Mumbai, India; patent pending) was used to create free ARG. The size of the punch was decided based on the preoperative measurement of the MH. No endodiathermy or laser was done to the donor site before using the punch. The rest of the steps for the graft transfer and tamponade were the same as above.

Postoperatively, patients were prescribed topical moxifloxacin and homatropine and were asked to maintain prone position for 1 week. They were initially seen at 1, 3, and 6 weeks. The surgical success was determined at the sixth week visit. Subsequent follow-ups and timing of silicone oil removal were at the treating surgeon's discretion. SDOCT was done at 1, 3, and 6 weeks in most patients. Some patients also underwent visual field and mfERG.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) for Windows (version 24.0). Data entries were performed in Excel sheets using [®]Microsoft Excel for Windows. Categorical variables were described as frequency (percentage), and mean \pm standard deviation was used for continuous parameters. Snellen's visual acuity was converted to logarithm of the minimum angle of resolution



Figure 1: Intraoperative photo of the right eye of the patient who had undergone multiple previous retinal detachment surgeries showing a recurrent retinal detachment with contraction, multiple breaks, and a stretched, large full-thickness macular hole (a). The postoperative photo of the right eye shows closed MH and attached retina with silicone oil *in situ* (b). The OCT scan shows well-integrated autologous retinal graft with restoration of both outer and inner retinal layers and normalization of the foveal contour (c). Scissors were used in this case for graft harvesting. MH = macular hole, OCT = optical coherence tomography

Table 1: Demographic details of the study cohort

Parameter	Finesse Flex loop, <i>n</i> =5 (22.7%)	Scissors, <i>n</i> =10 (45.5%)	Retinal punch, <i>n</i> =7 (31.8%)	<i>P</i>
Age (mean±SD), years	55.4±7.16	49.3±18.04	61.57±5.38	0.82
Type of surgery				
ARG	4 (80%)	5 (50%)	7 (100%)	
ARG + RRD	1 (20%)	5 (50%)	0	
Time from previous surgery (mean±SD), months	4.4±6.02	2.17±3.3	2.11±0.45	
Macular hole horizontal basal diameter (mean±SD), μm	1325.8±123.35	1273.11±207.6	727.14±57.65	
Lens status				
Phakic	3 (60%)	3 (30%)	1 (14.3%)	
Pseudophakic	2 (40%)	6 (60%)	6 (85.7%)	0.250
Aphakic	0	1 (10%)	0	
Tamponade				
Gas	5 (100%)	3 (30%)	7 (100%)	
Silicone oil	0	7 (70%)	0	
Autograft site				
Superotemporal	1 (20%)	6 (60%)	7 (100%)	
Superonasal	4 (80%)	3 (30%)	0	0.039
Superior 12 o'clock area	0	1 (10%)	0	

ARG=autologous retinal graft, RRD=rhegmatogenous retinal detachment, SD=standard deviation

Table 2: The comparison of pre- and postoperative visual acuity

Vision	Mean±SD	Mean difference (95% CI)	<i>P</i>
Loop			
Pre-op	1.08±0.31	(0.1-0.74)	0.043
Post-op	0.66±0.13		
Scissors			
Pre-op	1.51±0.61	(0.46-1.14)	0.005
Post-op	0.71±0.52		
Retinal punch			
Pre-op	1.17±0.21	(-0.09 to 0.24)	0.269
Post-op	1.1±0.27		

CI=confidence interval, SD=standard deviation

(LogMAR) units for statistical analysis. Chi-square test was applied for independence and was used to test the association between two qualitative variables. Kruskal-Wallis test was used to compare the median difference among three autograft acquisition groups, and Wilcoxon signed rank test was used to compare the pre- and postoperative visual acuity within each group. *P* value <0.05 was considered statistically significant.

Results

The study included 22 eyes of 22 patients, of which 11 (50%) were males. The mean age of the patients was 54.59 ± 13.68 (range 12–67) years. ARG was harvested using the Finesse loop in five eyes (22.7%), the retinal punch was used in seven eyes (31.8%), and intraocular scissors were used in 10 eyes (45.4%). ARG alone was done in 16 eyes (72.73%), while ARG was performed along with RD repair in six eyes (27.3%). The follow-up ranged from 6 to 18 months. The demographic details are given in Table 1.

The average preoperative minimum diameter of MHs was 1103.67 ± 310.09 (range 650–1529) μm. Also, 14% C₃F₈ gas was used as tamponade in 15 eyes (68.1%) and silicone oil was used in seven eyes (31.8%). All eyes with MH and RD received silicone oil tamponade. All eyes (100%) with ARG and RRD had attached retina at the final follow-up [Fig. 1].

MH closure at 6 weeks was seen in 16 eyes (72.7%) in our study. BCVA improvement was observed in 18 eyes (81.8%), while it remained unchanged in three eyes (13.6%) and worsened in one eye (4.5%) over a follow-up of 6 months. BCVA improvement was noted in all eyes (100%) in which Finesse loop and intraocular scissors were used to harvest the ARG. However, only three eyes (42.8%) in the group where retinal punch was used had improvement in vision. Postoperative improvement in BCVA at 6 weeks compared to baseline was statistically significant in the intraocular scissors group (*P* = 0.005) and in the Finesse loop group (*P* = 0.043), but not in the retinal punch group (*P* = 0.269) [Table 2].

The inner retina showed good integration with normalization of foveal contour in all but four eyes [Fig. 2]. However, restoration of outer retinal layers, namely, the external limiting membrane (ELM) and ellipsoid zone (EZ), after ARG was noted in two eyes (40%) in the Finesse loop group, three eyes (30%) in the intraocular scissors group, and in four eyes (57.1%) of the retinal punch group. The difference among these groups was not statistically significant (*P* = 0.507). Postoperative graft shrinkage was observed in two eyes each where intraocular scissors (20%) and retinal punch (28.57%) were used [Fig. 3]. One eye (14.29%) in the punch group also had total dislodgement and subsequent graft loss. No complications were observed in the membrane loop group.

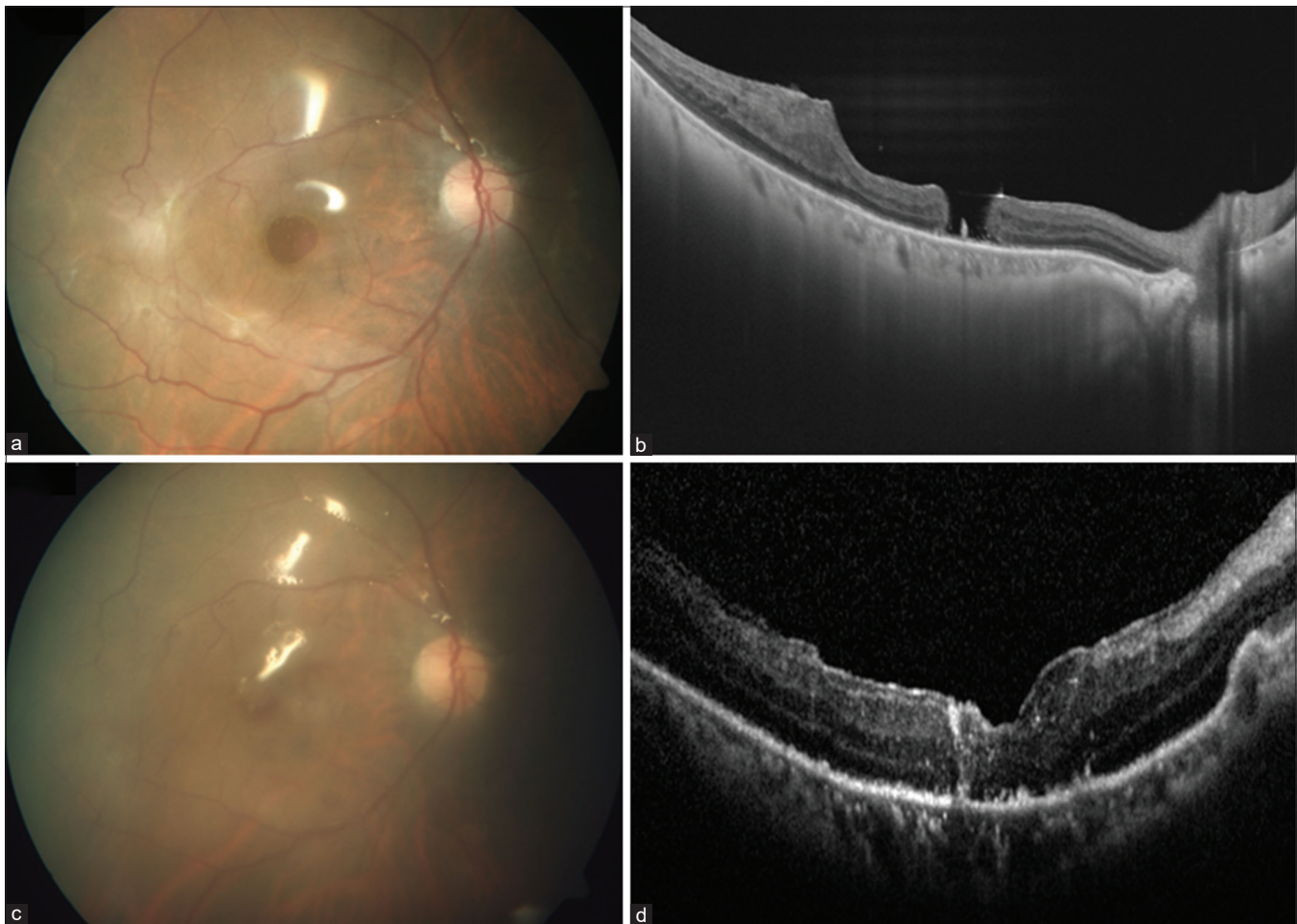


Figure 2: The right eye of a patient shows recurrent retinal detachment inferiorly with a large open MH under silicone oil. Preretinal membranes can be seen temporal to MH (a). The patient underwent surgery with autologous retinal graft. Postoperatively, the retina was attached and the MH was closed (b). The OCT scan shows good integration of the inner layers of the graft. A few hyperreflective spots are seen possibly due to the surgery-induced inflammation (c). The membrane loop (Finesse) was used for graft harvesting. MH = macular hole, OCT = optical coherence tomography

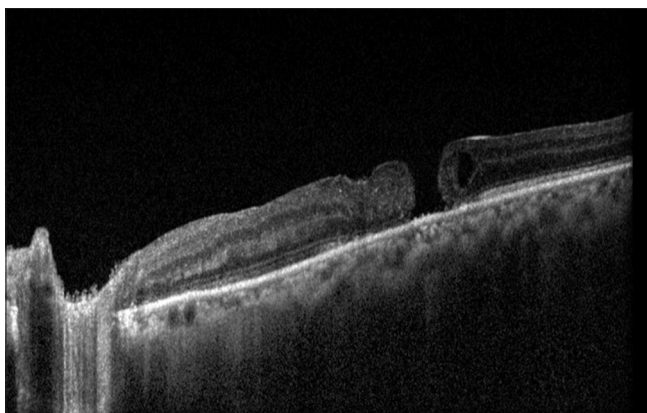


Figure 3: This OCT scan shows retraction of the temporal edge of the retinal graft causing the macular hole to reopen. Retinal punch was used for graft harvesting. OCT = optical coherence tomography CI = confidence interval, SD = standard deviation

Discussion

An MH that fails to close in the first attempt despite adequate ILM peeling poses a challenge. Large chronic holes or those

in high myopic eyes are more prone to failure.^[3] The inverted flap technique, advocated by Michalewska *et al.*,^[22] was seen to be more successful in closing such holes. The success rate of ILM peeling alone was seen to vary from 78% to 81% versus 89% with inverted ILM flap in large MH of >800 μm in size.^[23,24]

The free ILM flap technique has a limited success rate. A study comparing the outcome after three different techniques, namely, a free ILM flap, an inverted flap, and ILM peeling alone, found the success rate to be 86% after a free flap and 92% after an inverted flap.^[25]

As another option, the area of previous ILM peeling can be extended. But ILM peeling is known to induce several morphological and functional changes. The ILM, which is considered to be a basement membrane of the Müller cells, is connected to the foot plates of the photoreceptors. ILM peeling disrupts these connections and can lead to nerve fiber layer disassociation and swelling.^[26] In a meta-analysis, it was found that fovea sparing ILM peeling had better visual outcomes.^[26] Apart from these changes, significant shortening of papillofoveal distance with foveal displacement toward the disk was noted in a study.^[27] A larger area of ILM peeling was seen to be associated with reduced sensitivity in the central

macula.^[28] After transplantation of a free autologous ILM flap, the MH is seen to close by formation of fibrous tissue lacking any retinal photoreceptor or neurologic elements. Thus, the visual recovery and quality is likely to be inferior than that seen with a retinal autograft which provides a bridging scaffold and also seals off fluid movement from the vitreous cavity to the subretinal space.^[11,29]

The hole closure rate was 72.7% in this study, with 81.8% showing visual improvement. In a large multicenter, international collaborative study, among 41 eyes with refractory MH, the closure rate with autograft was 87.8%, and 52.3% showed improved vision.^[30] The retinal autograft technique is especially useful where the retina is contracted or less extensible due to scarring or tethering. It would also be useful in the eye with extensive macular degeneration and loss of underlying RPE, such as in high myopic degeneration.^[20,21] It is preferable in cases where the conventional methods are likely to be unsuccessful, for example, MH associated with macular telangiectasia.^[31]

The technique shows minor variations in the reports, but almost all authors have used the scissors to harvest the graft. We have used three different techniques with the purpose of making the harvesting of the graft as atraumatic to the donor tissue as possible. If the recurrent MH is associated with retinal detachment, harvesting the graft with the use of scissors is easy. But difficulty can be encountered in attached retina. In this study, two novel techniques were used. In one technique, the membrane loop (FINESSE Flex loop, Alcon) with a retractable thin nitinol loop with small tines on the undersurface was used. The loop is at an angle to the shaft, making it easy to use it as a scraper on the surface of the retinal tissue. The tine engages the membrane tissue, and it is generally used for ILM peeling. In this study, it was used to engage the edge of the graft and peel it from the RPE. This can give more atraumatic harvesting without causing damage to the edges of the graft. It allows smoother and complete harvesting of the retinal tissue with minimal distortion of the retina and minimal chances of retinal or choroidal bleeding. The rigidity of the loop can be controlled by retracting it further.

The retinal punch was designed by one of the authors (RKB). It can be customized as per the requirement and can give a precise size of the graft with minimal damage to the graft edges. With this technique, the size of the graft is limited. The graft might be just fitting or slightly larger. Risk of graft contracture might be more. But the advantage of the punch lies in a clear-cut edge without damage to the cells at the cut edge. No prior endodiathermy or laser is required for the donor site. Thus, the photoreceptor cells will retain better viability and probably have better uptake with the surrounding edges at the host site. Scissors would crush the cells at the edge between the two blades. Similarly, with the Finesse loop, the viable cells at the edges might be better preserved. The BCVA results showed no significant improvement in the retinal punch group, probably due to the number of cases with graft contracture and graft loss. But no statistically significant difference was observed in the functional restoration of the ELM and EZ in all the three techniques. Further studies are required to evaluate this aspect in detail.

A 15%–20% larger graft is desirable as it may undergo shrinkage postoperatively. Chen *et al.*^[17] reported good

outcome in all seven eyes with large MHs (>1000 μ m) with concomitant recurrent retinal detachment and proliferative vitreoretinopathy. A 20% larger graft was harvested after endodiathermy using scissors and transported to the MH under PFCL. The donor area was stained with Indocyanine green dye (ICG) to identify the inner and outer surfaces of the retina. Wu *et al.* used graft of the same size.^[13] They also used autologous blood clot over the retinal autograft with the hypothesis that the blood clot will act as glue and help keep the retinal graft in place. However, the contracting blood clot is likely to exert traction on the retinal graft and displace it. In their series of six eyes, the graft was displaced in two eyes. However, Chang *et al.*^[29] argue that blood contains multiple growth factors which can theoretically promote collagen synthesis, fibroblast proliferation, and increase the chances of hole closure.

The graft can be taken to the MH under air/fluid or PFCL. The advantage of PFCL is that it can stabilize the posterior retina as well as the graft and prevent migration or loss of the graft. However, it is more difficult to maintain the orientation of the tissue inside the PFCL phase and the graft tends to get crumpled. It is imperative to keep a watch on the way the graft is getting folded or use two intraocular forceps in a bimanual manner to hold the graft at two places and keep it stretched out between the two holding points to prevent folding onto itself. Thus, the orientation can also be maintained. However, reversal of the polarity might not affect the outcome much. Chen *et al.*^[17] showed a case wherein despite reversed polarity of the autograft, the MH closed and the patient gained useful vision which was the best among the case series.

It is noted that in adults, the peripheral retina contains the Müller cells that retain the progenitor properties. These cells have the capacity to migrate to the outer nuclear layer, proliferate, and replace the lost photoreceptor cells. Yamada *et al.*^[32] suggest that the graft should preferably be harvested from a peripheral site outside the arcades.

Among the various techniques for persistent MHs, such as tamponade alone, AMG, autologous blood, and retinal graft, Szurman *et al.*^[18] found no technique which was superior to the other. Comparable results were noted in all. Retinal graft can also be the primary option for large, chronic holes.^[33–35] Despite being a free graft, it gets incorporated in the surrounding tissue with excellent perfusion^[19] and recovery of physiological function, as seen by improved retinal sensitivity at the fovea on Humphrey visual field test (HVF) and improved responses on the mfERG in our two cases. Lumi *et al.*^[15] also found good functional recovery following ARG.

Intraoperatively, the microscope-integrated optical coherence tomography (MIOCT) might be helpful in confirming correct placement and size of the graft.^[20,36]

So far, only one study has reported the formation of a choroidal neovascular membrane under the grafted tissue.^[20] The authors of this study observed this complication in two out of five cases. They postulated the cause to be intra- or postoperative inflammation coupled with minor trauma to the RPE in the MH base.

This study has a few limitations. It is a retrospective account, and the number of cases is less. Different surgeons have employed slightly different techniques.

Conclusion

In conclusion, this study reaffirms that ARG is an effective surgical option for large MHs with or without retinal detachment as the primary treatment or after failed ILM peeling surgery. No significant difference in anatomic outcomes were noted among the three techniques of graft harvest in our study. However, the membrane loop was associated with the least number of complications, while the retinal punch was associated with poorer functional success and more graft shrinkage. Larger prospective studies are required to further analyze these aspects to provide robust evidence in times to come.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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