INVERTED INTERNAL LIMITING MEMBRANE FLAP TECHNIQUES AND OUTER RETINAL LAYER STRUCTURES

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Purpose: To examine the influence of the inverted flap (IF) internal limiting membrane (ILM) technique in macular hole (MH) closure on outer retinal layers after MH surgery.

Methods: Retrospective study. Postoperative position of ILM, recovery rate of external limiting membrane and ellipsoid zone, and best-corrected visual acuity were evaluated. The Inserted group, where the IF is placed inside the hole, was compared with the Cover group, where the IF completely covers the hole.

Results: Sixty-two eyes of 58 patients who underwent vitrectomy and ILM peeling with the IF technique for large MHs (>400 μm) with successful MH closure and a follow-up of 12 months were evaluated. In the 24 eyes of the Inserted group, there was no regeneration of external limiting membrane or ellipsoid zone after 12 months. In the 38 eyes of Cover group, external limiting membrane recovered in 55.3% of patients 1 month after surgery, and in 86.1% after 12 months. The ellipsoid zone layer was present in 58% of the patients.

Conclusion: Poorer anatomical and visual results were associated with the IF technique where ILM insertion occurs compared with ILM placed over the hole. These findings suggest that insertion of the ILM in the hole might prevent outer retinal layers realignment and visual recovery in MH surgery.

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Macular hole (MH) surgery has evolved from the initial studies of Wendel et al1 and became a treatable disease with pars plana vitrectomy allowing for anatomical closure.

With the introduction of internal limiting membrane (ILM) peeling, tangential traction was reduced and a higher rate of closure was achieved, with less recurrence.2 Park et al3 showed, in 58 eyes, that pars plana vitrectomy with ILM peeling was superior to vitrectomy alone in closing MHs. However, in large holes or myopic eyes, the failure rate was still high.4,5

Since the introduction in 2010 of the inverted flap (IF) technique in MH surgery by Michalewska et al,6 the success of surgery in hole closure has increased in large MH (>400 μm). The IF technique was also important in surgical repair of myopic MHs.7

The ILM flap techniques reported have differed mostly in how the ILM flap is positioned over the hole. Many of these differences are probably due to the difficulty in controlling the position of the flap during the fluid–air exchange. Therefore, the IF ILM technique is not unique nor exact showing some variations.8 Different techniques have been described in the literature for the ILM flap manipulation and positioning during the IF technique.8

Some surgeons intentionally insert the peeled ILM in the hole, after careful detachment around and up to the hole. Others choose to trim the excessive ILM using the vitrectomy probe, leaving one large ILM flap anchored on the border of the hole and inverted over the hole, with or without the help of perfluorocarbon9 or viscoelastic.10,11 If the inverted ILM stays in place, a small closed space over the MH allows for realignment of the external limiting membrane (ELM) and ellipsoid zone (EZ).12 However, with fluid–air exchange, this flap may not stay in place, over the hole, but rather stuck inside the hole.13

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After hole closure, the central scotoma gets less noticeable, and there is visual improvement. However, the functional improvement depends on the integrity of the outer retinal layers. External limiting membrane recovers first, and the EZ defects recover later. Both have defects that may decrease with time.

In this study, we examined the influence of the IF ILM techniques in MH closure on the outer retinal layer structures after MH surgery.

Patients and Methods

Setting and Patients

This was a retrospective study of 62 eyes of 58 patients with large MH, who underwent surgery between January 2015 and June 2017 at the Department of Ophthalmology of Hospital de Santa Maria, Lisbon, Portugal. Only closed MHs with a minimum follow-up of 12 months were included in the study. Exclusion criteria were maculopathy other than MH, previous retinal surgeries, other retinal diseases, or an axial length greater than 26.0 mm. Mean follow-up time was at least 12 months after surgery. This study was approved by the Ethics Committee of Hospital de Santa Maria. The tenets of the Declaration of Helsinki were followed. All subjects have given written informed consent to the surgical and the study procedures.

Collected Data

Preoperative data included age, sex, and a complete ophthalmic examination. Best-corrected visual acuity was measured using Snellen visual acuity charts and converted to the logarithm of the minimum angle of resolution (logMAR) for statistical analysis. Hand motion was considered as logMAR 3.0 and counting fingers as logMAR 2.0.

Data Availability

All data used to support the findings of this study are at the Ophthalmology Department, Hospital Santa Maria, Centro Hospitalar Universitário Lisboa Norte and available from the corresponding author upon request.

Surgical Procedure

A standard surgical procedure consisted of 23- or 25-gauge, three-port pars plana vitrectomy. Except in pseudophakic patients, every patient underwent combined cataract surgery, to reduce confounding results. Standard small-incision phacoemulsification and implantation of a standard foldable intraocular lens were associated with vitrectomy. In every eye, Brilliant Peel Dual (Geuder, Heidelberg, Germany) assisted ILM peeling was performed, in an area of approximately 3 mm, engaged with end grip intraocular forceps. With this technique, a flap is created and then peeled in a rosette way all around the hole and trimmed until the border of the macula, always leaving a flap large enough to invert and cover the hole, with or without the help of perfluorocarbon liquid or viscoelastic. In 10 of these eyes, the ILM was peeled around hole, and an attached flap is intentionally placed into the hole. Surgery was followed by fluid–air exchange and 15% SF6 (sulfur–hexafluoride) gas. All patients were instructed to maintain a face-down position for at least 5 days. All surgeries were performed by the four experienced surgeons (M.Y.F., E.N., H.P., and C.M.-N.).

Optical Coherence Tomography

All MHs were staged according to recent optical coherence tomography (OCT)-based classification and only full-thickness MHs with hole diameter superior to 400 μm were considered for the study. Retinal images were acquired using Spectralis spectral domain-OCT (Heidelberg Engineering, Heidelberg, Germany), using the eye-tracking feature software with posterior pole images centered on the fovea (61 acquisitions, 120 μm interval). The status of the foveal EZ and ELM were examined for each eye to test their integrity, intact, or disrupted. The intact eyes had a regular and continuous hyperreflective line corresponding to the EZ or ELM—Figure 1. The disrupted eyes were characterized by hyporeflective discontinuities in the EZ or ELM line—Figures 2 and 3. These classifications were achieved by agreement of two authors (M.Y.F. and N.G.F.).

Postoperative Follow-up

In postoperative observations, at Day 1, Month 3, and Month 6, a thorough ophthalmic examination was performed, including best-corrected visual acuity and OCT. Hole status, macula diameters, and ELM and EZ

Fig. 1. Optical coherence tomography of closed MH, with ILM over the hole in Closure group.
integrity at the fovea were measured by spectral OCT at 1 and 12 months after surgery.

Statistical Analysis

Results are expressed as medians (interquartile range) or n (%). Two groups were compared: Inserted group and Cover group. Within the Cover group, evolution time of the MH was also compared. Best-corrected visual acuity was compared before and after surgery. Between-group analyses were performed with the Mann–Whitney U test for continuous variables and the \( \chi^2 \) test for discrete variables. For comparisons before and after surgery, the Wilcoxon signed-rank test was used. Statistical significance was established at \( P < 0.05 \).

Results

Baseline Characteristics

Sixty-two eyes were submitted to surgery and four patients had bilateral MH. Twenty-four eyes resulted in inserted ILM (10 intentionally inserted and 14 due to misdirection of the ILM flap with fluid–air exchange), and 38 eyes resulted in hole cover by the ILM. Age, sex, eye and hole dimension did not differ significantly between the Inserted and Cover groups—Table 1. There were no differences in age or hole dimension between intact or disrupted layers. Within the Inserted group, there was also no difference in age or hole dimension between intentionally inserted or inadvertently inserted ILM.

| Table 1. Baseline Characteristics According to Two Groups: Inserted and Cover |
|-----------------------------|-----------------------------|---|
|                            | Inserted \((n = 24)\)       | Cover \((n = 38)\) | \( P \) |
| Age, years                 | 74 (13)                     | 68 (11)            | 0.05 |
| Female gender, n (%)       | 8 (38.1%)                   | 19 (55.9%)         | 0.27 |
| Right Eye, n (%)           | 13 (54.2%)                  | 13 (34.2%)         | 0.19 |
| Hole dimension, micron     | 579.0 (191.0)               | 597.5 (280.0)      | 0.44 |

Age and hole dimension: median (IQR). \( P \) values obtained from the Mann–Whitney test or the \( \chi^2 \) test, as appropriate. IQR, interquartile range.

Best-Corrected Visual Acuity Before and After Surgery

All analyzed groups showed an increased postsurgery best-corrected visual acuity—Table 2. There was disappearance of central scotoma in all groups.

External Retinal Layers (External Limiting Membrane + Ellipsoid Zone) Alignment 12 Months After Surgery

When comparing the Inserted group with the Cover group considering both external retinal layers (ELM + EZ), disrupted or intact at 12 months, the Inserted group showed 100.0% disrupted layers and the Cover group showed 71.1% intact layers—Figure 4.

External Limiting Membrane and Ellipsoid Zone Alignment at 1 and 12 Months After Surgery

All eyes in the Inserted group showed interrupted ELM and EZ at 1 and 12 months after surgery with foveal hyperreflective lesions in the inner retina as assessed by OCT. The Cover group showed an increase in realignment of ELM from Month 1 to 12 (55% to 86%), with 0% realignment of EZ at Month 1, which increased to 57.9% at Month 12—Figure 5. Reconstruction of the ELM preceded restoration of the foveal EZ in all cover technique cases.

Evolution Time of the Macular Hole Before Surgery

In the Inserted group, both ELM and EZ were 100% interrupted at both 1 and 12 months, regardless of the evolution time of the MH before surgery. In the Cover group, realignment of ELM was superior when the evolution time was \(<6\) months compared with \(>12\) months at both 1-month and 12-month assessments—Figure 6A—while there were no differences regarding EZ realignment—Figure 6B.
Complications
No complications in the form of retinal detachment or glaucoma occurred during this study.

Discussion
The technique of inverted ILM flap was described by Michalewska et al.,6 which was shown to provide superior anatomical and functional outcomes in cases of large MHs. This involves preserving a flap of the ILM connected to the border of the MH and then inverting this to cover the MH. According to the OCT images in the original study by Michalewska et al, the inverted ILM flap covering of the MH was important for hole closure.6,11,17

Even with the best surgical technique, the inverted ILM may 1) flip back during fluid–air exchange, even if it is in the proper position, which will result in surgical failure; 2) dip into the hole and become in contact with the inner lining of the hole, or 3) stay over the hole on the intended position.

The first situation can happen, even with all the care taken during surgery, slow fluid–air exchange after inverting the flap over the hole, or even using a viscoelastic cap to fold and secure the ILM into the MH before air exchange.18

The second case may be intentional to avoid flip back, or occur after surgery in postoperative face-down position, and it cannot be prevented. In fact, some surgeons intentionally gently tuck the flap into the MH to secure the free end under the hole edge during fluid–air exchange.8,13 This insert or tuck in technique was also described by other authors, such as Rizzo et al.,13 who also found, in a retrospective study on 620 eyes of 570 patients, that vitrectomy, ILM peeling, and IF technique are more effective than the standard ILM peeling technique. These authors peeled the ILM around the hole and detached it from the retina up to the edge of the hole. Excessive ILM was trimmed using the vitrectomy probe, and the ILM flap anchored on the edge of the hole was inverted and inserted into the hole. Although the hole will close if the flap is inserted into the hole, either intentionally or not, the outer retinal layers will not realign, at least during the first 12 months after surgery, as shown by our results.

The third situation, when the inverted ILM stays over the hole on the intended position, is apparently the ideal achievement because the hole will close, with better visual outcomes and outer retinal layers realignment, as shown by our results, and supported by the results obtained by Shin et al.9 These authors avoided packing the MH with the folded ILM, resulting in a multilayered membrane, as observed in OCT, and used perfluorocarbon to guarantee a single-layered ILM to provide a more regular structure for glial proliferation and aid in regeneration of outer retinal layers in closing the MH. Park et al.19 also compared the ILM insertion technique with the IF technique and concluded that both techniques were effective in closing large MHs, although the IF was superior in recovery of photoreceptors layers and better postoperative visual acuity.

In our study, we peeled about 3 mm of the ILM around the hole, trimmed until the border with care taken to leave one piece of ILM inverted over the hole. This attached and inverted ILM was left free and maintained in position in fluid–air exchange, with or without perfluorocarbon liquid or viscoelastic to maintain the ILM in position. Even with these precautions taken, 14 eyes resulted in ILM tuck into the hole, preventing the realignment of photoreceptors. The eyes with IF and one layer of ILM completely covering the hole achieved a closed space and ELM and EZ restored after 12 months.

Based on these results, it seems that ILM anchored in the hole prevents centripetal movement of photoreceptors,
therefore inhibiting realignment of MLE and EZ, either by obstruction or excessive gliosis. Hu et al reported that OCT after the inverted ILM flap technique revealed foveal hyperreflective lesions suggestive of excessive gliosis in the fovea. In this study, foveal hyperreflective lesions are apparent after ILM inserted in the hole, and the ELM and EZ at this site are disrupted. Should it result in scar formation, this may limit the recovery of ELM and the EZ.

In the report of Wakabayashi et al, a hyperreflective lesion replacing all intraretinal layers at the fovea was found in all cases that showed disruption of the ELM line. By contrast, when the ELM line was restored, the reflective lesion was at the inner retina above the ELM line or was absent. In our study, we found hyperreflective material within the MH in all cases of the fill in group.

When the hole is covered with the ILM flap, there may be glial activation with Muller cell gliosis associated with bridging the gap between the edges of the retinal hole, and glial and retinal pigment epithelium cells might play an important role. Humoral factors such as angiogenic factors may incite realignment of photoreceptors and ELM and EZ and closing of the hole. Shiode et al studies of idiopathic MH have found that the migration and gliosis of Muller cells are induced in environments where ILM acts as a scaffold surrounded by a dried environment rather than being surrounded by vitreous fluid, that may result in nonclosure of MH, even if ILM is in position. Other authors, such as Boninska et al, refer that in cases where only a thin ILM flap was noted over the MH after the surgery, regeneration of retinal

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**Fig. 5.** External limiting membrane and EZ alignment at 1 and 12 months after surgery in the inserted and cover groups.

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**Fig. 6.** Evolution time of the MH before surgery in the inserted and cover groups. A. External limiting membrane realignment; (B) EZ realignment. White: evolution time < 6 months; grey: evolution time 6–12 months; black: evolution time > 12 months.
tissue starting from the ELM was followed by realignment of the EZ layer.

Finally, the evolution time of the MH before surgery seems to have a role in the realignment of ELM in the Cover group, as shown by our results, since realignment of ELM was superior when the evolution time was <6 months compared with >12 months at both 1-month and 12-month assessment. However, evolution time of the hole does not seem to be a factor neither for the realignment of EZ in Cover group nor for the realignment of both ELM and EZ in Inserted group because in this last case, 100% of the eyes were interrupted at both 1 and 12 months.

In conclusion, our results show that the ELM and the EZ recovery rates after ILM flap with flap closure were higher than those obtained using the ILM peeled and inserted in the hole. Every effort should be made to maintain an IF on top of the retina, covering the hole, because this may facilitate the reconstruction of the outer retinal layer structures after MH surgery. Nevertheless, even in surgeries where the ILM is carefully placed over the hole, after the IF technique, ELM flap position may be difficult to control, due to fluid–air exchange, intraocular gas, and the patient maintaining the recommended face-down position. Placing viscoelastic over the ILM flap after fluid–air exchange may prevent slippage of the flap.

The evolution time of the MH seems to have a role in the realignment of ELM when there is flap closure, with no effect on outcomes in the case of fill in. Nevertheless, these results suggest that these patients should undergo surgery as soon as possible, to achieve the best possible outcomes.

This study had some limitations such as the low number of surgeries, especially the low number of surgeries with ILM inserted in the hole. Ethically, we could not continue inserting the ILM in the hole as the results were much better with ILM over the hole. Also, there were four different surgeons performing surgeries which increases the variability.

**Key words:** internal limiting membrane, macular hole, outer retinal layers.

**References**