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ILM peeling technique influences the degree of a dissociated optic nerve fibre layer appearance after macular hole surgery.

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Abstract

Purpose

To assess the effect of two different internal limiting membrane [ILM] peeling techniques carried out during surgery for idiopathic macular hole on the postoperative extent of a dissociated optic nerve fibre layer appearance [DONFL]

Methods

Prospective data collection of surgical records and videos and pre- and post-operative imaging of a consecutive series of patients undergoing surgery for idiopathic macular hole by two surgeons. One surgeon used a forceps pinch peel technique to peel the ILM and the other surgeon using a diamond dusted membrane scraper. The extent of any DONFL was measured using spectral domain optical coherence tomography and blue reflectance imaging at 3 months post-operatively. A proportion of the ILMs removed were examined with transmission electron microscopy.

Results

Fifty seven patients were studied with 41 in the forceps group and 16 in the scraper group. The groups were well matched with no significant difference in any pre-operative parameters. Some degree of DONFL was observed on the 3 month blue

reflectance images in 88% of the forceps group and 100% of the scraper group [p=0.14]. There was a significant difference in the total number of depressions in the nerve fibre layer typical of DONFL on OCT between the two groups [p=0.001] and general regression analysis showed that the peeling technique used was the only significant association with the degree of DONFL observed. Electron microscopy showed large patches of cellular debris on the retinal side of the peeled ILM in 3 out of 4 of the scraper group and 1 out of 12 cases of the forceps group.

Conclusion

ILM peeling technique and possibly other surgeon specific factors appear to influence the extent of DONFL observed after ILM peeling macular hole surgery

Key words: Dissociated optic nerve fibre layer, Internal limiting membrane, peeling, diamond dusted membrane scraper, forceps, idiopathic macular hole

Introduction

Peeling of the internal limiting membrane [ILM] of the retina has been shown to improve the surgical closure rate of macular holes treated with vitrectomy and gas tamponade. [1] It is known however that ILM peeling results in some surgical trauma with a variety of observable effects following surgery described, including a distinctive change in the appearance of the inner retina known as a disassociated optic nerve fibre layer [DONFL]. [2] DONFL was first observed after epiretinal membrane (ERM) peeling surgery but was subsequently recognised to be specific, and confined to the area where the ILM had been peeled. [2-5] The precise mechanism is unknown but a number of authors have proposed that it is related to trauma to the Muller cell endplates which abut, and partially form the retinal side of the ILM. [2,6] Although many authors have described its occurrence and imaging features, no study to date has assessed factors affecting its severity. This study aimed to assess the effect of two different peeling techniques on the subsequent extent of DONFL in a cohort of patients undergoing idiopathic macular hole surgery. Furthermore in a subgroup of patients we analysed the ILM removed at the time of surgery with transmission electron microscopy (TEM) to assess whether there were any histological differences in the ILM between the two groups.

Method

Consecutive patients undergoing surgery by two surgeons for idiopathic macular holes over a one year period were included in the study. Patients with traumatic macular holes, high myopia [>6 dioptres] and previous retinal surgery were excluded from the study. Ethical approval was obtained and informed consent to participate was gained from participants. Patients with less than 3 months follow up were excluded. All patients underwent trans-conjunctival 25g vitrectomy using wide field non-contact viewing with combined phacoemulsification and IOL implantation if phakic. Posterior hyaloid face separation was achieved with aspiration. In cases of stage 4 holes, with a Weiss ring present the presence of residual vitreous was checked for with diluted triamcinolone staining. Brilliant Blue G [ILM Blue, Dorce International, The Netherlands] was used to stain the macula for 5 seconds in all cases. A macular contact lens was used to view the peeling procedure. The ILM was peeled by surgeon one using a pinch technique and 25g end gripping forceps [Grieshaber revolution DSP ILM forceps, Alcon Grieshaber AG, Switzerland]. Surgeon two used a diamond dusted membrane scraper [DDMS, Tano diamond dusted membrane scraper, Synergetics inc, USA] to initiate and complete the peeling. A peel radius of approximately 1-1.5 disc diameters was aimed for in all cases. Both surgeons were right handed and initiated the peel inferior to the fovea at a distance of approximately 1000 microns from the foveal centre and peeled in a clockwise direction. After creating a flap with the DDMS, the ILM was then folded back on itself and a brushing motion, restricted to the everted flap only, used to complete the peel. In a subgroup of patients, after removing the ILM from the eye it was immediately placed in glutaraldehyde and processed for electron microscopy. Either 25 % SF₆ or 20% C₂F₆ gas was used as a tamponade and the patients instructed to position face down for 3 days. All surgeries were videoed for later

analysis with particular care taken to achieve carefully focused and centred images post peeling. Patients were reviewed at 2 weeks and 3 months post operatively. Preoperative and postoperative BCVA at 3 months was measured using a standard Snellen acuity chart and converted to LogMAR scores for the purposes of statistical analysis.

Patients underwent Spectral domain optical coherence tomography [SD OCT] and blue reflectance [BR] imaging on the Heidelberg Spectralis immediately preoperatively and at 3 months post-operatively. The BR images were 30 degree by 30 degree images and saved as 768 by 768 bitmap images. A radial line scan pattern SD OCT was used preoperatively centred on the macular hole and used to assess the preoperative minimum linear diameter of the holes using the Spectralis measuring tools. For the post-operative OCT a 20 by 30 degree horizontal line scan pattern was used centred on the fovea with a line spacing of 240 microns. All scans used a 25 ART setting enabling multisampling and noise reduction over 25 images.

The intra-operative video following ILM peeling was used to record the number of petechial retinal haemorrhages that occurred after peeling and the extent of the ILM peel was measured from the images using the vertical disc diameter as a reference. The actual dimension of the disc was measured from the pre-operative Spectralis images. The point where the ILM peel was initiated was marked and graded by the surgeon intra-operatively as:

- 0) No observable change in retinal surface visible
- 1) Subtle retinal whitening visible in nerve fibre layer
- 2) As 1 but with superficial retinal haemorrhages
- 3) As 2 but with punctate deeper retinal haemorrhage
- 4) Observable RPE or retinal disruption with larger retinal haemorrhage.

Any other points of surgical trauma were also recorded and graded using the same scale.

Post-operatively the OCT images were used to grade the hole as open or closed. Holes were considered closed, indicating anatomical success, if there was complete circumferential hole rim reattachment without a full thickness foveal neurosensory retinal defect demonstrated on OCT.

The presence of a DONFL appearance on blue reflectance imaging at 3 months post-operatively was recorded and OCT images used to grade the extent. The number of focal depressions characteristic of DONFL in the retinal surface were counted in each of the central 13 slices of the 3 month post-operative OCT and summed to produce a total DONFL score. [Figure 1 and 2] The presence of a typical retinal nerve fibre layer [i.e. an arcuate defect in the nerve fibre layer radiating from the optic disc to a localised area of the macula] was specifically sought in the post-operative blue reflectance images.

For the method of counting DONFL depressions the intra-observer reliability was measured in 20 randomly chosen OCT images with the count repeated on the same

images on two occasions separated by 3 months. The intraclass correlation coefficient was 0.91 signifying high repeatability.

Electron microscopy

Samples were fixed in 2% glutaraldehyde in 0.1M sodium cacodylate buffer. The ILM was enrobed in low-melting point agarose [4%] to form a small block [this made the ILM easier to handle]. After secondary fixation in 2% osmium tetroxide, the samples were dehydrated in graded acetone, embedded in epoxy resin and polymerised at 60°C. Ultrathin sections [70nm] were taken at 2 levels through the block, stained with uranyl acetate and lead citrate and viewed on a Philips CM100 TEM.

Detailed examination of the tissue was then performed to determine the occurrence and size of any cellular debris on the retinal surface of the ILM. Debris was divided up into the following categories. [Figure 3]

- 1) 'Small' - cellular fragments less than 2microns in size with no recognisable cell organelles
- 2) 'Whole cells' - large fragments greater than 2 microns with recognisable cellular features e.g. nucleus, mitochondria
- 3) 'Layers' - a number of cells forming a monolayer with recognisable cellular structures

For estimation of the amount of cellular debris, images were taken at x7900 from 14 randomly sampled areas of the ILM. To quantify the amount of debris on the retinal side of the ILM a grid of lines [line length 2µm] was superimposed on each image. The number of intercepts between the grid line and retinal surface were counted. Another grid [line length 1µm] was then superimposed on each image and the number of intercepts between the grid lines and any retinal debris were counted. The percentage of retinal surface covered by cellular debris was taken as the number of intercepts on debris/ [number of intercepts on surface x 2] * 100.

Statistical analysis

Descriptive and statistical analysis was performed using Minitab 16 statistical package. Pre, intra and post-operative variables are presented in terms of mean, standard deviation and range or percentage as appropriate.

Two-sample t-tests and Chi squared tests were used to compare continuous and non-continuous variables between the two peeling technique groups respectively. Statistical significance was considered with a p-value of 0.05 or less.

General regression analysis was then used to determine the effect of relevant demographic and intra-operative variables with the postoperative outcome of the total DONFL score. The regression analysis model included the following variables: age, sex, the occurrence of combined surgery, pick up point trauma, ILM peel technique and the type of gas used.

Results

There were 57 patients in total included: 41 patients in the forceps group and 16 in the scraper group. The groups were well matched for age, sex distribution and size and stage of the macular holes [table 1]. The surgical procedures were also well

matched for the addition of combined surgery and the extent of the peel performed although there was more C2F6 used in the scraper group. [Table 1]
 Petechial haemorrhages related to ILM separation from the underlying retina were observed in 27 [66%] patients in the forceps group and 12[75%] of the scraper group and the mean number and occurrence of retinal haemorrhages did not differ between the two groups. Similarly there was no significant difference in pick up point trauma between the groups [table 1].

Table 1: Summary of patient characteristics

	Forceps peel n=41	Diamond dusted membrane scraper peel n=16	p
Age (years) mean(SD)(Range)	71(7.1)(53-84)	73(5.5)(65-81)	0.29
Sex (female) N (%)	32 (78%)	13 (81%)	0.79
MLD (microns) Mean (SD)(range)	399(203)(125-850)	408(97.7)(253-624)	0.81
Gass Stage (%2,3,4)	52,33,15	43,43,14	0.71
Pre Op BCVA (Logmar) Mean(SD)(range)	0.97(0.26)(0.5-1.8)	0.92(0.18)(0.6-1.3)	0.43
Gas used SF6/C2F6 (%SF6, %C2F6)	27/14 (66%/34%)	5/11 (31%/69%)	0.037*
Combined phaco surgery (n, %)	29 (71%)	9 (56%)	0.29
Total number of superficial haemorrhages observed Mean(SD)(range)	1.8(2.4)(0-11)	3(2.4)(0-8)	0.10
Peel initiation point trauma (n for 0,1,2,3,4 grades)	30,5,3,3,0	7,4,3,2,0	0.21
Extent of peel (microns) Mean(SD)(Range)	16.4(11.2)(4.5-53.2)	18.6(5.0)(10.1-28.4)	0.299
Post op BCVA (Logmar) Mean(SD)(range)	0.38(0.28)(0-1)	0.43(0.14)(0.2-0.6)	0.34
Closure (n, %)	39 (95%)	16 (100%)	0.37
DONFL visible on Blue reflectance imaging (n, %)	36(88%)	16(100%)	0.14
Total DONFL score (n) Mean(SD)(range)	14.9(10.4)(0-33)	34.5(17.6)(13-82)	0.001*

Macular hole closure was achieved in 95% of the patients in the forceps group and 100% of the scraper group and there was no significant difference in visual acuity outcomes between the two groups.

There was some degree of a DONFL appearance detected on blue reflectance imaging in 88% of the forceps group and 100% of the scraper group. (Figure 4) One patient in the forceps group had a narrow retinal nerve fibre layer defect extending from the initial pick up point to the disc. There were none detected in the scraper group.

There were significantly more DONFL lesions detected in the scraper group.

Using general regression analysis the use of forceps for peeling was associated with a significantly decreased DONFL score. [p=0.001]. There were no other significant associations detected for the other included variables. [Table 2]

Table 2: General Regression analysis

Variable	Coefficient	P value
Age	-0.111	0.441
Sex	-0.431	0.693
Combined Surgery	-0.851	0.394
Pick up points	-1.034	0.258
Technique (forceps : DDMS)	-3.435	0.001*
Gas Type		
SF6	1.268	0.594
C2F6	4.445	0.073
C3F8	-1.997	0.673

DDMS Diamond dusted membrane scraper

The final 4 cases in the scraper group and 12 in the forceps group had TEM performed on the excised ILM. All 16 specimens had evidence of debris on the retinal side of the ILM. This was graded as small fragments only in 9 of the forceps group and none in the scraper group. Whole cells were present in 3 of the forceps and all 4 of the scraper group – these were confluent as a layer in small areas in 3 of the 4 patients in the scraper group but in only one of the forceps group.

[p=0.019]

The total extent of the retinal side covered by retina debris fragments ranged from 16-28% [Mean 22.3, SD5.7] in the scraper group and 21-36% [Mean 28.5, SD6] in the forceps group. [p=0.11]

Discussion

DONFL is the term that has been given to the characteristic appearance of the inner surface of the macula after ILM peeling.[2] DONFL is only generally visible from one month post-surgery and then increases in appearance up to 3-6 months post op.[7] It is more visible on blue light or red free imaging than colour photography but near universally present on SD OCT as apparent depressions in the nerve fibre layer which can extend into the inner plexiform layer.[7-10] It is thought to be related to Muller cell end plate trauma as the inner processes of these cells abut and are attached to

the ILM forming a layer between the nerve fibre layer and the ILM.[2,6] Trauma to these processes, which is known to occur during ILM peeling [11] could result in changes in the regular bundling of the nerve fibre layer as well as a volume reduction in the ganglion cell layer where the Muller cell bodies reside.[12] The nerve fibre layer is thought to be largely intact and true nerve fibre layer defects are rare but have been described at ILM pick up points with a forceps peeling technique.[6,12,13] To go along with this functional defects associated with DONFL have been described infrequently [4,5,14] although the true impact of DONFL is difficult to define and some authors have found functional defects including para-central scotomas and generalised depressions in retinal sensitivity associated with a DONFL.[7,15,16] Minimising its extent would be desirable especially as ILM peeling, having been shown to improve the success rate in macular hole surgery, is now routinely carried out.[1] There have been several papers characterising the extent of DONFL but there has been little study into what influences its occurrence and severity. We quantified the extent of the DONFL defects on SD OCT and found a significant difference between the forceps peeling group and the scraper group. Initially we attempted to grade the extent of DONFL from the BR images. We found however that image quality varied significantly which affected the ability to grade the images and furthermore the sensitivity of the blue reflectance images in detecting DONFL was less than with SD OCT. We therefore used a method to quantify DONFL based on counting depressions in the inner retina in each of the central 13 horizontal slices of the SDOCT scans. This technique correlated with the extent of DONFL visible on eyes with high quality BR images (data not shown) and had an acceptable level of repeatability. In future studies the use of en face OCT techniques may allow more precise quantification of the extent of DONFL.[6,9,10]

Two different surgeons were involved with the study each using their preferred technique. It should be noted that surgeons use DDMS in different ways. Many only use the scraper to initiate an ILM flap and then use forceps to complete the peel. The surgeon in this study used the scraper to initiate and complete the peel. Both surgeons used the same ILM stain namely Brilliant Blue G and used a protocol of staining for 5 seconds before washing out the stain. This is important as differing stains have been associated with possible variations in the plane of separation of the ILM from the retina with greater Muller cell remnants attached to the peeled ILM if no stain is used compared to ICG and ICG compared to BBG. [17,18,19,20]. If DONFL is related to Muller cell trauma then this change in cleavage plane with different dyes may alter the extent of DONFL observed and hence confound any analysis. The groups were well matched in terms of patient demographics, pre-operative characteristics and other aspects of the surgery although the scraper group had a higher use of C2F6 as opposed to SF6. We believe there are several possible reasons for the difference in the extent of DONFL observed between the techniques. Direct surgical trauma between the scraper and the bare NFL surface after the ILM has been peeled may explain the results [21]. However care was taken to avoid this and the absence of any observed nerve fibre layer defects tracking back to the disc on either red free imaging or SDOCT as well as the equivalent degree of retinal haemorrhages between the two groups would be inconsistent with this. It has also been shown that the scrapes made during peeling with a diamond dusted scraper do not penetrate the ILM itself. [22] The angle of peel with the scraper after initiation of

the peel and eversion of the flap will be close to 180 degrees as compared to approximately 140-160 degrees with forceps. Theoretical modelling of ERM peeling has suggested that the optimum angle of peel will vary according to the stiffness of the material being peeled and its attachment to its underlying tissue and it is possible that the more acute angle of peel with the scraper will alter the plane of cleavage from the retina. [23] A further possibility is the pressure applied to the retina during the scraping movements. Intraoperative OCTs have shown that the retina is compressed to a considerable extent during the scraping motion and this may alter the plane of cleavage of the ILM from the retina or cause more direct trauma to the Muller cells themselves. [24] It is known that the ILM does not abut directly on the nerve fibre layer but instead on an arborizing area of Muller cell processes.

Histologically we found that in 3 out of 4 cases in the scraper group cases examined with TEM, the ILM had large patches of retinal debris whereas this was not found in any of the forceps group. Similarly recognisable cellular fragments were found in all 4 scraper cases and only 3 of the 12 forceps cases. The total extent of the retinal surface of the ILM covered by retinal cellular debris however was similar between the groups perhaps suggesting that the plane of retinal separation of the ILM was only altered in places supporting the hypothesis that it is pressure applied during the scraper movement in localised areas that is responsible for the confluent and larger areas of retinal debris.

It is also possible that specific surgeon differences and experience resulted in the difference in the incidence of DONFL observed and retinal debris differences rather than the technique of peeling itself. We did not systematically record the time taken to complete the peeling procedures which could conceivably affect the results from light toxicity. We however did not observe any RPE or outer retinal changes typical of this on either OCT or autofluorescent imaging. We attempted to quantify surgical trauma by quantifying the number of observable petechial haemorrhages occurring as the ILM was separated from the retinal surface and also scoring any trauma at the ILM peel initiation point. Recently Diaz et al have highlighted that pick up point trauma can be related to inner retinal changes following ILM peeling.[25] There was no significant difference between the number of petechial haemorrhages or pick up point trauma between the groups and careful analysis of the location of DONFL lesions did not show any specific co-localisation with the pick-up points as previously highlighted. Furthermore there was only one patient with a classical retinal nerve fibre layer defect detected and that was in the forceps group. We are however unable to say whether some of the differences in DONFL between the groups could be related to surgeon factors and further study is needed into the effect of inter surgeon differences in the extent of DONFL. Certainly if surgeon factors are important then any future study of DONFL should be restricted to one surgeon using one technique.

In conclusion the appearance of DONFL has been widely described after ILM peeling but to date no studies have described any clear risk factors for its occurrence and severity. Here we describe a clear difference in the severity of DONFL on SD OCT

after MH surgery with ILM peeling by one surgeon using a forceps technique compared to another surgeon using a DDMS technique to initiate and complete the peel. We therefore advice against using a DDMS for ILM peeling in the way described.

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Legend for figures

Fig. 1

Composite image showing: a, pre-operative SDOCT of stage 2 335 micron hole without VMT. b, 3 month postoperative Blue reflectance image showing subtle DONFL appearance. c, Horizontal line scan through foveal centre. d, example of eccentric line scan.

Fig. 2

Higher magnification images of line scans in Figure 1

a Central line scan with edge of ILM marked- short arrow, DONFL depressions-long arrows.

b Eccentric line scan with same arrow coding

Fig. 3

Classification of retinal debris used

a Small less than 2 micron in diameter fragments

b Larger fragments of whole cells showing identifiable cell organelles

c Layers of cells with recognisable cellular structures

Fig.4

Representative examples of blue reflectance images after a DDMS peel (left) and forceps peel (right). Note marked DONFL appearance after the DDMS peel and more subtle appearance after the forceps peel.