Title: Foveal sparing internal limiting membrane peeling for idiopathic macular hole; effects on anatomical restoration of the fovea and visual function

Short title: Foveal sparing ILM peeling for macular holes

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Ethical/legal considerations

The study followed the tenets of the Declaration of Helsinki, with approval from a UK Health Research Authority ethics committee (18/WM/02). Informed consent was obtained from the subjects after explanation of the nature of

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Patient anonymity and informed consent

Informed consent was obtained from the subjects after explanation of the nature of

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D Steel is a consultant for Alcon
Abstract (200/200 words)

Purpose:

Muller cells appear to be important in maintaining foveal morphology through connections between their foot processes and the Internal limiting membrane (ILM). ILM peeling causes Muller cell trauma. We hypothesised that leaving a rim of unp peeled ILM around idiopathic macular holes (MH) undergoing vitrectomy surgery would improve postoperative foveal morphology and vision.

Methods:

Prospective pilot study of fovea-sparing ILM peeling in a consecutive cohort of patients with MHs over a 12-month period. SD-OCT and ETDRS letters visual acuity (BCVA) were assessed pre- and postoperatively, and foveal morphology and metamorphopsia postoperatively. The foveal sparing group (FSG) were compared to a second consecutive cohort who received standard ILM peeling (Control group, CG).

Results:

34 eyes of 34 patients were included in each group. Groups showed no significant preoperative differences. 34/34 holes were successfully closed with surgery in FSG and 32/34 in CG. FSG showed better postoperative BCVA (67.7 versus 63.8, p=0.003) and BCVA improvement (25.1 versus 20.2, p=0.03). FSG demonstrated thicker minimum foveal thickness (211 versus 173 microns, p=0.002) and less steep foveal depression (158 versus 149, p=0.002).

Conclusion:
Preserving non-peeled ILM around MHs resulted in a high closure rate, improved foveal morphology and better postoperative BCVA. An appropriately powered randomised controlled study is warranted.

The rate of idiopathic macular hole (MH) hole closure after vitrectomy for macular hole has risen over the last 20 years largely due to the widespread adoption of peeling the internal limiting membrane (ILM) of the retina, which has been shown to improve hole closure rates in several studies.¹

The ILM is a relatively rigid structure²,³ and its removal reduces the compliance of the peri-hole retina to facilitate hole closure and eliminate all tangential traction.²,⁴,⁵
However, several potential detrimental effects on the inner retina have been demonstrated after ILM peeling and irregularities in the inner retinal surface have been described both related to forceps trauma and the coincidental avulsion of Muller cell end feet processes during forced ILM separation. Furthermore, there are a number of morphological alterations in foveal shape which have been described and attributed to ILM peeling.6–9 The exact effect of any of these on visual function is uncertain.

It is known that the umbo of the fovea is important for optimum visual function. It is also thought that the Muller cells at the fovea with their extended funnel shape, higher refractive index than the surrounding tissue, and orientation along the direction of light propagation may act as optical fibres to maximize light transmission through the mesh-like inner retina to the photoreceptors.10

Therefore, we postulated that preserving the ILM which surrounds the MH during surgical ILM peeling could reduce the trauma inflicted upon Muller cells and, as a result, optimise foveal anatomical restoration and function following macular hole surgery whilst still maintaining a high rate of hole closure.

This prospective pilot study aims to determine whether preservation of the peri-hole ILM during ILM peeling for MH surgery results in improved anatomical and functional restoration of the retina.

**Method**
We designed a prospective, non-randomised pilot study. Consecutive patients presenting to one surgeon with MHs over a 12-month period were recruited. Our inclusion criteria were as follows: duration of symptoms for less than 12 months and a minimum linear diameter (MLD) of less than 630 microns. We excluded MHs that were associated with myopia >6 dioptres, visually significant cataract, trauma, severe glaucoma, retinal detachment and stage 4 macular holes with complete vitreoretinal separation from the optic disc.

The study followed the tenets of the Declaration of Helsinki, with approval from a UK Health Research Authority ethics committee (18/WM/02). Informed consent was obtained from the subjects after detailed explanation of the study and its rationale.

All patients underwent transconjunctival 27-gauge (27g) vitrectomy (Constellation, Alcon, Fort Worth, USA) using wide-field non-contact viewing (Eibos, Haag-Streit, Switzerland) with combined phacoemulsification and intraocular lens (IOL) implantation if phakic. Posterior hyaloid face separation was achieved with aspiration. BBG (ILM Blue, DORC International, The Netherlands) was used to stain the macula in all cases. The dye was refluxed on to the macula retina using the vitrectomy probe and aspirated off following 5 seconds of contact time. A macular contact lens was used to view the peeling procedure. The ILM was peeled using a pinch technique. To achieve an area of foveal spared ILM, a series of overlapping circles of peeled ILM around the hole circumference was completed using 27g ILM forceps (Vitreq, The Netherlands). After this process was completed the ILM peeled area was extended to approximately 1-2 disc diameters around the hole if required, in an attempt to standardise the diameter of the peeled area. (Figure 1 and 2)
25% SF6 and 20% C2F6 gas was used as tamponade agents (SF6 in holes less than 400 microns and C2F6 in holes >400 microns in MLD). Patients were instructed to position face down for 1 day and then to avoid a supine position for 1 weeks. Each patient was reviewed at 2 weeks and 3 months following surgery. Pre- and post-operative best corrected visual acuity (BCVA) was measured at 3 months following the last interventional procedure using a ETDRS letter chart with optimal refraction. At 3 months postoperatively we measured metamorphopsia (MM) using D charts. In both instances the assessor was masked to the treatment group.

Patients underwent spectral-domain optical coherence tomography (SD-OCT) on the Heidelberg Spectralis (Heidelberg Engineering, Heidelberg, Germany) immediately prior to vitrectomy and at 3 months postoperatively using a 5 by 15-degree scan area centred on the macular hole, an ART of 16 and a line spacing of 30 microns. A postoperative 30 by 20-degree scan with ART 20 and 60-micron line spacing was also performed. The MLD and base diameter (BD) of the hole was measured as previously described using the Spectralis measuring tools. Using the ‘en face’ view, the area of ILM sparing surrounding the foveal centre was measured. The total area demarcated by the ILM peeled edge was also measured. (Figure 3)

A horizontal line scan passing through the centre of the fovea on the 5 by 15 postoperative line scan was saved and then imported into the freely available software ‘FOVEA’ by an observer masked to treatment group. The scan selected was the scan line where the foveal floor was thinnest. ‘FOVEA’ can segment out the shape of the foveal profile using a semi-automated methodology once the user has
outlined the retinal pigment epithelium (RPE) as a baseline. Various parameters are outputted to a txt file, including the maximum retinal thickness (nasal and temporal to the foveal centre) and the minimum retinal thickness at the foveal centre.

FOVEA also locates the points on the foveal slope which represent the maximum curvature. (Figure 4). To measure the angle between these points and the foveal centre we used a custom-made MATLAB program to extract these 3 points and measure the angle. The width of any external limiting membrane (ELM) and ellipsoid defect present on the image was measured. The distance from the centre of the hole to a vessel’s crossing point on the temporal border of the optic disc was measured before surgery, and from the same disc margin point to the centre of the thinnest part of the closed hole postoperatively using the measuring tools of the Spectralis.

The patients were compared to an immediately following consecutive cohort of patients with the same inclusion and exclusion criteria (termed the ‘control’ group for the remainder of this article). They had the exact same surgery as the Foveal sparring group (FSG) but underwent conventional ILM peel without sparing the fovea.

**Statistical analysis**

The study was conducted as a pilot to determine the feasibility of the technique and end points for future appropriately powered studies. As such, no power calculation was performed.

Shapiro-Wilk test was used to assess data for normality. Descriptive and statistical analysis was performed using Minitab 16 statistical package (Minitab Ltd, Coventry,
UK). Pre- and postoperative continuous variables are presented in terms of mean, standard deviation and range when normally distributed, and median, interquartile range (IQR) and range when non-normally distributed. Categorical variables are presented as percentages.

2-tailed independent T-tests and Chi-squared tests were used to compare continuous and categorical variables respectively between the two groups. Statistical significance was defined as a p-value of 0.05 or less.

Results
Thirty-four eyes of thirty-four consecutive patients who met our pre-defined inclusion criteria were treated with a foveal sparing ILM peel during a 12-month period and defined the FSG. The peeling procedure was completed in all patients when attempted. The mean age was 71 (SD±6.5) years and 23 (71%) were female. Patient baseline characteristics of patients and MH measurements are described in Table 1. There were no significant differences in any of the preoperative parameters. The mean size of preserved ILM at the foveal centre was 1.63mm² (range: 0.91 to 2.3), which is equivalent to an approximate mean diameter of 0.72mm.

All holes in the FSG closed whereas 32/34 (94.12%) closed in the control group. Due to non-normality of the pre BCVA, both the pre- and postoperative BCVA were compared using a Mann-Whitney Test. There was a non-significant difference in pre-op BCVA (p=0.92) but a significant difference in post op Va (p=0.003). Change in BCVA was compared using t-test and was significant (p=0.027). In order to confirm that the change in post op BCVA was not influenced by the pre-op BCVA an
Analysis of Covariance was performed with the pre-operative BCVA entered as a covariate. There was a significant difference in post op BCVA even controlling for pre op BCVA (p=0.015). The FSG therefore achieved better post-operative vision overall and greater improvement in vision following surgery. (Table 2)

The foveal floor was significantly thicker and less steep in the FSG. There were 14 patients with an ellipsoid defect at 3 months postoperatively in the FSG and 6 in the control group. When an ellipsoid defect was present, the size of the defect was greater in the control group (mean: 225.7 microns) than the FSG group (mean: 64.1 microns, p=0.02). There was no difference in the nasal and temporal retinal thicknesses, nor foveal width. There was also no between-group difference in the ‘change in fovea to optic disc’ distance following surgery.

Total D chart scores were not significantly different between the two groups (p=0.11)

Discussion

The results of this study suggest that sparing the foveal ILM when performing an ILM peel using the technique described in our methodology is associated with acceptable MH closure rates, improved foveal morphology and better post-operative vision when compared with standard (non-fovea-sparing) ILM peeling.

Tzyy-Chang Ho et al\textsuperscript{14} first described the concept of this surgical intervention in a series of 14 eyes, all less than 400 microns in size and all with vitreomacular traction (VMT). They demonstrated an improved visual acuity compared to a matched control cohort.\textsuperscript{14} In this study we present a larger and more diverse series of patients which included MHs up to 616 microns in MLD, with and without VMT. Ho et al also used
C3F8 in all eyes whereas 21 of the eyes in our series had tamponade with the short
acting gas SF6 and the others with medium acting gas C2F6. We also used an
alternative strategy to spare the foveal ILM which did not require the use of scissors.
Finally, we describe the post-operative shape of the fovea in greater detail than
previously reported.

Both series have identified improved postoperative visual acuity following fovea-
sparing ILM peeling. Although the exact mechanism for this is undetermined, a
potential mechanism can be hypothesised.

The photoreceptor layer at the foveola is entirely cones, and axons from these
densely packed central photoreceptors are laterally displaced. Where they meet their
corresponding bipolar cells and subsequent ongoing connections, they form a
thickened rim to the central pit. The displacement of fibres away from the foveal
centre is hypothesised to have occurred phylogenetically to reduce light obstruction
to the central densely packed cones to maximize visual acuity. Muller cells
ensheath the cone axons anatomically and also support their physiological functions.
In combination, the cone axons and Muller cells form the Henle fibre layer. Muller
cells follow an extended ‘Z’ course and have a higher refractive index than their
surrounding tissue. As a result, it has been suggested that they act as optical fibres
to ‘funnel’ light transmission through the mesh-like inner retina to the
photoreceptors. Therefore, to regain optimal vision after MH surgery, one can
suggest that this anatomical arrangement must be reformed.

The ILM functions to form the inner boundary of the retina and is considered the
basement membrane of the Muller cells, which abut it, and create an arborizing
pattern with their end feet and the convoluted retinal side of the ILM. When the ILM
is peeled, some end feet are avulsed with Muller cell damage.\textsuperscript{16} MHs are thought to occur predominantly as a result of dehiscences in the central foveolar. During MH formation the foveal centre cones are avulsed from the RPE and extend up the sides of the MH, but still maintain their Muller cell sheaths and therefore remain connected with the peri-macular hole ILM.\textsuperscript{17} By avoiding peeling this important region of ILM, we hypothesize that Muller cell integrity is better maintained and allows improved foveal shape reformation, and superior postoperative visual acuity as a consequence.

Other studies have simply categorised foveal shape after macular hole surgery as ‘U’, ‘V’ or irregular and found that U shaped foveas were associated with thicker foveal floors and better postoperative visual acuities. We used foveal floor thickness and central foveal angle to more precisely classify the foveal shape after surgery, and demonstrated that by sparing the foveal ILM, better foveal shape is maintained and is associated with improved visual acuity.

It is important to differentiate our described foveal sparing technique from ILM flaps. In ILM flap techniques the ILM is peeled right up to the hole rim, and hence the Muller cell trauma will still occur.\textsuperscript{18}

Although our series is small, a 100% rate of hole closure was achieved. Peeling of the ILM improved hole closure and this is thought to occur through a number of mechanisms. Despite being thin, the mechanical strength of the ILM is measured in the megapascal range which is similar to articular cartilage and is roughly 1000-times stronger than cell layers. The ILM contributes to at least 50% of the retina’s rigidity.\textsuperscript{2,3} Therefore, its removal reduces retinal compliance which aids hole closure and also causes the surrounding retina to move centripetally to decrease the distance between the fovea and the optic disc.\textsuperscript{7} By segmenting the central ILM, the fovea could be spared during the ILM peel whilst enabling centripetal movement to
still occur. Indeed, we demonstrated the same changes in nasal and temporal retinal thickness and fovea disc distance as previously described with no significant difference between the FSG and the control group. Peeling of the ILM also removes all inner retinal vitreous remnants, surface epiretinal membranes and the scaffold which enables epiretinal membrane formation. It is interesting that despite leaving a rim of ILM around the hole, where these affects could be envisaged to be most important, we still achieved a high closure rate. An area of non-staining is commonly seen with ILM-specific stains around the macular hole rim which potentially represents Muller cell process extension as a healing response.19 ILM flaps are thought to improve hole closure by acting as a scaffold and providing Muller cell fragments on the residual ILM surfaces which attract a glial migration into the hole.20 In foveal sparing ILM peeling, leaving the rim of ILM around the hole may act as a similar attractant to aid glial cell MH closure.

We measured post-operative MM using D charts. Although a small difference between the groups was found, statistical significance was not achieved. The overall degree of MM was small but similar to a previous study using the D chart.11 The extent of MM following surgery has previously been shown to relate to the size of the ILM peel. Less MM occurs following a larger ILM peel than a smaller peel.21 Post-operative MM also depends on the degree of horizontal as compared to vertical asymmetry in the fovea postoperatively.21 We did not have any significant difference in the total area of ILM peeled between the groups and did not measure vertical foveal profile, but it would appear that foveal sparing ILM peeling does not affect the extent of MM to any clinically relevant extent.

The study was designed as a pilot study and as such is limited by its sample size which predisposes to type II errors. Patients did not undergo randomisation to the
FSG group and control group which predisposes to bias in selection. However, all surgeries were performed by a single surgeon (DHS) in a consecutive manner. No patients were specifically selected to undergo each surgical intervention based on their characteristics. Therefore, it is appropriate to consider the two groups well-matched for variables that are known to affect visual outcome. It is important to note that stage 4 holes were excluded from the study, as they are known to be associated with increased amounts of peri-hole ERM, which we felt could have confounded the results.

**Conclusion**

In this pilot study of 34 consecutive patients with stage 2 and 3 MHs of less than 630microns in MLD, sparing of a rim of peri-macular hole ILM during surgery resulted in a high closure rate, a thicker minimal foveal thickness, improved foveal contour, improved reformation of the ellipsoid zone and better postoperative visual acuity than a comparable cohort of patients operated upon by the same surgeon. Further investigation in an appropriately powered randomised controlled study is warranted.

**References**


2. WOLLENSAK G, SPOERL E, GROSSE G, WIRBELAUER C. BIOMECHANICAL SIGNIFICANCE OF THE HUMAN INTERNAL LIMITING


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Table 1: Preoperative variables

<table>
<thead>
<tr>
<th></th>
<th>Foveal spare group, n=34</th>
<th>Control group, n=34</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>Age, years.</strong></td>
<td>71.6.5 (55-80)</td>
<td>70.9, 6.5 (59-82)</td>
<td>0.82</td>
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<tr>
<td>Mean, SD (minimum to maximum)</td>
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<tr>
<td><strong>Gender</strong></td>
<td>9 male (26%)</td>
<td>7 male (21%)</td>
<td>0.57</td>
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<td>N, percent</td>
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<tr>
<td><strong>Symptom duration prior to surgery, months.</strong></td>
<td>4.1, 2.7 (1-11)</td>
<td>4.3, 2.6 (1-12)</td>
<td>0.72</td>
</tr>
<tr>
<td>Mean, SD (minimum to maximum)</td>
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<tr>
<td><strong>Minimum linear diameter, microns.</strong></td>
<td>372.6, 135.8 (145-616)</td>
<td>357.1, 118.5 (122-615)</td>
<td>0.85</td>
</tr>
<tr>
<td>Mean, SD (minimum to maximum)</td>
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<tr>
<td><strong>Base diameter, microns.</strong></td>
<td>701.1, 234.6 (264-1105)</td>
<td>722.1, 232.1 (217-1151)</td>
<td>0.32</td>
</tr>
<tr>
<td>Mean, SD (minimum to maximum)</td>
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<tr>
<td><strong>Preoperative visual acuity, ETDRS letters.</strong></td>
<td>43.3, 9.1 (25-60)</td>
<td>43.6, 7.2 (30-55)</td>
<td>0.92</td>
</tr>
<tr>
<td>Mean, SD (minimum to maximum)</td>
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<tr>
<td><strong>Pseudophakic n, percent.</strong></td>
<td>5 (15%)</td>
<td>4 (12%)</td>
<td>0.72</td>
</tr>
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<td><strong>VMT. N, percent.</strong></td>
<td>8 (24%)</td>
<td>9 (26%)</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Tamponade choice. n, percent</strong></td>
<td>11 C2F6 (32%), 23 SF6 (68%)</td>
<td>12 C2F6 (34%), 22 SF6 (66%)</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>ILM Peel diameter</strong></td>
<td>4982.4, 559.6 (3536-5799)</td>
<td>4838.9, 658.8 (2951 - 5774)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Mean, SD, (minimum to maximum)</strong></td>
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Table 2: Postoperative variables and outcomes

<table>
<thead>
<tr>
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<th>Foveal spare group, n=34</th>
<th>Control group, n=34</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>Postoperative visual acuity, ETDRS letters</strong></td>
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<tr>
<td>Mean, SD, (minimum to maximum)</td>
<td>67.7, 10.2 (38-81)</td>
<td>63.8, 8.8 (37-76)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Change in visual acuity, ETDRS letters</strong></td>
<td>25.1, 9.1 (7 to 46)</td>
<td>20.2, 9.6 (-2 to 38)</td>
<td>0.027</td>
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<tr>
<td>Mean, SD, (minimum to maximum)</td>
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<tr>
<td><strong>Total D chart score</strong></td>
<td>0.25, 0.58 (0-2.6)</td>
<td>0.61, 1.01 (0-3.7)</td>
<td>0.11</td>
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<tr>
<td><strong>Foveal floor thickness, microns</strong></td>
<td>210.9, 41.7 (121.9 to 285.5)</td>
<td>173.4, 44.7 (57.6 to 262.2)</td>
<td>0.002</td>
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<tr>
<td>Mean, SD, (minimum to maximum)</td>
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<tr>
<td><strong>Ellipsoid defect when present, microns</strong></td>
<td>64.1, 96.6 (0-322)</td>
<td>225.7, 192.0 (0-1077)</td>
<td>0.02</td>
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<tr>
<td>Mean, SD, (minimum to maximum)</td>
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<tr>
<td><strong>ELM defect presence</strong></td>
<td>2</td>
<td>3</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Foveal width, microns</strong></td>
<td>1669.2, 268.2 (1062.7 to 2520.3)</td>
<td>1724.5, 399.4 (1182.6 to 2855.6)</td>
<td>0.55</td>
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<tr>
<td>Mean, SD, (minimum to maximum)</td>
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<tr>
<td><strong>Central foveal angle, degrees</strong></td>
<td>158.1, 10.7 (143.1 to 172.8)</td>
<td>149.5, 8.9 (121.0 to 168.9)</td>
<td>0.002</td>
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<td>Mean, SD, (minimum to maximum)</td>
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<tr>
<td><strong>Temporal foveal thickness, microns</strong></td>
<td>326.9, 35.9 (271.5 to 379.7)</td>
<td>326.3, 33.6 (271.5 to 379.7)</td>
<td>0.95</td>
</tr>
<tr>
<td>Mean, SD, (minimum to maximum)</td>
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<tr>
<td><strong>Nasal foveal thickness, microns</strong></td>
<td>342.1, 31.8 (273.8 to 386.8)</td>
<td>334.6, 33.2 (275.4 to 390.5)</td>
<td>0.40</td>
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<td>Mean, SD, (minimum to maximum)</td>
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<tr>
<td><strong>Change in fovea to temporal border of optic disc distance, microns</strong></td>
<td>-51.2, 135.6 (134 to -302)</td>
<td>-61.3, 98.4 (67 to -181)</td>
<td>0.77</td>
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<td>Mean, SD, (minimum to maximum)</td>
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Figure legends

**Figure 1**: Schematic diagram of the foveal sparing internal limiting membrane peeling technique used. A series of overlapping circles of peeled ILM around the hole circumference was completed sparing an approximately 500-micron rim of ILM around the macular hole rim. After this process was completed the ILM peeled area was extended to approximately 1-2 disc diameters around the hole if required (dotted black line).
Figure 2: Intraoperative fundal image captured as a video still during surgery at the completion of the foveal sparing ILM peel. Area of foveal sparing outlined in blue and macular hole in red.
Figure 3: Examples of 3-month postoperative SDOCTs of patients in the two groups. The program ‘FOVEA’ has been used to semi automatically segment out the foveal profile. The thicker foveal centre, more even and less steep foveal profile, and smaller ellipsoid defects can be appreciated in the foveal spare group examples shown.
Figure 4: Representative example of a foveal spare group patient compared with control group. Preoperative SDOCT (a), postoperative SDOCT (b) and en face OCT (c) with area of foveal spared ILM outlined in black with the peeled area clearly seen with a dissociated optic nerve fibre layer appearance. Right and left eyes are labelled accordingly.