The Dynamic Healing Process of Idiopathic Macular Holes after Surgical Repair: A Spectral-Domain Optical Coherence Tomography Study

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PURPOSE. To analyze progressive changes of the outer retina after vitrectomy for macular hole (MH) repair.

METHODS. Nineteen consecutive patients underwent vitrectomy for idiopathic MH. Spectral domain optical coherence tomography (SD-OCT) examinations were performed pre- and postoperatively during follow-up visits at 1, 3, 6, 9, and 12 months. Active eye-tracking technology ensured that the same scanning location was identified each time.

RESULTS. Ten eyes showed a normal external limiting membrane (ELM) at 1 month after surgery and 15 eyes at 3 months. The ELM was already continuous in 79% of the eyes with persistent outer foveal defects during follow-up. No eyes revealed a continuous inner segment/outer segment (IS/OS) line at 1 month, only one eye at 3 months, and 10 eyes at 12 months. No eyes had a disrupted ELM with an intact IS/OS line. Foveal cysts were visible in three eyes at 1 month and in eight eyes during follow-up. The cystic space gradually filled, resulting in a continuous IS/OS line in five of these eyes. Recovery of ELM, IS/OS, and outer nuclear layer (ONL) determined most of visual acuity improvement. The ONL appeared normal in the 10 eyes with an intact IS/OS line at last follow-up. By contrast, it was disrupted in 7 of 9 eyes with a final persistent outer foveal defect.

CONCLUSIONS. The ELM is the first structure to recover after MH closure. Foveal cysts may develop during follow-up, and in the presence of an intact ONL, they may gradually fill with complete recovery of the IS/OS junction. (Invest Ophthalmol Vis Sci. 2011;52:4439–4446) DOI:10.1167/iovs.10-6732

Today macular hole (MH) surgery results in successful hole closure and significant visual improvement in >85% of cases. However, postoperative visual acuity (VA) may occasionally be poor despite anatomic closure. In such cases, time-domain optical coherence tomography (OCT) often shows the presence of photoreceptor irregularities. Conventional time-domain OCT is inherently limited by the 10-µm axial resolution and the low acquisition speed, both of which reduce detailed analysis of pathologic changes. Using a prototype of ultrahigh-resolution OCT (UHR-OCT) with 3 µm of axial resolution, Ko and associates were the first to report on peculiar anatomic abnormalities at the junction between the inner and outer segments (IS/OS) of the photoreceptors in surgically closed MHS. Spectral-domain OCT (SD-OCT), with 5- to 7-µm axial resolution, allows clear detection of the IS/OS line and the external limiting membrane (ELM), the junction between the inner segment and the Muller cells. While both SD- and UHR-OCT have recently been used to describe the anatomic outcomes of surgically repaired MHS, the dynamic healing process of the outer retina was either not recorded at each follow-up visit or the follow-up time was limited to 6 months. Therefore, the purpose of this study was to analyze by SD-OCT the progressive outer retinal changes for 1 year in patients who underwent a standard vitrectomy procedure for MH repair.

METHODS

We studied 19 eyes in 19 consecutive patients (13 women, 6 men) who underwent vitrectomy for an idiopathic stage 3 to 4 MH at Sacco University Hospital from February 2008 to January 2010. All examinations and investigations adhered to the tenets of the Declaration of Helsinki. This study was approved by the Institutional Review Board Committee of Milan University Medical School at Sacco Hospital. The patients’ ages ranged from 55 to 83 years (mean age, 69.5; Table 1). Full-thickness MHS were diagnosed in each after complete preoperative ophthalmologic examination, including intraocular pressure measurement, lens clarity evaluation, refraction, and biomicroscopic examination of the fovea and vitreous. The best corrected visual acuity (BCVA) was measured using the standard Snellen eye chart. A combined confocal scanning laser ophthalmoscope and SD-OCT (Spectralis HRA + OCT, Heidelberg Engineering GmbH, Heidelberg, Germany) was used to confirm the MH status. The size was determined by measuring the largest diameter in fundus autofluorescence (FAF) imaging. Eight MHS were 400 µm or less, seven were 410–590 µm, and four were 600 µm or more. According to Gass’s classification, nine eyes had stage 3 MH, and 10 eyes had stage 4 MH (Table 1).

A 20-gauge pars plana vitrectomy with internal limiting membrane (ILM) peeling was performed in the first 16 cases, whereas the last three cases underwent a 25-gauge vitrectomy with ILM peeling. All the surgical procedures were performed by one of the authors (FB). Indocyanine green (0.07%, first six eyes) or Brilliant Blue G (Brilliant Peel, Fluoron; Geuder AG, Heidelberg, Germany) were used for staining the internal limiting membrane. A small bubble of perfluorocarbon liquid was placed over the MH when coloring with indocyanine green to prevent migration of the dye into the subretinal space. An air/fluid exchange with subsequent injection of 20% sulfur hexafluoride gas completed each surgical procedure. After surgery, strict face-down positioning was ordered for 3 to 5 days. Nine patients were already pseudophakic before surgery, one patient underwent a combined procedure, and phacoemulsification with placement of an intraocular lens was performed.
reduce movement artifacts. For OCT scanning, the software provides an “Automatic Real-Time” (ART) function to reduce noise and increase image quality. With ART activated, multiple frames (B-scans) of the same scanning location are performed during the scanning process, and images are averaged for noise reduction. In this study, 20 frames were acquired for each B-scan location, and the images were averaged for noise reduction. For MH analysis, raster scans ranging from 20° × 15° to 30° × 15° and consisting of 13 to 37 line scans were performed. The spacing between the B-scans ranged from 119 to 252 μm. An internal fixation light was used to center the scanning area on the fovea.

For each eye, the following features of the outer retina were analyzed at each follow-up visit: (1) outer foveal hyporeflective defects such as focal disruption/interruption in the normally hyperreflective IS/OS junction (Fig. 1A) and outer foveal cystic spaces (Fig. 1B), (2) abnormalities of the ELM (Fig. 1A), (3) persistent foveal detachments (Fig. 1C), (4) moderately reflective foveal lesions (Fig. 1D), and (5) alteration of the outer nuclear layer (ONL).

### Statistical Analysis of Visual Acuity and Anatomic Correlations

For all computations, commercially available software was used (R; R Foundation for Statistical Computing, Vienna, Austria). Changes of VA over time as well as association between it and the OCT findings were evaluated using multiple regression analysis. \( P < 0.05 \) was considered significant.

#### Time Dependence of VA

A time-complete model was analyzed with Walter variables\textsuperscript{18} composed of five columns, one for each time point T1, T3, T6, T9, and T12. In this model, each column is a dummy variable that tests if the VA changed significantly from the previous time point (i.e., T3 tests if the VA at 3 months is different from that at T1). The preoperative VA at T0 was used for the intercept. The patient effect was removed from the residual SE by adding a factor for each patient. The improvement of VA could be simply related to the mechanical realignment of photoreceptors. We tested this hypothesis with a time reduced model considering only the effect of T1. An \( F \) test was used to compare the two models.

#### SD-OCT Findings: Dependence of VA

A similar approach was used for evaluating the relationship between OCT findings and VA changes. First, a complete model tested the effects of the ELM, IS/OS, and ONL remodeling on VA. Here the intercept was defined as the “worst” situation: ELM absent, and IS/OS junction present at T6. (C) Persistent foveal detachment at T1. (D) Moderately reflective foveal lesion (*) overlying a cyst at T12.

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![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933461/)
FIGURE 2. Box plot of VA during follow-up. Within boxes: dark lines, medians; crosses, means. The improvement was significant at T1, T6, and T9.

FIGURE 3. Changes of the ELM (upper) and photoreceptor IS/OS (lower) junction during follow-up. The integrity of the ELM was restored more rapidly that the other anatomic features. Cont., continuous; Interr., interrupted; Cyst, presence of foveal cysts; F. Det., foveal detachment.

FIGURE 4. Case 12. Changes in the ELM and photoreceptor IS/OS junction during follow-up. (A) Preoperative FAF image of the MH (680 μm). (B) The corresponding SD-OCT shows a full-thickness stage 4 MH. The VA was 0.1. (C) At T1, the central FAF was normal. (D) Corresponding T1 SD-OCT image showed an already intact and rectilinear ELM and an unstructured IS/OS junction (between asterisks) with two small interspersed cystic spaces. The VA was 0.1. (E) At T3, the SD-OCT image showed that the IS/OS junction was more structured, and only a residual small cyst remained. The VA was 0.1. (F-H) At T6 (F), T9 (G), and T12 (H), there was a progressive reduction in the size of the cyst. These changes were accompanied by improvements in VA to 0.4 at T6 and T9 and to 0.6 at T12. The extent of the IS/OS disruption decreased during follow-up to almost a complete recovery. The overlying outer nuclear layer was intact at each time.
ELM with the IS/OS junction (ELM-IS/OS) and with the ONL (ELM-ONL). An F test was used to compare the reduced with the complete models.

**RESULTS**

All MHs closed after the first surgery, as confirmed by SD-OCT. At the 1-month follow-up visit, three patients (cases 7, 15, and 16) had a small peripheral superior retinal detachment that was successfully treated with revision of vitrectomy, cryocoagulation of the retinal break, and gas tamponade (20% sulfur hexafluoride) without affecting the final anatomic and functional outcomes. The VA improved significantly from 0.2 ± 0.1 (mean ± SD) preoperatively to 0.7 ± 0.2 at T12 (Table 1). The mean change in VA was 5.5 lines. Mean final VA did not differ significantly between cases with ICG (6 eyes) and Brilliant Blue G (13 eyes) -assisted peeling of ILM: 0.73 and 0.64, respectively (Welch two-sample t-test; P = 0.41). During follow-up, the improvement in VA was significant at T1, T6, and T9 compared with the preceding follow-up visits (Fig. 2). The “time-complete” statistical model showed that approximately 80% of VA variance was explained by the elapse of time (R² = 0.7991). The mean preoperative VA was approximately 0.25 after removing the patient effect. At T1 it increased to 0.40, at T6 it increased by another 0.1, and at T9 it reached 0.66. When only the effect of T1 was tested, it was still significant, but the time-reduced model accounted for a smaller amount of the variance (R² = 0.6168) than did the time-complete model. The two models were significantly different (F test, P < 0.00001); therefore, the hypothesis that VA recovery is only a matter of mechanical realignment of photoreceptors after surgery was rejected.

With regard to the postoperative features of the outer retina, the ELM was restored more rapidly than the other anatomic features (Fig. 3). It was continuous in 10 out of 19 eyes at the T1 follow-up visit (Fig. 4) and in 15 eyes at T3. It is noteworthy that the ELM appeared already continuous in 15 of the 19 eyes with persistent outer foveal hyporeflective defects such as disruption/interruption of the IS/OS junction and the outer foveal cystic space during follow-up (Figs. 4 –7).

Foveal hyporeflective defects changed over time (Fig. 3). Disruption/interruption at the IS/OS junction gradually decreased although restoration of the integrity took longer than reestablishment of the ELM. Thus no eyes revealed a continuous IS/OS line at T1, only one eye out of 19 at T3, five eyes at T6, eight eyes at T9, and 10 eyes at T12. It is noteworthy that there were no eyes with a continuous IS/OS line and an interrupted ELM anytime during follow-up. Foveal cysts (Fig. 4)
were visible in three eyes at T1 and in eight eyes (Figs. 5–7) during later follow-up periods as a subsequent stage to the disruption/interruption of the IS/OS line. The cystic spaces gradually filled, resulting in a continuous IS/OS line in five of these 11 eyes, leaving six eyes with persistent cysts. The cyst in one eye was resolved by T6 (Fig. 6F), in two eyes by T9, and in two eyes by T12 (Fig. 7H). The ELM was always continuous and rectilinear overlying the cysts (Figs. 4–7). Again, the final integrity of the IS/OS line did not differ between cases with ICG (6 eyes) and Brilliant Blue G (13 eyes) -assisted peeling of ILM (Fisher’s exact test; \( P = 1 \)).

Persistent foveal detachment occurred in only two eyes at T1 (cases 11 and 14; Fig. 6). Both had a small preoperative MH (<400 μm), and both showed a continuous, curvilinear ELM at T1 and a continuous IS/OS junction by T6 and T3, respectively.

Moderately reflective foveal lesions that partially or completely replaced the normal foveal architecture were present in 10 out of 19 eyes during follow-up. In one of these, they were no longer visible by T6 (Fig. 7), and in two they were no longer visible by T9 (Fig. 8). All three of these eyes showed a normal ONL and a continuous IS/OS line at the last follow-up visit. We did not find any relationship between the basal hole diameter and the development of moderately reflective foveal lesions. Alterations of the outer nuclear layer were always present in the 10 eyes with moderately reflective foveal lesions. All 10 eyes with a recovered IS/OS line at T12 had a normal-appearing ONL (Figs. 6–8). In contrast, only two of the nine eyes with a final persistent outer foveal hyporeflective defect revealed the presence of an intact ONL (Fig. 4).

Statistical analysis of the complete model of changes in the ELM, IS/OS, and ONL revealed that the OCT findings explained most of the VA variations during the 1-year follow-up (\( R^2 = 0.7963 \)). When the ELM became continuous, the VA increased by approximately 0.12, and this was significant (\( P = 0.025 \)). When the ONL was present, VA increased by 0.11, and this was also significant (\( P = 0.038 \)). Finally, a continuous IS/OS junction increased VA by >0.3 (\( P < 0.01 \)). Among the three reduced models, all of them were significantly different from the complete model: \( R^2 = 0.6602 \) for ELM (\( P < 0.01 \)), \( R^2 = 0.7863 \) for ELM-IS/OS (\( P = 0.04 \)), and \( R^2 = 0.6894 \) for ELM/ONL (\( P < 0.01 \)). This suggests that the combined recovery of ELM, IS/OS, and ONL determined most of VA improvement.

**FIGURE 6.** Case 11. (A) Preoperative FAF image of the MH (390 μm). (B) The corresponding SD-OCT showed a full-thickness stage 3 MH. The VA was 0.3. (C) In the FAF image at T1, foveal autofluorescence recovered to almost normal levels. (D) The corresponding SD-OCT image showed a persistent foveal detachment with an already intact, curvilinear ELM. The VA was 0.7. (E) The SD-OCT image at T3 showed that the ELM was flattened and the outer foveal defect had the appearance of a cyst. The outer nuclear layer overlying the cyst was normal. The VA was 0.7. (F) In the SD-OCT image at T6, the IS/OS line had recovered completely and was maintained at T9 (G). The VAs at both times were 1.0.
DISCUSSION

As determined in histopathologic specimens, Muller cell gliosis is the most important factor in healing of MHs after surgery.\textsuperscript{19,20} Although important, these observations were made on single specimens at fixed times during the healing process of a MH after surgery. SD-OCT images can improve our understanding of the sequence of events involved in the in vivo anatomic reconstruction and provide new insights into the clinical relevance of changes of certain morphologic features. Some recent reports on the anatomic and visual outcomes of surgically repaired MHs using UHR- or SD-OCT lacked two important features to investigate the dynamic healing process of the outer retina: serial examinations at each follow-up visit in all the patients\textsuperscript{10–14} and limited follow-up time.\textsuperscript{15}

Michalewska et al.\textsuperscript{21} were the first to report on a large series of patients with proper serial SD-OCT examinations. They indeed showed a continuous improvement of macular morphology that was correlated with improved VA. On the other hand, it was a retrospective study with a SD-OCT that lacked two important features to investigate the dynamic healing process of the outer retina: serial examinations at each follow-up visit in all the patients.\textsuperscript{10–14} As in Michalewska’s study, we performed serial SD-OCT examinations at 1, 3, 6, 9, and 12 months but with the aid of an active eye-tracking technology. The ideal procedure to evaluate continuous changes in macular morphology after MH closure would be to scan the same location at each follow-up visit. With active eye tracking, the system automatically follows eye movements and locks each OCT B-scan to the fundus image. In our series, for each patient the first complete volume scan acquired at the preoperative visit was set as a reference scan. The “AutoRescan” function of the instrument ensured that the same scanning location was identified by the tracking program on follow-up visits. The same scanning location during follow-up is not related to the location of the fixation light but to the eye-tracking system and its reference points. The reference points (landmarks) for the eye tracker are anatomic structures at the confocal scanning laser ophthalmoscope fundus image. As long as there is an overlap of the fundus image for the first and the follow-up examinations that allows the identification of enough landmarks to align the two images automatically, than the automatic rescan is feasible. There might be a slight variation of the position of the follow-up scans compared to the baseline examination. Therefore structural changes might also be due to differences in the scanning position. However, due to the active real-time eye tracker of the system we used, this uncertainty should be much smaller than the spacing between the B-scans. B-scan spacing in our series varied from 119 to 252 μm. Since the smallest MH diameter was 350 μm, we were reasonably confident of being within the area of the healing MH with follow-up scans.
In the present study, we found that the ELM became normalized before the outer foveal hyporeflective defects were resolved. In fact, it was already continuous in 79% of the eyes with persistent disruption/interruption of the IS/OS line or with outer foveal cysts. Fifty-three percent of the eyes showed a normal ELM already 1 month after surgery and 79% at 3 months. Our findings are consistent with the discrepancy between ELM and IS/OS restoration after MH surgery recently reported by Wakabayashi et al.14; however, their SD-OCT data were collected first at 3 months after surgery and again with SD-OCTs that lacked an active eye-tracking technology during image acquisition. The reason for such a quick normalization of the ELM is not known. The ELM represents a series of junctional complexes between Muller cells and rod and cone photoreceptor cells. It might be that this junction is needed throughout the affected area before regeneration of photoreceptor outer segment can occur.

Consistent with previous reports,9,10,14,15,21 we found that foveal hyporeflective defects changed over time. There were no eyes with a continuous IS/OS line at T1, only one eye at T3, and 10 eyes at T12, the last follow-up visit. In the current series, no eyes had a disrupted ELM with an intact IS/OS line. This is consistent with a recent SD-OCT– based report by Wakabayashi et al.22 on foveal microstructure after retinal detachment repair. They also found no eyes with a disrupted ELM in the presence of an intact IS/OS line. Based on their findings, the integrity of the ELM seems critical for the potential restoration of the photoreceptor microstructure after retinal detachment, and we suggest that this integrity starts the healing process of closed MHs. Foveal cysts were visible at T1 (three eyes) and during follow-up as a subsequent stage to disruption/interruption of IS/OS line (eight eyes). Between 6 and 12 months, these cystic spaces gradually filled, resulting in a complete recovery of the IS/OS junction in five of these 11 eyes. The development of cystic spaces after the reapproximation of the MH edges and during follow-up, as well as the reduction of these cysts during the study period, suggest a regrowth of photoreceptor outer segments rather than a simple anatomic realignment of the photoreceptors.

The redevelopment of photoreceptor outer segments is also supported by the complete statistical model on time and VA that accounts for more of the variability in the VA ($R^2 = 0.7991$) than did the reduced model ($R^2 = 0.6168$). This means that VA recovery was not due only to mechanical photoreceptor realignment related to the surgical intervention that was achieved 1 month after surgery. Consistent with previous studies,11–15,21,22 we also found that restoration of the ELM and retinal detachment repair.
IS/OS is important for the functional outcome. However, the simultaneous presence of ELM, IS/OS, and ONL defined most of VA improvement, suggesting that a combined recovery of these anatomic layers is needed for a complete healing process. This is consistent also with the finding that ONL was normal in the 10 eyes with an intact IS/OS line at the last follow-up visit. In contrast, it was disrupted in seven of the nine eyes with a final persistent outer foveal defect. Therefore, an intact ONL is necessary to achieve a complete restoration of the photoreceptor microstructure. Terms like persistent foveal detachment and outer foveal cyst have been used indifferently to define outer foveal defects.15 We believe they represent different findings. In the present study, persistent foveal detachments were detected in only two eyes with small MHs (case 11 and 14; Fig 6), 1 month after surgery, and both were flattened at the 3-month follow-up. In the dynamic healing process that results in the closure of MHs, this feature could be considered the immediate step after reapproximation of the edges of small MHs. The curvilinear nature of the ELM and the short duration of the foveal detachments seem to support this assumption. In contrast, the outer foveal cysts may develop later during follow-up, have a rectilinear-continuous ELM, and last for long time, and represent true defects of photoreceptor outer segment.

In the current series, moderately reflective foveal lesions partially or completely replacing the normal foveal architecture were found in 10 eyes during follow-up. This finding has been described previously,10–12,14,15 and most likely it represents a glial cell proliferative response that occurs at the foveal defect after MH repair.19,20 Interestingly, three out of the 10 eyes did not reveal any of these lesions at the last follow-up visit. In contrast with previous studies,12,14 we could not find a relationship between the basal hole size and the development of moderately reflective foveal lesions. One likely explanation could be the small number of patients in our study. Alternatively, a larger preoperative hole size might also cause the discrepancy. In our series, the median preoperative hole diameter was 430 μm, much smaller than that reported by Oh et al.12 and Wakabayashi et al.14 Whether glial closure is a consequence of larger MHs or an impediment to better healing of the IS/OS defect requires further study.

The main limitation of our study is the small number of patients. However, countering this limitation is that the same surgical technique was performed in every patient by the same surgeon, and seven follow-up visits were completed for 1 year after surgery. Additionally, axial motion artifacts in the SD-OCT images were minimized by an active eye-tracking system that for the first time allowed the same scanning location to be imaged on each of the following visits.

In conclusion, the present study supports previous findings11–15,21,22 that recovery of the ELM and IS/OS is important for VA improvement. Additional new findings are that (1) the ELM is the first structure to recover after MH closure (even at 1 month after surgery), (2) foveal cysts may develop during follow-up and in 45% of cases gradually fill, resulting in a complete recovery of the IS/OS junction, (3) moderately reflective foveal lesions may also progressively regress in almost one-third of cases, and (4) an intact ONL seems to be necessary to achieve a complete restoration of the photoreceptor microstructure. The ultrastructural correlate of the restoration of the IS/OS line observed by OCT remains still uncertain. Future studies will explore if it reflects regrowth of hypertrophic photoreceptor outer segments or other mechanisms.

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References