Hole edge morphology by intraoperative OCT predicts restoration of the retina microstructure in macular hole surgery

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Abstract
Objectives: To investigate the association between iOCT feature in macular hole surgery and the restoration of the retina microstructure and visual outcomes. Methods: Fifty-three eyes of 53 patients with macular hole were recruited in this retrospective study. All eyes were assessed with intraoperative OCT after internal limiting membrane peeling. We divided the patients into three groups: foveal flap group, hole-door group, and negative group according to morphological changes on the edge of hole using iOCT after ILM peeling. We compared the anatomical and functional surgical outcomes between these groups. Results: The negative group had significantly poorer BCVA and restoration of the ELM than other two groups (P=0.002, 0.012) at 6 months postoperatively. In the hole size ≤400um group there were no statistical difference of ELM restoration and BCVA postoperatively between the groups. While in hole size > 400um group, The hole-door group and fovea flap group had significantly better final visual acuity and restoration of ELM than the negative group (P=0.013, P=0.005) Conclusion: This study describes a novel intraoperative sign using iOCT. This sign is a useful predictor of postoperative in restoration of the retina microstructure and visual outcomes of the macular hole especially in large macular hole.

Introduction
Current surgical techniques for macular hole (MH) that ensure satisfactory anatomic outcomes with success rates of 85% to 95% have been reported in a large number of studies. Spectral domain optical coherence tomography (SD-OCT) allows for the examination of the retina microstructure. Recent studies using SD-OCT reported favorable visual outcomes after MH surgery related to restoration of the external limiting membrane (ELM) and inner segment/outer segment junction. Several studies in the literature have indicated that the recovery of the ELM might be the most critical aspect for visual function improvement in the early postoperative period. Several researchers used OCT measurements in attempts to evaluate prognostic factors in MH surgery, including minimum and base hole diameter (MHD, BHD), hole form factor (HFF), macular hole index (MHI), and tractional hole index (THI). However, the results are variable, and none of them is a satisfactory predictor of postoperative restoration of the retina microstructure and visual.
outcomes of the MH. We considered that this variability may be due to the poor reproducibility of OCT measurements.

Various studies have proved the usefulness and feasibility of intraoperative OCT (iOCT) in vitreoretinal surgery[13]. iOCT was identified as adding valuable information related to surgical anatomic features and the surgical procedure, and this information directly impacted the surgical procedure. Several authors have recently shown iOCT to be useful in patients undergoing membrane peeling in MH surgery. Moreover, alterations in MH geometry on iOCT have been visualized that may have important implications for postoperative care and positioning[14,15]. However, the relationship of these intraoperative changes with the MH closure rate and anatomic normalization were less analyzed.

In this study, we describe morphological changes on the edge of the macular hole using iOCT after ILM peeling during vitrectomy for MH. In addition, we divided the features in to three categories and investigated the association between iOCT features and the restoration of the retina microstructure and postoperative visual outcomes of the MH.

Materials And Methods

Study Design

This is a retrospective study of consecutive patients undergoing 23-gauge pars plana vitrectomy for MH by a single surgeonL.J-Sat Eye Hospital of Wenzhou Medical College from July 2015 to July 2018. All patients had given written informed consent prior to the surgery. All the procedures were approved by the institutional review board of our hospital and adhered to the Declarations of Helsinki.

Patient Selection

All patients with MH who underwent 23-gauge pars plana vitrectomy were included in the study. Exclusion criteria: previous vitreoretinal surgery, history of penetrating trauma, degenerative myopia, and final follow-up period less than 6 months, the eyes that underwent pars plana vitrectomy for MH without the help of iOCT or using other techniques (inverted flap technique) during the study period. According to morphological changes on the edge of the hole using iOCT after ILM peeling, all patients were divided into three groups: the foveal flap group, the Hole-door group refer to Kumar V ‘s study[21], and the negative group. The iOCT showed that the foveal flap which existed preoperatively
was adherent to the hole edge after ILM peeling were defined as the Foveal flap, and the iOCT showed vertical pillars of tissue at the edges of the hole projecting into the vitreous cavity, which did not have a foveal flap preoperatively were defined as the Hole-door. In the negative group, neither foveal flap nor Hole-door features were observed through iOCT.

**Surgical Technique**

The surgery was performed under retrobulbar anesthesia in all patients using 23-gauge pars plana vitrectomy. After core vitrectomy, posterior vitreous detachment was induced using the suction power of a 23-gauge vitrectomy cutter in the optic disc area. The macular area was spared. Using the same 23-gauge cutter, the posterior hyaloid membrane was cut while sparing and isolating the posterior hyaloid in the macular area. After staining the posterior pole with indocyanine green (0.025mg/ml), the ILM was grasped with an ILM forceps and peeled off in a circular manner for approximately two-three disc diameters around the MH. Air-fluid exchange followed by C3F8 endotamponade was performed.

All eyes underwent BCVA, optical coherence tomography(OCT) examination, anterior segment, and fundus examination. The Snellen chart was used for BCVA examination and converted to the minimum resolution logarithm (logMAR) visual acuity. All OCTs were done using a commercially available SD-OCT device (Spectralis HRA OCT; Heidelberg Engineering, Heidelberg, Germany). The iOCT images obtained (OptovueiVue, USA) before and after ILM peeling were analyzed for qualitative changes. The primary outcome measures were anatomic success and restoration of the photoreceptor layer of the MH on OCT. The functional outcome of surgery was evaluated by best corrected visual acuity (BCVA) at the last follow-up.

The postoperation BCVA and anatomical morphology of photoreceptor layer observed on SD-OCT images obtained in the postoperative follow-up period were compared among the three groups. Moreover, the restoration of the photoreceptor layer was assessed via the reconstruction of the continuous back reflection line corresponding to the ellipsoid zone (EZ) and the ELM. Two independent observers (H.C,J.F) evaluated the imaging, with a consensus used to resolve disagreements. All data were analyzed using the SPSS 22.0 statistical software (SPSS Inc, Chicago, IL),
and a P-value 0.05 was considered statistically significant. The visual acuity was presented with LogMAR throughout the whole study, and the descriptive analysis was used for all the ocular parameters discussed in the current study. The one-way analysis of variance with post-hoc exam was used for the Preoperative BCVA or Postoperative BCVA between the three groups throughout the study period, while the Chi-squared Test was applied to evaluate the difference of Macular hole closure, ELM restoration, or EZ restoration.

Results
Fifty-three eyes of 53 patients matched the study criteria and were included in the study. The mean age of the patients was 66.04±6.50 years (range 47–78 y), and 36 patients were female, 17 patients were male. The mean preoperative BCVA was 1.10±0.77, and the mean MHD was 420.19±170.79μm. Depending on the hole edge feature on iOCT, the patients were divided into three groups: the negative group included 24 eyes, the fovea flap group included 14 eyes, and the hole-door group included 15 eyes. At the baseline examination, there was no statistically significant difference between the three groups regarding age (p = 0.151), sex (p = 0.252), mean preoperative BCVA (p = 0.287), and MHD (p = 0.268). Table 1.

Macular Hole Closure, iOCT features, and Visual Acuity Change After Surgery
All the eyes had MH closure after vitrectomy; 52 eyes had type 1 MH closure, and only one eye had type 2 MH closure. The mean postoperative BCVA was 0.44±0.46, which significantly improved compared with preoperative BCVA (P<0.001). The restoration of ELM and EZ could be observed 6 months after surgery in 77.4% (41 of 53) and 37.7% (20 of 53) of the patients after surgery.

iOCT features and Visual Acuity Change After Surgery between three Groups
The baseline and final visual acuity and anatomic outcomes between the three groups are summarized in Table 1. There was no significant difference in the rate of MH closure between the three groups after surgery at 6 months postoperatively.
The mean visual acuity at 6 months postoperatively was 0.68±0.60 in the negative group, 0.24±0.15 in the Hole-door group, and 0.25±0.15 in the foveal flap group. The Hole-door group and foveal flap group had significantly better final visual acuity than the negative group (P = 0.002). The restoration of ELM could be observed 6 months after surgery in 58.3% (14 of 24) of the patients in the negative group, 93.3% (14 of 15) in the Hole-door group, and 92.9% (13 of 14) in the foveal flap group. Restoration of the EZ was observed at 6 months postoperatively in 33.3% (8 of 24) of the patients in the negative group, 33.3% (5 of 15) in the Hole-door group, and 50% (7 of 14) in the foveal flap group. The negative group had significantly poorer restoration of the ELM than the other two groups (P = 0.012). Table 1.

**Macular Hole Size and BCVA and Microstructure Changes of the Fovea After Surgery**

Subgroup analysis divided patients into an MHD greater than 400 μm group and an MHD 400 μm or less group.

In the MHD 400 μm or less group, the ELMs were completely restored in all groups, and restoration of the EZ was observed at 6 months postoperatively in 77.8% (7 of 9) of the patients in the negative group, 62.5% (5 of 8) in the Hole-door group, and 50% (4 of 8) in the foveal flap group. There were no statistical difference between the ELM, EZ restoration, and BCVA postoperatively of the three groups (P = 0.516, P = 0.179). Table 2.

In the MHD greater than 400 μm group, the restoration of ELM could be observed 6 months after surgery in 33.3% (5 of 15) of the patients in the negative group, 85.7% (6 of 7) in the Hole-door group, and 100% (6 of 6) in the foveal flap group. Restoration of the EZ was observed at 6 months postoperatively in 13.3% (2 of 15) of the patients in the negative group, 28.6% (2 of 7) in the Hole-door group, and 33.3% (2 of 6) in the foveal flap group. The negative group had significantly poorer restoration of the ELM than the other two groups (P = 0.005), while there was no statistical difference between the EZ restoration of the three groups (P = 0.569). The Hole-door group and the foveal flap...
group had significantly better final visual acuity than the negative group (P = 0.013). (Table 3)

**Representative Case Examples**

*Fig. 1* Case 1 was a representative foveal flap case, a 63-year-old woman showing MH with foveal flap preoperatively (Figure 1); iOCT showed that the foveal flap was preserved after ILM peeling; At 6 months postoperatively, OCT shows hole closure with full recovery of ELM and EZ; BCVA was 0.6

*Fig. 2* Case 2 was a representative hole-door case, a 60-year-old woman showing MH without foveal flap preoperatively (Figure 2); iOCT showed vertical pillars of tissue at the edges of the hole projecting into the vitreous cavity after ILM peeling; At 6 months postoperatively, OCT shows hole closure with full recovery of ELM and EZ; BCVA was 0.4

*Fig. 3* Case 3 was a representative control case, a 67-year-old woman showing MH without foveal flap preoperatively (Figure 3); iOCT showed neither foveal nor vertical pillars of tissue at the edges of the hole after ILM peeling. At 6 months postoperatively, the image shows hole closure, a lack of restoration of the ONL, but with the hyperreflective bridging tissue; BCVA was 0.15.

**Discussion**

Several authors have recently shown MH geometry changes on iOCT after ILM peeling in MH surgery. However, the relationship of these intraoperative changes with the MH closure rate and anatomic normalization were less analyzed. Our study found that there were three types of iOCT features at the hole edge observed after ILM peeling. The morphological characteristics of iOCT are closely related to the prognosis of MH surgery, in that foveal flap and Hole-door changes in iOCT during surgery served as a positive predictor of MHs that acquired better functional and anatomic results after surgery than the group in which these features were present.

Though the nature of the foveal flap is still unknown, it is considered to be the early stage of operculum. As PVD progresses, the foveal flap is separated from the retinal tissue and detached in the form of an operculum. Histopathological observation of the operculum shows the presence of retinal tissue, implying that the fovea flap is a part of the retinal tissue which distraction by posterior vitreous cortex. There was a report regarding preserving the foveal flap for the treatment of
MH in which good functional and anatomic outcomes were achieved. Although preservation of the flap could be identified by the surgeon under the microscope during the procedure, the use of iOCT may be helpful for making more objective assessments during surgery. Our study observed the morphology of the foveal flap after ILM removal using iOCT, and found that all flaps were preserved as confirmed by iOCT during the surgery, and these patients all had a better prognosis. We assumed that the preserved foveal flaps may be helpful in the faster closure of MHs via more quickly covering up defects in the inner retina and functioning as a scaffold for tissue proliferation, also produce an environment for the photoreceptors to be restored at the fovea. To the best of our knowledge, this study is the first to use iOCT to describe the feature of fovea flap after ILM peeling and to demonstrate its beneficial effect for prognosis after MH surgery.

However, in the patients who did not have fovea flap iOCT also showed vertical pillars of tissue at the edges of the hole projecting into the vitreous cavity after ILM peeling in eight patients. This phenomenon is similar to the “hole-door” feature described in previous literature, they conclude Hole-door sign can predicts postoperative Type-1 closure of MH. In Our study, we found that patients with this phenomenon have a better recovery of the foveal microstructural and visual outcome compared with the negative group, while there is no difference in the closure rate of MH. The different mean diameter of holes in selected cases may be the main reason, an average of 420um in our study, while 501um in their study. These tissue pillars may be composed of redundant retinal tissue, subclinical epiretinal membranes, or small residual pieces of ILM attached to the edges of the hole. The mechanism may be similar to the invert ILM flap technology, providing mechanical support to bridge the gap, more quickly cover up defects in the inner retina; Moreover, they are believed to be helpful in the faster closure of MHs and recovery of the photoreceptors.

Visual recovery after MH closure may depend on the microstructural recovery of the fovea, particularly the outer retina. A restoration of the outer nuclear layer (ONL) with recovery of the ELM and the EZ lines over the closed MH was associated with better BCVAs in 50% of the eyes. By contrast, a lack of restoration of the ONL but with the hyper reflective bridging tissue at the closed
MH indicated that the MH was closed with scarred tissue or migrated glial tissue including collagen components derived from Müller cells. The restoration of the ELM in the foveal flap group and the hole-door group was higher than in the negative group, and correspondingly, the foveal flap and hole-door groups had better visual acuity. Our research proves favorable visual outcomes after MH surgery related to restoration of the ELM, and this is similar to previous reports. This observation indicated that the recovery of the ELM might be the most critical component for visual function improvement in the early stage after MH surgery.

The size of the hole is closely related to the prognosis and a study showed that patients with smaller MHs had superior final visual acuity and better restoration of the outer retinal structure after MH surgery. Although the current management options for small or medium MHs have been reviewed extensively, treatment options for large, recurrent, or persistent MHs have yet to come to a general consensus because of the lack of randomized clinical trials with sufficiently large sample sizes.

Regarding preoperative MH size, Liu L et al stated that simply dividing patients into a larger than 400 μm group or 400 μm or less group might be more clinically significant for the purpose of preoperative counseling of patients about prognosis after MHs surgery. This study shows that the intraoperative feature at the hole edge may be the predictor of prognosis of MH in MHD greater than 400μm. Inverted ILM flap technique has been widely used in the treatment of refractory macular hole, including large macular hole. These phenomena found by iOCT may be similar to the inverted ILM flap which is assumed that the facilitates gliosis and functions as a scaffold for tissue proliferation, assisting in the closure of MHs and restoration of outer retinal structure.

This study has the following limitations. It was a retrospective study and involved a small number of cases, which limited the statistical strength of the analysis. In addition, the imaging system used in this study was not integrated into the microscope, which may impact the overall functionality of iOCT in these cases.

Both research systems and commercial systems have been described that provide microscope-integrated technology. A number of studies of microscope-integrated iOCT systems have provided
early data suggesting the feasibility and potentially significant usefulness of this technology. The 3-year results of the DISCOVER study found that posterior segment surgeons seemed to prefer static imaging over time in the subsequent years of the study. In 69% of procedures, posterior segment surgeons preferred viewing images on the display screen as well, which increased from year 1 to subsequent years. These improvements may be related to greater OCT detail and subtle changes on the OCT required in on-screen review than in real-time OCT. Therefore, non-microscope-integrated iOCT can be used for static image analysis, and it is still of important clinical value in guiding the treatment of MH.

Conclusions
In conclusion, this study describes a novel intraoperative sign using iOCT. This sign is a useful predictor of postoperative restoration of the retinal microstructure and visual outcomes of the MH especially in large MHs, and provides useful insights into the pathophysiology of ILM peeling in MH surgery.

Abbreviations
iOCT: Intraoperative Optical Coherence Tomography;
MH: Macular Hole;
EZ: the Ellipsoid Zone;
ELM: External Limiting Membrane;
BCVA: Best Corrected Visual Acuity.

Declarations

Acknowledgements
Not applicable.

Authors’ contributions
JWT contributed to the concept and study design. The patient was enrolled from LJS. CH, ZL and PDM collected the data, made data interpretations, CH drafted the manuscript. All the authors were involved in the critical revision of the manuscript, supervision of the manuscript and final approval of the submission.
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Availability of data and materials
The datasets for the analysis of the current study are readily available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Eye Hospital of Wenzhou Medical University, and written informed consent was obtained from all patients.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Tables
Due to technical limitations, tables are only available as a download in the supplemental files section.

Figures
Figure 1

Case 1 was a representative foveal flap case, a 63-year-old woman showing MH with foveal flap preoperatively (Figure 1); iOCT showed that the foveal flap was preserved after ILM peeling; At 6 months postoperatively, OCT shows hole closure with full recovery of ELM and EZ; BCVA was 0.6
Case 2 was a representative hole-door case, a 60-year-old woman showing MH without foveal flap preoperatively (Figure 2). IOCT showed vertical pillars of tissue at the edges of the hole projecting into the vitreous cavity after ILM peeling; At 6 months postoperatively, OCT shows hole closure with full recovery of ELM and EZ; BCVA was 0.4
Case 3 was a representative control case, a 67-year-old woman showing MH without foveal flap preoperatively (Figure 3), iOCT showed neither foveal nor vertical pillars of tissue at the edges of the hole after ILM peeling. At 6 months postoperatively, the image shows hole closure, a lack of restoration of the ONL, but with the hyperreflective bridging tissue; BCVA was 0.15.

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