Focal Macular ERGs in Eyes after Removal of Macular ILM during Macular Hole Surgery

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PURPOSE. The removal of the internal limiting membrane (ILM) for traction maculopathy has recently been advocated. However, it is generally believed that the ILM plays an important role in retinal function, because it is the basal lamina of the Müller cells that are involved in the generation of the electroretinogram (ERG) b-wave. To date, there has been no objective assessment of retinal function on removing the ILM. In this study, the changes of each component of the focal macular electroretinograms (FMERGs) were investigated in eyes before and after the ILM was removed in the macular area during surgery for idiopathic macular holes (IMHs).

METHODS. FMERGs were elicited by a 15° stimulus centered on the fovea and monitored by an infrared fundus camera. FMERGs were recorded from 49 eyes of 48 patients with IMHs before and 6 weeks after anatomically successful macular surgery. Whether an eye had or did not have the ILM removed was randomly determined. The ILM was removed in 30 eyes (ILM-off group) and was not removed in 19 eyes (ILM-on group). Six months after surgery, the same examination was performed in 27 eyes of the ILM-off group and in 15 eyes of the ILM-on group. The amplitudes and implicit times of the a- and b-waves and the mean amplitudes and implicit times of the first three oscillatory potentials (OP1 to OP3) were compared before and after surgery within and between the groups.

RESULTS. Visual acuity increased significantly after surgery in both groups. In the ILM-on group, the amplitude of the a- and b-waves and the OPs increased significantly 6 months after surgery (P = 0.0093, P = 0.0019, P = 0.0024, respectively, paired t-test). In the ILM-off group, the a-wave amplitude and mean OP amplitudes were significantly larger 6 months after surgery (P = 0.0077, P = 0.0030, respectively, paired t-test). The b-wave amplitude, however, did not change significantly. The percentage increase in the b-wave amplitude 6 months after surgery was significantly higher in the ILM-on group (44.0%) than in the ILM-off group (15.0%; P = 0.037, t-test).

CONCLUSIONS. The removal of the ILM had no adverse effect on visual acuity. However, the selective delay of recovery of the FMERG b-wave 6 months after surgery suggests an alteration of retinal physiology in the macular region. (Invest Ophthalmol Vis Sci. 2001;42:229–234)

The functional role of the internal limiting membrane (ILM) has not been determined. However, it is generally believed that the ILM plays an important role in retinal function, because it is the basal lamina of the Müller cells and is connected to the end feet of the Müller cells. In addition, the anionic sites on the ILM may act as a charge barrier between the retina and the vitreous cavity.1,2

In macular hole surgery, the surgical removal of the ILM in the macular region has been performed often lately, because it appears to increase the closure rate of macular holes.3–5 The stripping of the ILM for traction maculopathy has been endorsed because no proliferative response and no adverse effect on subjective visual function occurs after removal of the ILM.6,9 We have also been removing the ILM during surgery for idiopathic macular holes (IMHs) and have not seen any clinical adverse effects after ILM removal (N = 76 eyes). However, there has not been a prospective randomized controlled study with a large sample. The earlier results were limited to the evaluation of subjective visual functions shortly after surgery, and there has been no objective assessment using, for example, the electroretinogram (ERG). There are many factors to be considered before ILM removal is undertaken. Particularly, attention should be focused on changes of the b-wave of the macular ERG induced by the removal of the ILM, which has been reported to include the end feet of the Müller cells that are involved in the generation of the b-wave. In a recent report, a patient with a dominantly inherited Müller cell disease, Müller cell sheen maculopathy, showed impaired full-field b-waves.10,11 This dystrophy is a diffuse disease affecting the whole inner retina, whereas the removal of the ILM during macular hole surgery is limited to the macular area. Thus, it is necessary to examine the physiology of the macular area with focal macular electroretinography (FMERG).

We have been conducting a layer-by-layer analysis in various types of macular diseases with FMERGs using long-duration stimuli.12–18 This technique allows us to separate the on and off responses and record the photopic a- and b-waves and the oscillatory potentials (OPs), which are independent of the off response. Using these procedures, we have studied the early and late effects of removing the ILM of each component of the FMERG. This study addresses two questions: first, whether the removal of the ILM has any effect on the FMERGs and, second, whether the removal of the ILM during macular surgery can be physiologically justified.

METHODS

Surgery was performed by three surgeons on 89 consecutive eyes with IMHs from January 1998 through December 1999 in our institution, and in all cases holes were closed with a single operation. The operation time, manipulation of the macula, use of infusion during the insertion of the intraocular lens, and amount of the removal of the vitreous cortex after lens removal varied among the surgeons. To minimize this surgical variation, we selected the operations performed by a single surgeon (HT), who used a single operative technique. Of the 89 eyes, 49 eyes of 48 patients were subjected to vitrectomy combined with lens removal by this surgeon, and the statistical analysis was performed on these 49 eyes. In addition, the evaluation of visual acuity (VA) after 6 months in this group was thought not to be...
influenced by nuclear cataract, because phacoemulsification and intraocular lens insertion were performed simultaneously.

Each eye was randomly placed into two groups prospectively according to the day of the week that the surgery was performed. All the patients underwent the same procedure including phacoemulsification, core vitrectomy, removal of posterior hyaloid membrane, and intraocular lens insertion. The management of the tissue around the macular hole varied in the two groups. In the ILM-off group, the ILM was removed with ILM forceps as a single piece of curled, glistening membrane in the area of approximately 15° or slightly less than 15°. In the ILM-on group, only scraping of the retinal surface around the macular holes with a diamond-dusted eraser or brush-back style needle was performed, and no membranous structure was removed. After fluid air exchange, 0.6 ml of pure perfluoropropane was injected into the vitreous cavity in both groups.

Thirty eyes were placed in the ILM-off group and 19 eyes in the ILM-on group. This distribution did not differ significantly from a chance placement in the two groups (χ² = 27.47, df = 1, P = 0.116). One patient had a macular hole bilaterally, and the ILM was removed from the right eye but not from the left eye.

FMERGs were elicited by a 15° stimulus positioned on the fovea and monitored by an infrared fundus camera. FMERGs were recorded from the 49 eyes with IMH before and 6 weeks after macular hole surgery. Six months after surgery, the same examination was performed on 27 eyes of the ILM-on group and on 15 eyes of the ILM-off group. FMERG was not performed at 6 months after surgery in another eye. Six patients were followed up by the referring ophthalmologists and did not return or returned at a later time for examination, and two eyes were observed for 5 months after the operation.

The amplitudes and implicit times of the a- and b-waves and the mean amplitudes and implicit times of first three oscillatory potentials (OP1 to OP3) were compared between the two groups. The changes in the amplitude and implicit time of a-, b-waves and OPs were also compared between the two groups. The system and the responses elicited by spot stimuli 15° and smaller have been shown to be local responses. The changes in the amplitude and implicit time of a-, b-waves and OPs were compared before and after surgery in both groups. The changes in the amplitude and implicit time of a-, b-waves and OPs were also compared between the two groups. The system and the assessment of recording the FMERG under direct fundus observation have been described in detail. Briefly, an infrared television fundus camera installed with the stimulus light, background illumination, and fixation target, was used to monitor the exact locus of the stimulus on the macula. The size, frequency, and intensity of the stimulus spot, and the level of background illumination were adjustable. The background light was projected into the eye from the fundus camera at an angle of 45°. Additional background illumination outside the central 45° produced homogeneous background illumination for nearly the entire visual field.

The Burian–Allen bipolar contact lens electrode was used for the FMERG recordings. This electrode allowed not only low noise recordings but also permitted a clear view of the fundus that was displayed on a television monitor. The luminance of white stimulus light and background light was 29.46 candelas (cd)/m² and 2.89 cd/m², respectively. After the patient’s pupils were fully dilated with a combination of 0.5% tropicamide and 0.5% phenylephrine hydrochloride, the FMERGs were recorded with 5-Hz rectangular stimuli (100 msec light on and 100 msec light off). The stimulus spot was centered on the fovea. A total of 512 responses was averaged by a signal processor. A time constant of 0.03 seconds and a 100-Hz high-cut filter on the amplifier was used to record the photopic a- and b-waves, and the time constant was reduced to 0.003 seconds for recording the OPs. The amplitude of the a-wave was measured from the baseline to the peak of the a-wave. The amplitude of the b-wave was measured from the trough of the a-wave to the peak of the b-wave. The amplitude of each OP wavelet was measured from the baseline, drawn as a first-order approximation between the troughs of successive wavelets, to its peak. The FMERGs elicited by this method have been shown to be generated by the cone system, and the responses elicited by spot stimuli 15° and smaller have been shown to be local responses.

This research was conducted in accordance with the institutional guidelines of Nagoya University and conformed with the tenets of the World Medical Association Declaration of Helsinki. After providing sufficient information on other treatment options including observation alone and macular ERG examinations, informed consent was obtained from each patient for the surgery.

RESULTS

Figure 1 shows representative FMERG recordings before and 6 weeks and 6 months after surgery in a patient in the ILM-on group (Fig. 1, left) and another after ILM removal (ILM-off, Fig. 1, right). In the ILM-on eye, the b-wave and OPs amplitudes were larger 6 weeks after surgery, and there was further in-
TABLE 1. Preoperative Clinical Findings

<table>
<thead>
<tr>
<th></th>
<th>ILM-Off Group</th>
<th>ILM-On Group</th>
</tr>
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<tbody>
<tr>
<td>Number of eyes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before and 6 weeks after surgery</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>Six months after surgery</td>
<td>27</td>
<td>15</td>
</tr>
<tr>
<td>Age (y)</td>
<td>66.1 ± 1.3</td>
<td>67.2 ± 2.3</td>
</tr>
<tr>
<td>Visual acuity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LogMAR</td>
<td>0.91 ± 0.06</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td>Snellen</td>
<td>20/160–1</td>
<td>20/125+1</td>
</tr>
<tr>
<td>Size of macular hole (disc diameters)</td>
<td>0.29 ± 0.02</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Operation time (min)*</td>
<td>60.5 ± 2.0</td>
<td>61.4 ± 2.4</td>
</tr>
<tr>
<td>Duration of gas tamponade (d)</td>
<td>15.4 ± 0.8</td>
<td>15.3 ± 0.9</td>
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</table>

Data are mean ± SE.

* Operation time was between the beginning of vitrectomy after phacoemulsification and the closure of sclerotomy including intraocular lens insertion.

Changes after 6 months. In the ILM-off eye, in contrast, the b-wave and OPs amplitudes showed a marked decrease at 6 weeks after surgery but recovered after 6 months to the same level as that before surgery.

The patients' age, preoperative VA, size of macular hole evaluated relative to the vertical disc diameter, operation time from the beginning of vitrectomy after phacoemulsification and the closure of sclerotomy, and the duration of gas tamponade are shown in Table 1. In addition, the mean ± SEM of the amplitude, implicit times of the a- and b-waves, and the mean amplitude of the OPs of the preoperative FMERGs are compared for the two groups in Table 2. These data demonstrate that there were no statistical differences between the two groups before surgery.

Postoperative VA

The best corrected VA in all eyes except one increased 6 months after surgery (Table 2, Fig. 2, left). One eye had VA of 20/50 before and 6 months after surgery. The log of the minimum angle of resolution (log MAR) was used for statistical analysis of the VA measured with a Japanese standard VA chart.

Six weeks after surgery, both the ILM-on and ILM-off groups showed a significant increase in the best corrected VA (P < 0.0001, P < 0.0001, respectively, paired t-test), and no significant difference was found between the two groups. After 6 months, there was a further increase of the best corrected VA for both groups (P < 0.0001, P < 0.0001, paired t-test). No significant difference was found between the two groups (Fig. 2, right).

Focal Macular ERGs

In the ILM-on group, the amplitude of b-wave and the mean amplitude of OPs increased significantly 6 weeks after surgery (P = 0.0429, P = 0.004, respectively, paired t-test). Six months after surgery, the amplitude of all three components (a- and b-waves, and OPs) were significantly larger than before surgery (P = 0.0093, P = 0.0019, P = 0.0024, respectively, paired t-test; Fig. 3, left). The implicit times of the a- and b-wave and the mean of OPs were unchanged after surgery (Fig. 3, right).

In ILM-off group, the amplitude of the a- and b-waves, and the mean amplitude of the OPs were unchanged 6 weeks after surgery compared with those before surgery. Six months after surgery, the a-wave and the mean OPs were significantly larger (P = 0.0077, P = 0.0030, respectively, paired t-test) than before surgery. The mean amplitude of b-wave was slightly larger; however, the increase was not statistically significant 6 months after surgery (Fig. 3, left). The percentage increase in the amplitude of the b-wave 6 months after surgery was significantly greater in the ILM-on group (44.0% ± 11.2%, mean ± SEM) compared with the ILM-off group (15.0% ± 7.9%; P = 0.037, t-test; Fig. 4, left).

The implicit times of the a- and b-wave were significantly delayed in the ILM-off group compared with those before surgery (Fig. 3, right; P = 0.0202, P = 0.0016, respectively, paired t-test). The difference in the implicit times of the b-wave

TABLE 2. Changes of Best Corrected VA and FMERGs before and after Vitrectomy

<table>
<thead>
<tr>
<th></th>
<th>ILM-Off Group</th>
<th>ILM-On Group</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>6 Weeks</td>
</tr>
<tr>
<td>Visual acuity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LogMAR</td>
<td>0.91 ± 0.06</td>
<td>0.51 ± 0.05</td>
</tr>
<tr>
<td>Snellen</td>
<td>20/160–1</td>
<td>20/60–1</td>
</tr>
<tr>
<td>a-Wave Amplitude (µV)</td>
<td>1.17 ± 0.06</td>
<td>1.32 ± 0.09</td>
</tr>
<tr>
<td>Implicit time (msec)</td>
<td>20.4 ± 0.29</td>
<td>21.3 ± 0.45</td>
</tr>
<tr>
<td>b-Wave Amplitude (µV)</td>
<td>3.04 ± 0.21</td>
<td>3.15 ± 0.20</td>
</tr>
<tr>
<td>Implicit time (msec)</td>
<td>44.8 ± 0.45</td>
<td>46.2 ± 0.50</td>
</tr>
<tr>
<td>Oscillatory potentials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean amplitude (µV)</td>
<td>0.71 ± 0.06</td>
<td>0.79 ± 0.08</td>
</tr>
<tr>
<td>Mean implicit time (msec)</td>
<td>31.5 ± 0.28</td>
<td>31.7 ± 0.37</td>
</tr>
</tbody>
</table>

Data are mean ± SE, and times are in relation to surgery. Statistical significance was determined in preoperative data versus 6 weeks and 6 months after surgery using a paired t-test.

* Significant difference between 6 weeks and 6 months after surgery.
before and early after surgery was significantly greater in the ILM-off group (1.43 ± 0.41 msec) than that in the ILM-on group (2.00 ± 0.51 msec; *P* < 0.0194, *t*-test; Fig. 4, right). Six months after surgery, the implicit times of the a- and b-waves returned to the same level as before surgery. A change in the mean implicit times of the OPs before and after surgery was not found.

**DISCUSSION**

The ILM is the basal lamina of the Müller cells, which are involved in the generation of the b-wave of the ERG. The removal of the ILM has been endorsed as an alternative to the meticulous epiretinal membrane removal or to biochemical support in macular hole surgery. However, the effects of ILM removal on retinal function remain unknown.

An ultrastructural study has shown that the prefibrous tissue attaches not only on the back surface of vitreous cortex but also extends around and inside the macular hole. One of the key factors in closing IMHs is the elimination of the traction around the macular hole as completely as possible. Thus, it has been suggested that removing the ILM may eliminate almost all traction and lead to a higher probability of macular hole closures. Another effect of removing the ILM may be the induction of sufficient surgical trauma to induce gliosis that helps keep the macular hole closed.

It is interesting to note that specimens obtained during macular hole surgery or surgery for other macular disease such as vitreomacular traction syndrome were found to include the ILM inadvertently removed. In reports of the epiretinal membranes removed during vitrectomy, the eyes in which the specimen included the ILM showed lower postoperative VA. Unfortunately, there are no studies on the natural history of the retina after the loss of the ILM. Eckardt et al. reported that good anatomic results were achieved with the intentional removal of the ILM during macular hole surgery. However, they also reported an important observation that canals leading from the inner to the outer surface of the ILM contained Müller cell processes with clear signs of necrosis or degeneration.

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933218/)  
**FIGURE 2.** *Left:* Best corrected VA before and 6 months after surgery plotted as the minimum angle of resolution (logMAR) values and Snellen VA. VA increased after surgery in all eyes except one. *Right:* Mean ± SEM of the best corrected VA before and 6 weeks and 6 months after surgery. At 6 weeks after surgery, both groups showed a significant increase compared with that before surgery (**P** < 0.0001, paired *t*-test), and no significant difference was demonstrated between the two groups (*t*-test). After 6 months, the best corrected VA was further increased compared with that at 6 weeks after surgery (**P** < 0.0001, paired *t*-test), and no significant difference was demonstrated between the two groups (*t*-test). N.S., not significant.

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933218/)  
**FIGURE 3.** *Left:* Amplitudes of focal macular ERGs before, 6 weeks after, and 6 months after surgery. In the ILM-on group (●), the b-wave and mean OP amplitudes increased significantly compared with that before surgery. Six months after surgery, the amplitude of all three components (a- and b-waves and mean OPs) increased significantly compared with that before surgery. In the ILM-off group (○), the amplitudes of all three components were unchanged 6 weeks after surgery compared with those before surgery, and after 6 months, the a-wave and mean OP amplitudes are increased significantly compared with that before surgery. The b-wave amplitude in ILM-off was slightly larger; however, the increase was not statistically significant (**P** < 0.05; **P** < 0.01; paired *t*-test). N.S., not significant.
pathogenic significance of these findings remains unknown; however, they suggest possible damage to the Müller cells.

Because the electrical potential changes in the Müller cells contribute to the b-wave of the ERG, it was reasoned that defects of the ILM in the macular region should affect the macular ERGs. Thus, we recorded FMERGs using a 15° stimulus that is approximately the same size as the removed ILM during macular hole surgery. However, it is possible that a small area of the ILM was still attached in the recorded area.

In photopic ERGs, the best evaluation of the b-wave is performed by separating the on- and off-components with long-duration stimuli. This is because the positive deflection recorded by brief stimuli is a combination of the b- and d-waves. Thus, in this study, the positive deflection reflects the pure b-wave without contamination by the d-wave.

The delay in the implicit time of the b-waves in the ILM-off group was found early after surgery. The delayed implicit times recovered to preoperative times 6 months after surgery, which suggests that the surgical effects of removing the ILM on inner retinal function are temporary. In peeling the ILM, great care was taken to avoid causing retinal injury that would lead to macular edema. The ILM was peeled off as a single membrane in the ILM-off group without difficulty by using ILM forceps. Some specimens were identified as the ILM histologically; however, most membranes were determined to be the ILM from the texture of glistening membrane. In the surgery of eyes with epiretinal membranes, a retinal whitening is sometimes noted during surgery after the removal of epiretinal membrane with the ILM. In this study, no fundus change such as a whitish edematous appearance was found in the macular region after removing ILM for the successful closure of IMH. Histopathologically, the ILM regenerates very little in the defective area, whereas the rim of the defective area has some regenerative capacity.20 The limited recovery of the b-wave may not depend on a defect of the ILM but may be due to the recovery of the Müller cells.

To investigate the effect of ILM removal on retinal function, eyes with idiopathic macular holes are quite suitable, because the alterations are uniform and uncomplicated compared with eyes with epiretinal membranes or with diabetic macular edema. Whether the ILM should be removed during the surgery for IMH or other tractional maculopathies must be considered for each eye. There may be certain clinical advantages of removing ILM for the successful closure of IMH.

Although this was not a purely randomized, controlled study, the results demonstrated a limited and delayed recovery of the b-wave amplitude of the FMERG in the ILM-off group in a relatively short period of 6 months after surgery. These findings suggest some dysfunction or physiological changes in the Müller cells. Further long-term follow-up with electrophysiological and clinical examinations of eyes with the ILM removed will demonstrate the final condition of the Müller cells.

**References**


