Ophthalmologica

DOI: 10.1159/000509878
Received: 4/29/2020 6:22:45 PM
Accepted: 6/22/2020 12:16:04 PM
Published(online): 7/3/2020

The Effect of Internal Limiting Membrane Cleaning on Epiretinal Membrane Formation after Vitrectomy for Proliferative Diabetic Retinopathy
Mehta A. Rana-Rahman R. Klaassen I. Rees J. Steel D.H.

ISSN: 0030-3755 (Print), eISSN: 1423-0267 (Online)
https://www.karger.com/OPH
Ophthalmologica

Research Article

The Effect of Internal Limiting Membrane Cleaning on Epiretinal Membrane Formation after Vitrectomy for Proliferative Diabetic Retinopathy

Alexander Mehta[a], Romeela Rana-Rahman[b], Ingeborg Klaassen[c], Jon Rees[d], David H Steel[a,b]

[a] Newcastle University, Newcastle-upon-Tyne, UK

[b] Department of Ophthalmology, Sunderland Eye Infirmary, Sunderland, UK

[c] Ocular Angiogenesis Group, Amsterdam UMC, Amsterdam, The Netherlands

[d] Faculty of Health Sciences and Well Being, University of Sunderland, Sunderland, UK

Short Title: Internal Limiting Membrane Cleaning after Diabetic Vitrectomy

Corresponding Author:

Professor David Steel,

Department of Ophthalmology

Sunderland Eye Infirmary

Queen Alexandra Road

Sunderland, SR2 9HP, UK

Tel: +44191 565 6256

E-mail: david.steel@ncl.ac.uk

Number of Tables: 4

Number of Figures: 2

Word count: 4207

Keywords:

Internal limiting membrane, Epiretinal membrane, Diabetic vitrectomy, Proliferative diabetic retinopathy, Vitreous proteomics

Abstract

Purpose

We hypothesised that cleaning the internal limiting membrane (ILM) with a flexible nitinol loop following diabetic vitrectomy without peeling may reduce the common occurrence of postoperative epiretinal membrane (ERM) formation.

Methods

Consecutive patients undergoing vitrectomy for proliferative diabetic retinopathy by one surgeon from 2015-2019 were studied and divided into two cohorts: the control group underwent standard surgery; the ILM-Clean group underwent additional cleaning of the macular retina using a flexible nitinol loop post-vitrectomy. Masked comparison of ERM on optical coherence tomography was performed at 3 months and visual acuity (VA) was measured until 12 months postoperatively.

Results

Baseline demographics, clinical features and protein levels were similar between cohorts. The ILM-Clean group (n=56) had fewer clinically significant ERM compared to the control group (n=50) (4%vs.20%;p=0.01) and a significantly lower proportion of the ILM-Clean group required revision surgery (2%vs.14%;p=0.02). VA in the ILM-Clean group was significantly better than the control group at 3 months (0.35vs.0.50logMAR;p=0.02) but not at 12 months (0.34vs.0.43logMAR;p=0.17).

Conclusion

ILM cleaning with a flexible nitinol loop following diabetic vitrectomy resulted in significant reduction in ERM formation and reduced necessity for revision surgery. There was significant improvement in VA at 3 months but not over longer follow-up.

Introduction

Epiretinal membrane (ERM) formation is a well-known association of proliferative diabetic retinopathy (PDR) and can lead to a variety of tractional consequences with reduced vision. Its occurrence relates to a range of effects secondary to raised glucose levels including changes in vitreous structure, an abnormal adhesion between the vitreous cortex and internal limiting membrane (ILM) of the retina, and a pro-proliferative cytokine mix in the vitreous cavity [1,2]. Vitrectomy surgery is a proven and successful treatment for the complications of PDR where the surgical aims of treatment include removing vitreous haemorrhage and relieving vitreoretinal traction [3]. However, ERM is observed postoperatively after diabetic vitrectomy in 20–50% of cases, is associated with macular thickening, can affect visual outcome and has been reported to require revision surgery in 7–22% of cases [4–8]. The exact cause of this is uncertain although surgical and proteomic risk factors have been described [9,10]. ILM peeling has been proposed as an effective strategy to reduce the occurrence of significant ERM after surgery [11,12] but its use has been questioned because of its potential to cause harm [13]. We carried out a prospective study to assess the effect of ILM cleaning, without ILM peeling, using a flexible nitinol loop on the occurrence of ERM after surgery for PDR. Vitreous proteomic assays were carried out on a range of relevant proteins in all cases and peeled ILM was examined by transmission electron microscopy after ILM cleaning in a subset of cases.

Materials and Methods

Data from consecutive patients undergoing vitrectomy for PDR by one surgeon over a 42-month period from 2015 to 2019 was retrospectively analysed. The cohort was divided into two cohorts within recruitment occurring in both cohorts over 21 months. In the first cohort, which in henceforth referred to as the control cohort, the ILM was not cleaned, and in the second cohort it was cleaned with a Finesse flex loop (Alcon Grieshaber, Schaffhausen, Switzerland), otherwise surgery was identical.

Indications for surgery included vitreous haemorrhage, tractional retinal detachment with and without a rhegmatogenous component. Patients were excluded if there had been previous vitrectomy, there was a prior history of diabetic macular oedema (DMO) requiring treatment with retinal photocoagulation or intravitreal therapies within the preceding 2 years, silicone oil

tamponade was required, there was less than 3 months follow up or postoperative spectral domain optical coherence tomography (SDOCT) was not performed 3–4 months postoperatively.

All surgeries were carried out using the Alcon Constellation 25g Ultravit system (Alcon, Fort Worth, Texas, USA) with wide angle viewing using a standardised technique. After core vitrectomy, delamination and removal of all posterior hyaloid face and fibrovascular membranes was carried out. This was done primarily with the vitreous cutter alone, and intravitreal scissors were used only if necessary, as previously described [14,15]. Careful inspection to detect the presence of vitreoschisis was carried out and staining of residual vitreous gel using diluted triamcinolone (TMC) was used in all cases. Any vitreous remnants and epiretinal membrane detected was peeled using the vitreous cutter and forceps as necessary.

In a second cohort of patients, after staining with TMC, and after peeling of membranes as above, a Finesse flex loop was used to gently brush the retina, to remove any residual epiretinal tissue present, concentrating on the macula area within the vascular arcades. Particular care was taken to brush all areas extending for a disc diameter in radius around the foveal centre in a systematic way using radial and concentric brush directions, dictated by surgical ease. The procedure was performed regardless of the presence of discernible vitreous remnants identified by TMC, however the presence of patches of discernible vitreous remnants with strands or sheets of membrane after TMC application was recorded (see supplementary video).

Endolaser retinal photocoagulation was carried out to complete any deficiencies in previous laser. Retinal breaks were treated with argon laser retinopexy. Sulphur hexafluoride gas or air were used as postoperative tamponade where needed. Preoperative anti-VEGF therapy was used in selected patients relating to the activity and extent of the neovascularization. Combined phacovitrectomy was carried out in cases with visually significant cataract obscuring the operative view.

The primary outcome was the presence and severity of ERM on SDOCT at 3–4 months postoperatively. Patients underwent SDOCT (30 by 30° horizontal grid protocol with 60-micron line spacing) using a Spectralis HRA®+ SDOCT (Heidelberg Engineering, Heidelberg, Germany). The SDOCTs were graded by two independent observers masked to the intervention group for the presence of ERM, which was defined as a hyper-reflective inner retinal band. The presence of any foveal (within central $1mm^2$) and eccentric (outside central $1mm^2$ but within a standard 6mm Early Treatment Diabetic Retinopathy Study (ETDRS) circle) ERM was recorded and graded as absent (score 0), present (score 1), or present and associated with retinal plication and/or peg-like attachments (score 2) (as shown in Fig. 1) and making a total maximum score of 4, as reported previously [16]. Central macular thickness (CMT: average retinal thickness over the central 1mm diameter subfield,

and macular volume were recorded, as was the presence of intraretinal cysts. ERM was designated as clinically significant if the following criteria were met: 1) ERM involving the foveal centre associated with a change in foveal architecture and plication. 2) Eccentric ERM if associated with retinal plication and in continuity with an area of central retinal thickening.

A variety of pre-, intra- and postoperative characteristics of the patients were recorded, including age, gender, type and duration of diabetes and glycosylated haemoglobin (HbA1c) level at the time of vitrectomy. The amount of preoperative panretinal photocoagulation (PRP) was graded as equal to, or more than, ETDRS full scatter, less than standard ETDRS full scatter or no preoperative PRP [17]. The extent of any retinal haemorrhage was recorded as more, less or the same as the standard 2a photograph used in the ETDRS study and the presence of any preretinal haemorrhage recorded [18] and graded as absent, extramacular or premacular. The extent of the vitreoretinal adhesion areas was estimated based on disc areas. The position of neovascularisation was recorded as none, disc attachment only, posterior pole attachment only or 1–4 quadrants of anterior vitreoretinal attachment. Tractional retinal detachment was recorded as absent, eccentric to macular or macular involving. The number of applications of intraoperative laser was recorded. Patients were followed up at approximately 3, 6 and 12months postoperatively as per routine care. The number, timing and indications for repeat surgery were recorded. Best corrected visual acuity (BCVA) using an ETDRS letter chart was recorded at baseline and 3, 6 and 12 months postoperatively, and converted to logMAR for analysis. LogMAR values corresponding to count fingers (CF), hand movements (HM), perception of light (PL) were substituted with 1.98, 2.28 and 2.70 respectively.

Proteomics

In all patients, a sample of undiluted vitreous (0.5–1.0ml) was aspirated with high cut rate into a 2ml syringe prior to initiation of full vitrectomy, and then frozen at -80°C. A range of cytokines [10,19] and growth factors based on previous work (namely vascular endothelial growth factor (VEGF) A, placental growth factor, connective tissue growth factor, monocyte chemoattractant protein 1 (MCP-1), interleukin 6 (IL-6), interleukin 8 (IL-8), angiopoietin 2, intercellular adhesion molecule 1, matrix metalloproteinase 2, matrix metalloproteinase 9, tissue necrosis factor α) were quantified by using a

customisable array-based multiplex immunoassay (Human Quantibody array, RayBiotech, Norcross, GA, USA) as previously described [19].

Electron Microscopy

After completion of recruitment to the main cohort, in four separate patients undergoing vitrectomy for PDR, and after ILM cleaning with the Finesse flex loop as per our protocol described above, the ILM was peeled and then retrieved for transmission electron microscopy.

Samples were fixed in 2% glutaraldehyde in 0.1M sodium cacodylate buffer and processed as previously described [20]. Detailed examination of the tissue was performed to determine the occurrence of any cellular debris on the vitreous surface of the ILM, and any detectable evidence of loop related surface marks such as grooving, or ILM disruption. The extent of any vitreous side epiretinal membrane was graded in extent as previously described [20].

Informed consent for the collection of the vitreous and ILM specimens was obtained from the subjects after explanation of the nature of the study. These were carried out in accordance with the ethical standards of an institutional research committee (National Health Service South East Coast–Surrey Research Ethics Committee – reference 12/LO/0130) and with the 1964 Declaration of Helsinki and its later amendments. Use of the retrospectively collected clinical data was classed as service evaluation under UK guidelines and as such did not require separate ethical review.

Statistical analysis

Descriptive and statistical analysis was performed using SPSS version 25. Patients demographic characteristics, pre- and post-operative variables are presented in terms of mean, standard deviation and range, median, interquartile range or percentage as appropriate. Two-sample unpaired t-tests were used to compare continuous variables or the Mann-Whitney test as appropriate. Associations between non-continuous variables were analysed using the chi-square test and Fisher's exact probability. Analysis of covariance was used to compare the improvement in vision postoperatively at 3-months between the two groups taking into account the preoperative vision as a covariate. Logistic regression was performed to assess factors associated with an ERM score of ≥1 postoperatively at 3 months using the forward method with a significant reduction in log likelihood

being used to retain factors in the model. Statistical significance was considered with a p-value of 0.05 or less.

Results

During the study period 157 primary vitrectomies for the complication of PDR were carried out. Silicone oil was used in 7 cases, SDOCT were unavailable for 13 eyes (9 in the control group and 4 in the ILM clean group), 21 cases had a prior history of treatment of DMO and 10 eyes were fellow eyes. 106 eyes of 106 patients were thus studied, with 50 in the control group and 56 in the ILM clean group. The mean age was 52 years (standard deviation 15, range 22 to 82) and 55 (52%) were male. The groups were well matched by their clinical features at baseline and as observed during surgery (Table 1) and vitreous protein levels (Table 2). All but two patients were white British in ethnicity. Follow up was completed in 44/50 (88%) and 38/50 (76%) of the control group and 49/56 (86%) and 44/56 (79%) in the ILM clean group at 6 and 12 months respectively.

In the ILM clean group there were patches of discernible vitreous remnants present as manifest by TMC staining during surgery in 17 of the 56 (30%) eyes. During the ILM cleaning, an unintentional ILM tear was created in 2 eyes, both at approximately 1500 microns from the foveal centre. In these cases, the torn ILM was locally removed only without peeling the ILM at the fovea.

The kappa coefficient for interrater reliability in grading ERM presence on SDOCT was 0.80 (95% confidence intervals: 0.66-0.94). There was a significant reduction in the severity of the foveal, extrafoveal and clinically significant ERM between the ILM clean and control groups. Similarly, the mean CMT and macular volume was reduced in the ILM clean group. There was a significant difference in postoperative visual acuity (VA) between the two groups at 3 months (mean logMAR 0.35 vs. 0.50, p=0.02) (Table 3). When the preoperative VA was included as a covariate the significant difference in postoperative vision persisted (p=0.008). The significant difference in postoperative VA persisted at 6 months (mean logMAR 0.33 (SD 0.27) vs. 0.46 (SD 0.40), p=0.05) but was non-significant at 12 months (mean logMAR 0.34 (SD 0.29) vs. 0.43 (SD 0.38), p=0.17).

There was a reduction in ERM in both patients with vitreous haemorrhage and macular tractional retinal detachment, but the effect was only significant in the vitreous haemorrhage group. There was no reduction in the occurrence of ERM in the 5 patients with combined tractional rhegmatogenous retinal detachment (CTRD) who all had ERM postoperatively (Table 4).

By the end of 12 months, follow up revision surgery had been required in 7 of the control patients – 5 for tractional ERM and 2 for postoperative vitreous cavity haemorrhage (POVCH). In the ILM clean cohort revision vitrectomy had been required in 1 for POVCH (p=0.02). In the control group, 3

patients were treated with postoperative anti-VEGFs and 3 Iluvien® for DMO. In the ILM clean group, 3 patients had postoperative anti-VEGFs and 1 Iluvien® for DMO. In both groups, 8 patients underwent cataract surgery in the first 12 months following initial vitrectomy (p=0.806).

Prediction of ERM by baseline features

Using the overall study population (n=106), 64 (60%) of these patients had at least some ERM identified either eccentrically or foveally (defined as a total score of 1 or more out of a maximum of 4) in the initial 3 months. The features identified by logistic regression as being predictive of ERM with a score ≥1 was the control group vs. ILM clean group (odds ratio 3.652 (1.476–9.036), p=0.005), number of intraoperative laser applications (odds ratio 0.999 (0.998–1.000), p=0.019) and the indication of vitrectomy being a CTRD (odds ratio 3.552 (1.208-10.440), p=0.021).

Transmission electron microscopy

In the ILM specimens examined by electron microscopy after ILM cleaning, the ILM surface was generally devoid of vitreous side epiretinal membrane, but occasional foci of cells and collagen were seen. We found no signs of ILM disruption but did find occasional and rare areas of focal thinning which may have been related to the ILM cleaning (as per Fig. 2).

Discussion

We found a significant reduction in ERM 3 months after vitrectomy for the complications of PDR in the patients who underwent ILM cleaning using the Finesse flex loop. The groups were well matched both by clinical features and vitreous protein levels. The reduction in ERM was associated with a reduction in retinal thickness compared to the comparator cohort without ILM cleaning, with a concomitant significant improvement in VA at 3 months. This difference was non-significant by 12 months but there was a significant reduction in the requirement for revision vitrectomy in the ILM cleaning group.

The use of TMC to detect residual vitreous attachment in vitrectomy for PDR has been described previously [21–23], but we are not aware of a study systematically examining the effect of ILM cleaning with a membrane scraping device in patients with PDR. Vitreoschisis and residual vitreous adhesion to the ILM surface are well known features of PDR and its associated vitreopathy [1,24–26]. Removal of this material can improve surgical results by reducing rebleeding and recurrent traction, and the particulate staining achieved by dilute TMC can aid in its identification. It can however be difficult to peel conventionally being tenuous with a tendency to shred with forceps. We chose to use the Alcon Grieshaber Finesse flex loop. This is a flexible nitinol loop that in its fully extended position is less rigid that the more established diamond dusted membrane scraper (DDMS). The flex loop has

a series of fine 15micron high tines which can be used to remove the remaining layer of posterior cortical vitreous. The tines are triangular shaped and approximately half the height of diamonds on a DDMS. Similarly, the loop has approximately half the rigidity of a DDMS reducing the chances of ILM trauma, abrasion and unintentional tearing, which only occurred in 2 cases. Interestingly examination of the 4 ILM specimens we studied after ILM peeling showed very few signs of inner surface trauma or scratches, as has been observed with the DDMS [27,28]. Other authors have used alternative instruments to remove vitreous remnants including polyvinyl alcohol sponges in the case of rhegmatogenous retinal detachments, which the authors referred to as vitreous wiping [29]. It is likely both instruments have the same effect. We cleaned the ILM in a systematic way even when there was no discernible adherent vitreous on the fovea as an adherent layer of epiretinal tissue has been shown to be highly prevalent in diabetic eyes with advanced retinopathy [30]. We found that in 30% of eyes there were macroscopic patches of vitreous remnants identified by TMC at the fovea.

The presence of ERM after surgery for PDR has been widely reported with its prevalence ranging from 20–53% [31–33] relating to case mix and the methods of detection. We found that over 70% of our initial control group had some evidence of ERM on SDOCT at 3 months after surgery, although this was only thought to be of clinical significance in 20%. Revision surgery was carried out in 5 (10%) of these cases, although it has been reported to be a common cause for revision surgery with or without associated retinal detachment [8] in some series. Risk factors for the occurrence of significant ERM after surgery have been reported as including the activity of the retinopathy, the extent of fibrovascular proliferation, the occurrence of postoperative vitreous cavity haemorrhage and residual fibrovascular stumps after surgery [9]. It has been noted that IL-6, IL-8 and MCP-1 are upregulated in vitreous samples with ERM recurrence undergoing revision surgery relative to the original surgery [10]. It has been postulated that its occurrence is related to the pathology of diabetic vitreopathy with residual epiretinal tissue acting as both a source and scaffold for recurrent ERM, with inflammatory and pro fibrotic mediators associated with both the PDR and the surgery itself stimulating proliferation and contracture of epiretinal remnants [9]. Pre-existing and surgically induced retinal holes may also contribute to the process by adding to the complexity of dissection and leaving residual epiretinal membranes, tissue trauma from hole creation and laser, and retinal pigment epithelium cell migration. In keeping with this, we found that the amount of intraoperative laser applications and the presence of a CTRD were predictive for the risk of clinically significant ERM occurring after surgery. Indeed, of the 5 eyes with CTRDs included in the total cohort, 4 developed clinically relevant ERMs after surgery, including one in the ILM clean group, although this case did not undergo revision surgery as it was felt to be too mild to warrant repeat intervention. We measured the levels of several vitreous proteins, which have previously been found to be abnormal in eyes with

PDR in previous studies at the time of vitrectomy surgery [19]. We did not find any of the proteins were predictive of postoperative ERM. Importantly however there was no significant difference in the levels between the two cohorts reinforcing their matching.

Other authors have proposed ILM peeling as a technique to reduce ERM formation in vitrectomy for PDR [11,12] with a reduction in the occurrence of ERM from 38–49% to 0–21%. There have been reports of reduced vision after ILM peeling in advanced diabetic retinopathy perhaps relating to the greater adherence of ILM to its underlying Müller cell endplates with greater resultant trauma in diabetic eyes [13,34,35]. Despite reducing macular thickness, ILM peeling did not improve VA significantly at 3 months in either of the two cited studies and also has not been shown to improve visual results in patients undergoing vitrectomy for diabetic macular oedema [36]. We therefore wished to assess whether ILM cleaning could give comparable results to ILM peeling without the associated risks and perhaps improved vision. ILM cleaning significantly reduced the occurrence and severity of ERM, including clinically significant ERM from 10 (20%) cases to 2 (4%) and no cases required revision surgery for ERM in the ILM clean cohort, compared to 5 in the control group. The central macular thickness was reduced as was the case after ILM peeling, and VA was improved at 3 months but not after 12 months when revision surgery had been completed and several patients had undergone treatment for macular oedema. The apparent improvement by 12 months in VA in the control group (3-month mean: 0.50 logMAR; 12-month mean: 0.43 logMAR) compared to the stability of the ILM-Clean group (3-month mean: 0.35 logMAR; 12-month mean: 0.34 logMAR) was due to the increased proportion of control group patients treated with revision surgery (14% vs. 2%), intravitreal medication (12% vs. 7%) or cataract surgery (16% vs. 14%).

ILM cleaning did not eliminate all ERM but there were only 4 (7%) cases with ERM involving the central 1mm in the ILM clean group compared to 9 (18%) in the control group. Similarly, Michalewska et al found that 21% of patients that had ILM peeling had ERM outside the area of the ILM peel [12]. We also observed that on the four ILMs examined with TCM after cleaning, there were small remnants of ERM still persistent albeit sparse. It would appear that it is difficult to completely clean the ILM of all epiretinal tissue. It is also worth noting that postoperative ERM is more common in CTRD cases perhaps relating to cellular migration through the retinal breaks. Similarly, the reduction in ERM in cases with macular tractional rhegmatogenous retinal detachments was non-significant perhaps relating to greater Müller cell activation.

The study has several limitations. It was not randomised but the groups were well matched for baseline clinical features including vitreous attachment, extent of intraoperative laser and vitreous cytokine levels known to be related to preretinal fibrosis. We did not have preoperative SDOCT scans

as the majority of the patients had vitreous haemorrhage at baseline so could not exclude a difference in macular thickening at baseline between the groups. In should be noted however that we excluded patients with known pre-existing maculopathy to reduce this risk. We excluded patients requiring silicone oil insertion and so cannot extrapolate our results to them. We only recorded ERM at one-time point postoperatively and results may have differed at other time points. ERM after vitrectomy has been shown to vary with the time postoperatively [32], but all the patients who required vitrectomy for ERM in the 12 months follow up had clinically significant ERM at the 3-month time point. Late ERM formation significant enough to require vitrectomy would appear to be rare. The population studied was overwhelmingly white Caucasian and results may differ in other racial groups. We did not have details of postoperative glucose control but preoperatively there was no significant difference in glycosylated haemoglobin preoperatively between the groups.

Conclusion

In conclusion, we report a significant reduction in the prevalence of ERM after vitrectomy for PDR by using a technique of ILM cleaning with a flexible nitinol loop. Repeat surgery for tractional maculopathy within 12 months postoperatively was reduced by means of the technique, and there was a significant improvement in postoperative VA at 3 and 6 months postoperatively. Patients with CTRDs had a higher incidence of postoperative ERM which was not prevented by ILM cleaning suggesting that other techniques should be considered in this group of patients in particular. Further evaluation to confirm these results in randomised studies is needed.

Statements

Acknowledgement (optional)

Nil

Statement of Ethics

Informed consent for the collection of the vitreous and ILM specimens was obtained from the subjects after explanation of the nature of the study. These were carried out in accordance with the ethical standards of an institutional research committee (National Health Service South East Coast–Surrey Research Ethics Committee – reference 12/LO/0130) and with the 1964 Declaration of Helsinki and its later amendments. Use of the retrospectively collected clinical data was classed as service evaluation under UK guidelines and as such did not require separate ethical review.

Conflict of Interest

D Steel reports payments for consultancy from Alcon, Roche and Gyroscope as well as grant support from Alcon for projects unrelated to the reported work. The other authors have no conflicts of interest to declare.

Funding Sources

This study was partially funded by Bayer plc (VP01). The grant supported the proteomic analysis.

Author Contributions

A Mehta drafted the initial manuscript and assisted with the data analysis, revised and approved the final version and prepared the manuscript for submission. R Rana-Rahman collected the data and approved the final manuscript. I Klaassen carried out the proteomic analysis and approved the final manuscript. J Rees carried out the data analysis and approved the final manuscript. D Steel conceived the idea, carried out the surgeries, drafted the manuscript and approved the final version.

References [Numerical]

1. Sebag J. Diabetic Vitreopathy (Guest Editorial). Ophthalmology. 1996 Feb;103(2):205–6.

2. Costa Ede P, Rodrigues EB, Farah ME, Sebag J, Meyer CH. Novel vitreous modulators for pharmacologic vitreolysis in the treatment of diabetic retinopathy. Curr Pharm Biotechnol. 2011 Mar;12(3):410–22.

3. Jackson TL, Johnston RL, Donachie PH, Williamson TH, Sparrow JM, Steel DH. The Royal College of Ophthalmologists' National Ophthalmology Database Study of Vitreoretinal Surgery: Report 6, Diabetic Vitrectomy. JAMA Ophthalmol. 2016 Jan;134(1):79–85.

4. Blankenship GW, Machemer R. Long-term Diabetic Vitrectomy Results. Report of 10 year follow-up. Ophthalmology. 1985 Apr;92(4):503–6.

5. Schiff WM, Barile GR, Hwang JC, Tseng JJ, Cekic O, Del Priore LV et al. Diabetic vitrectomy: influence of lens status upon anatomic and visual outcomes. Ophthalmology. 2007 Mar;114(3):544–50.

6. Oshima Y, Shima C, Wakabayashi T, Kusaka S, Shiraga F, Ohji M et al. Microincision vitrectomy surgery and intravitreal bevacizumab as a surgical adjunct to treat diabetic traction retinal detachment. Ophthalmology. 2009 May;116(5):927–38.

7. Yorston D, Wickham L, Benson S, Bunce S, Sheard R, Charteris D. Predictive clinical features and outcomes of vitrectomy for proliferative diabetic retinopathy. Br J Ophthalmol. 2008 Mar;92(3):365–68.

8. Gupta B, Sivaprasad S, Wong R, Laidlaw A, Jackson TL, McHugh D et al. Visual and anatomical outcomes following vitrectomy for complications of diabetic retinopathy: the DRIVE UK study. Eye. 2012 Apr;26(4):510–6.

9. Hsu YR, Yang CM, Yeh PT. Clinical and histological features of epiretinal membrane after diabetic vitrectomy. Graefes Arch Clin Exp Ophthalmol. 2014 Mar;252(3):401–10.

10. Yoshida S, Kobayashi Y, Nakao S, Sassa Y, Hisatomi T, Ikeda Y et al. Differential association of elevated inflammatory cytokines with postoperative fibrous proliferation and neovascularization after unsuccessful vitrectomy in eyes with proliferative diabetic retinopathy. Clinical Ophthalmol. 2017 Sep;11(1):1697–1705.

11. Chang PY, Yang CM, Yang CH, Chen MS, Wang JY. Pars plana vitrectomy for diabetic fibrovascular proliferation with and without internal limiting membrane peeling. Eye. 2008 Dec;23(4):960–5.

12. Michalewska Z, Bednarski M, Michalewski J, Jerzy N. The role of ILM peeling in vitreous surgery for proliferative diabetic retinopathy complications. Ophthalmic Surg Lasers Imaging Retina. 2013 May;44(3):238–42.

13. Romano MR, Romano V, Vallejo-Garcia JL, Vinciguerra R, Romano M, Cereda M et al. Macular hypotrophy after internal limiting membrane removal for diabetic macular edema. Retina. 2014 Jun;34(6):1182–9.

14. Guthrie G, Magill H, Steel DH. 23-gauge versus 25-gauge vitrectomy for proliferative diabetic retinopathy: a comparison of surgical outcomes. Ophthalmologica. 2015 Feb;233(2):104–11.

15. Steel DH, Connor A, Habib MS, Owen R. Entry site treatment to prevent late recurrent postoperative vitreous cavity haemorrhage after vitrectomy for proliferative diabetic retinopathy. Br J Ophthalmol. 2009 Dec;94(9):1219–25.

16. Wong Y, Steel DHW, Habib MS, Stubbing-Moore A, Bajwa D, Avery PJ et al. Vitreoretinal interface abnormalities in patients treated with ranibizumab for diabetic macular oedema. Graefes Arch Clin Exp Ophthalmol. 2017 Apr;255(4):733–42.

17. Early Treatment Diabetic Retinopathy Study Research Group. Treatment techniques and clinical guidelines for photocoagulation of diabetic macular edema. Early Treatment Diabetic Retinopathy Study Report Number 2. Ophthalmology. 1987 Jul;94(7):761–74.

18. Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs–an extension of the modified Airlie House classification. ETDRS report number 10. Ophthalmology. 1991 May;98(5 Suppl):786–806.

19. Klaassen I, de Vries EW, Vogels IMC, van Kampen AHC, Bosscha MI, Steel DHW et al. Identification of proteins associated with clinical and pathological features of proliferative diabetic retinopathy in vitreous and fibrovascular membranes. PLoS One. DOI: 10.1371/journal.pone.0187304.

20. Steel DH, Dinah C, Madi HA, White K, Rees J. The staining pattern of brilliant blue G during macular hole surgery: a clinicopathologic study. Invest Ophthalmol Vis Sci. 2014 Aug;55(9):5924–31.

21. Matsumoto H, Yamanaka I, Hisatomi T, Enaida H, Ueno A, Hata Y et al. Triamcinolone acetonide-assisted pars plana vitrectomy improves residual posterior vitreous hyaloid removal: ultrastructural analysis of the inner limiting membrane. Retina. 2007 Feb;27(2):174–9.

22. Enaida H, Hata Y, Ueno A, Nakamura T, Hisatomi T, Miyazaki M et al. Possible benefits of triamcinolone-assisted pars plana vitrectomy for retinal diseases. Retina. 2003 Dec;23(6):764–70.

23. Sakamoto T, Miyazaki M, Hisatomi T, Nakamura T, Ueno A, Itaya K et al. Triamcinolone-assisted pars plana vitrectomy improves the surgical procedures and decreases the postoperative blood-ocular barrier breakdown. Graefes Arch Clin Exp Ophthalmol. 2002 Jun;240(6):423–9.

24. Sebag J. Vitreoschisis. Graefes Arch Clin Exp Ophthalmol. 2008 Mar;246(3):329–32.

25. Chu TG, Lopez PF, Cano MR, Freeman WR, Lean JS, Liggett PE et al. Posterior vitreoschisis: An echographic finding in proliferative diabetic retinopathy. Ophthalmology. 1996 Feb;103(2):315–22.

26. Schwartz SD, Alexander R, Hiscott P, Gregor ZJ. Recognition of vitreoschisis in proliferative diabetic retinopathy: A useful landmark in vitrectomy for diabetic traction retinal detachment. Ophthalmology. 1996 Feb;103(2):323–8.

27. Hirakata A, Inoue M, Oshitari K, Okada AA, Nagamoto T, Tano Y. Histopathological examination of internal limiting membrane surface after scraping with diamond-dusted membrane scraper. Acta Ophthalmol. 2010 Nov;88(7):e293–4.

28. Mahajan VB, Chin EK, Tarantola RM, Almeida DR, Somani R, Boldt HC et al. Macular Hole Closure With Internal Limiting Membrane Abrasion Technique. JAMA Ophthalmol. 2015 Jun;133(6):635–41.

29. van Overdam KA, van Etten PG, van Meurs JC, Manning SS. Vitreous Wiping, a new technique for removal of vitreous cortex remnants during vitrectomy. Acta Ophthalmol. 2019 Aug;97(5):e747–e752.

30. Gandorfer A, Rohleder M, Kampik A. Epiretinal pathology of vitreomacular traction syndrome. Br J Ophthalmol. 2002 Aug;86(8):902–9.

31. Messmer E, Bornfeld N, Oehlschläger, Heinrich T, Foerster MH, Wessing A. Epiretinal membrane formation after pars plana vitrectomy in proliferative diabetic retinopathy. Klin Monbl Augenheilkd. 1992 Apr;200(4):267–72.

32. Im JC, Kim JH, Park DH, Shin JP. Structural Changes of the Macula on Optical Coherence Tomography after Vitrectomy for Proliferative Diabetic Retinopathy. Ophthalmologica. 2017 Oct;238(4):186–95.

33. Yang CM, Yeh PT, Cheng SF, Yang CH, Chen MS. Macular appearance after diabetic vitrectomy for fibrovascular proliferation: an optical coherence tomography study. Acta Ophthalmol. 2010 Mar;88(2):193–8.

34. Kumagai K, Hangai M, Ogino N, Larson E. Effect of internal limiting membrane peeling on long-term visual outcomes for diabetic macular edema. Retina. 2015 Jul;35(7):1422–8.

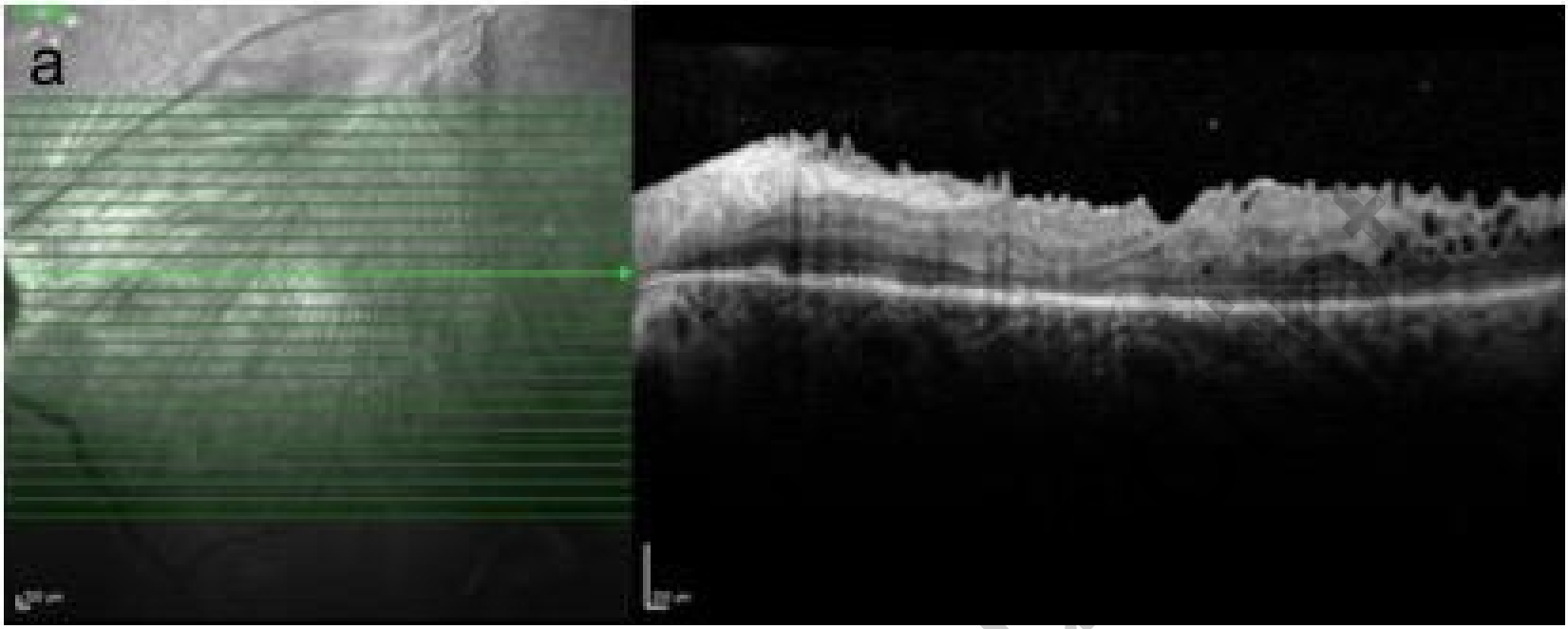
35. Yanyali A, Horozoglu F, Celik E, Nohutcu AF. Long-term outcomes of pars plana vitrectomy with internal limiting membrane removal in diabetic macular edema. Retina. 2007 Jun;27(5):557–66.

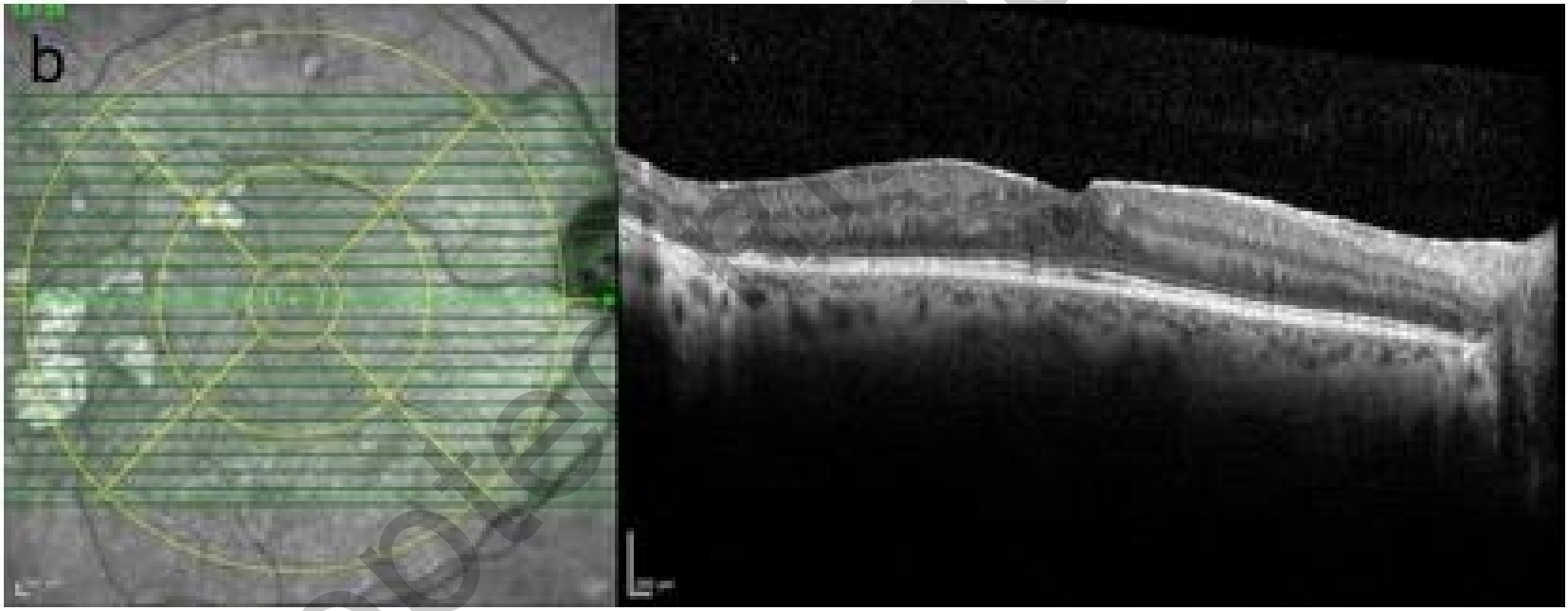
36. Rinaldi M, dell'Omo R, Morescalchi F, Semeraro F, Gambicorti E, Cacciatore F et al. ILM peeling in nontractional diabetic macular edema: review and metanalysis. Int Ophthalmol. 2018 Dec;38(6):2709–14.

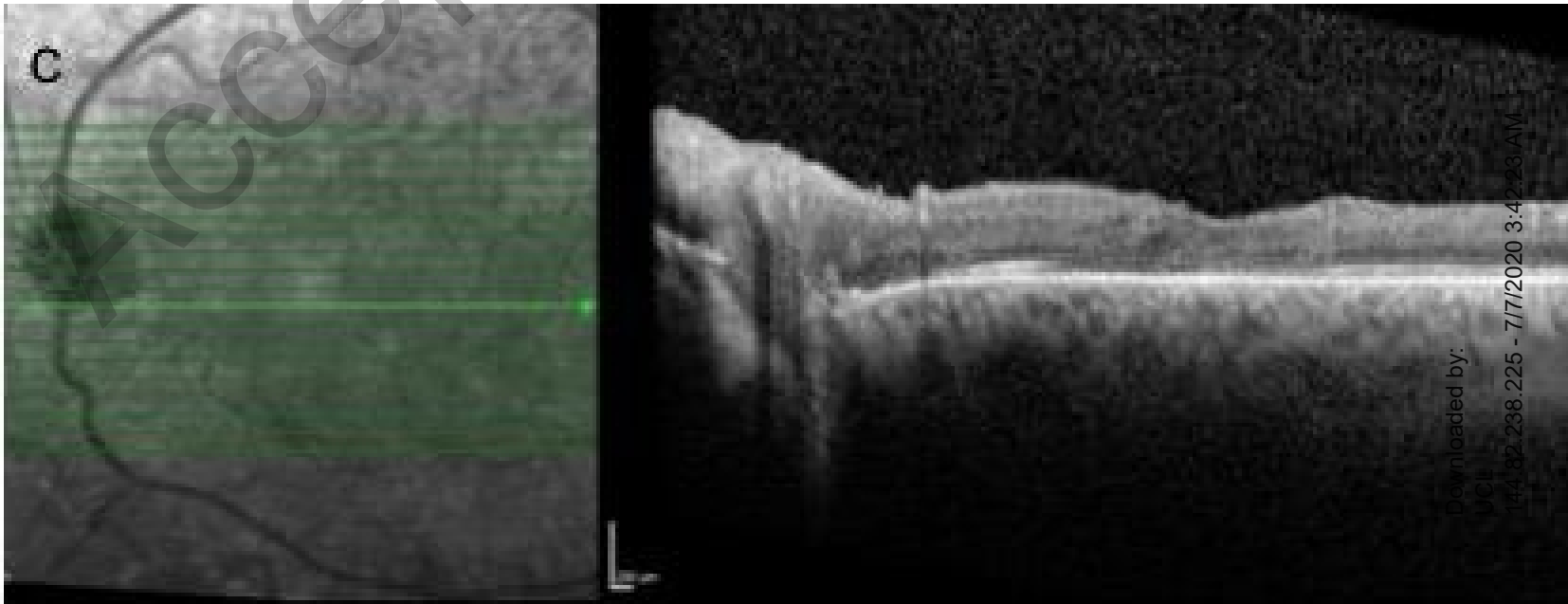
Figure Legends

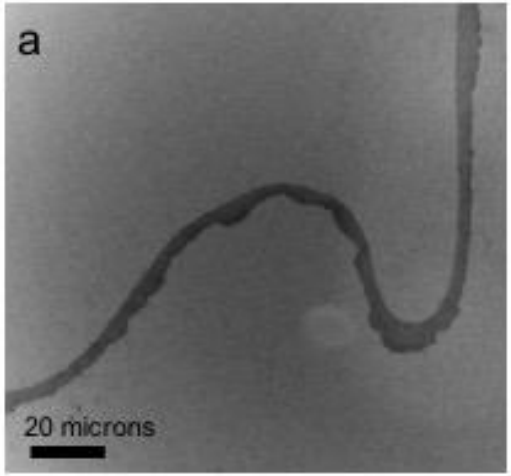
Fig. 1. Grading of ERM on SDOCTs at 3 months postoperatively. Panel 'a' illustrates an ERM with plication and involvement of the central fovea. Panel 'b' shows a linear ERM with foveal involvement. Note in this image an ETDRS grid has been overlaid on the infrared image to allow accurate detection of the extent of the ERM. Panel 'c' shows an eye with no discernible ERM.

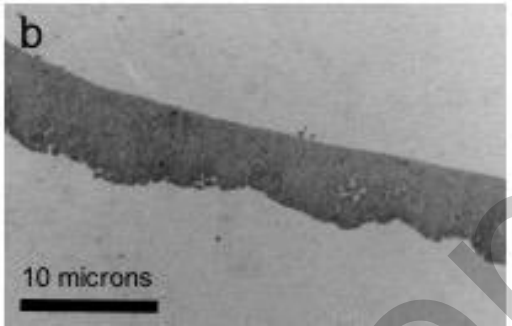
Fig. 2. Transmission electron microscopy images of peeled ILM after ILM cleaning. Panel 'a' shows a segment of ILM with clean and smooth vitreous side of the ILM and irregular retinal side. Panel 'b' is a higher power view of 'a'. There were infrequent areas of ILM with focal irregularities in the vitreous side (arrows) (Panel 'c'). There were also occasional areas of cellular remnants (arrow) (Panel 'd').

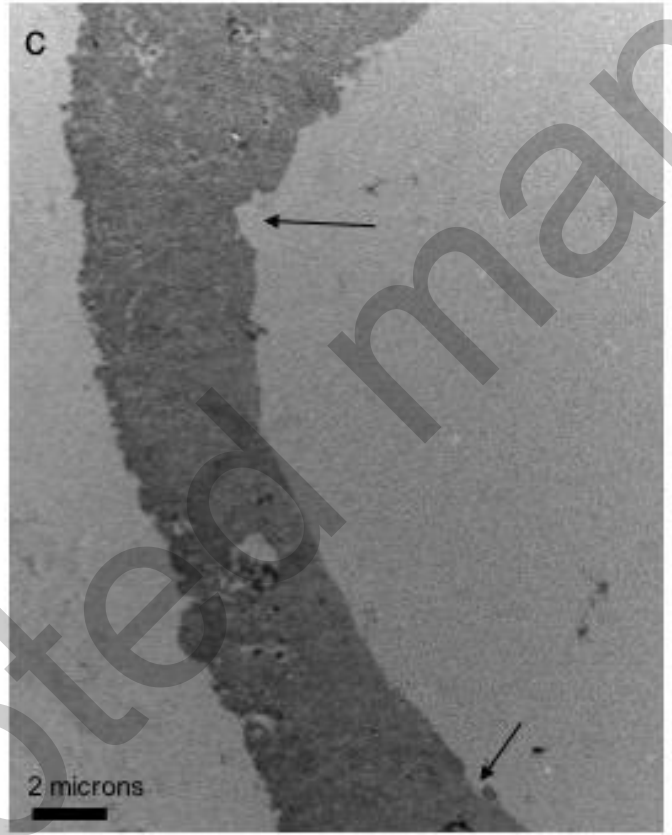












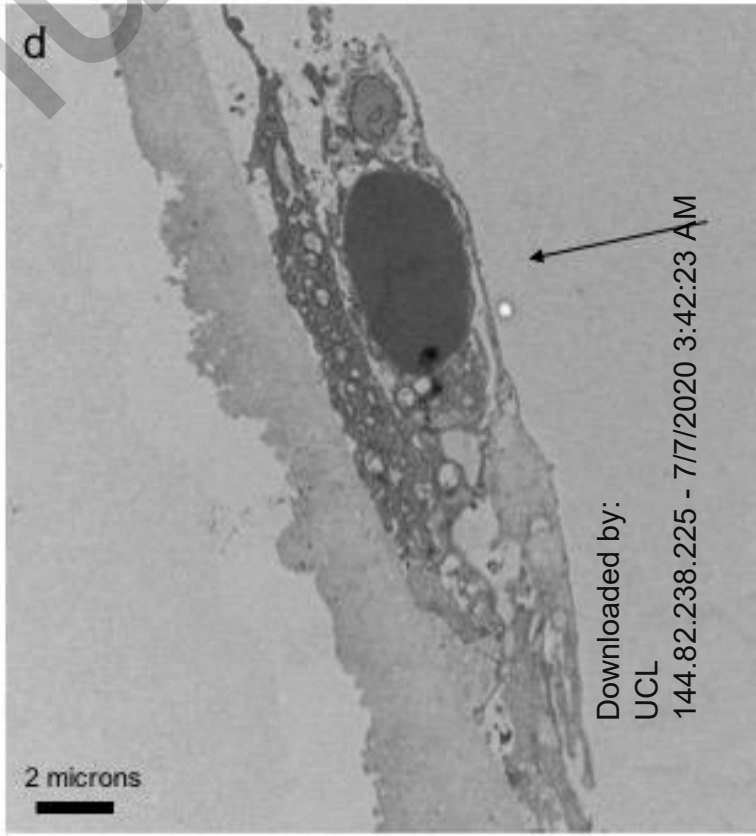


Table 1 Comparison of demographic and clinical features between patients who underwent additional inner limiting membrane cleaning and those that did not following vitrectomy for Proliferative Diabetic Retinopathy in valid study eyes (n=106)

Feature	ILM Clean Group (n=56)	Control Group (n=50)	p
Age, years mean, SD, (min–max)	54.3,15.4 (27–81)	51.6,14.4 (22–82)	0.37
Gender n, (%)	Male – 29 (52%)	Male – 26 (52%)	0.98
Indication for vitrectomy: VH, MT, CTRD n, (%)	VH – 40 (71%) MT – 14 (25%) CTRD – 2 (4%)	VH – 39 (78%) MT – 8 (16%) CTRD – 3 (6%)	0.47
Type Diabetes (Type1, Type 2) n, (%)	Type1 – 32 (57%)	Type1 – 19 (38%)	0.07
Duration Diabetes, Years mean, SD, (min–max)	23.3, 10.7 (8–51)	20.5, 9.0 (4–51)	0.14
HbA1c (mmol/l) mean, SD, (min–max)	74.6, 13.9 (42–115)	79.8, 20.6 (37–140)	0.13
Preoperative visual acuity, logMAR mean, SD, (min–max)	1.43, 0.53 (0.3–2.3)	1.37, 0.54 (0.2–2.0)	0.58
Preoperative PRP extent (None, less than standard ETDRS scatter (1), equal to or more than standard ETDRS scatter (2)) n, (%)	None – 3 (5%) 1 – 6 (10%) 2 – 47(85%)	None – 2 (4%) 1 – 7 (14%) 2 – 41(82%)	0.92
Extent of retinal haem (less than ETDRS standard photo 2a (1), equal to 2a (2) or greater than 2a (3)) n, (%)	1 – 1 (2%) 2 – 28 (50%) 3 – 27 (48%)	1 – 4 (8%) 2 – 14 (28%) 3 – 32 (64%)	0.84
Pre retinal haemorrhage (None, extrafoveal (1), foveal involving (2)) n, (%)	None – 37 (66%) 1 – 16 (29%) 2 – 3(5%)	None – 38 (76%) 1 – 6 (12%) 2 – 6 (12%)	0.07
Presence of any traction retinal detachment (None, extra–macular (1) and macular involving (2)) n, (%)	None – 23 (41%) 1 – 24 (43%) 2 – 9 (16%)	None – 27 (54%) 1 – 12 (24%) 2 – 11 (22%)	0.39
Location of vitreoretinal adhesion (None, disc only (1), posterior retinal only (2), posterior and 1 quadrant anteriorly (3), posterior and 2 or more anterior quadrants (4)) n, (%)	None – 2 (4%) 1 – 5 (9%) 2 – 43 (59%) 3 – 10 (18%) 4 – 6 (11%)	None – 3 (6%) 1 – 4 (8%) 2 – 32 (64%) 3 – 5 (10%) 4 – 6 (12%)	0.82
Total disc areas of vitreoretinal attachment median, interquartile range, maximum	6, 5, 20	4, 5.5, 25	0.27
Tamponade used (None vs gas) n, (%)	Gas – 5 (9%)	Gas – 8 (16%)	0.38

Baseline lens status n, (%)	Phakic – 50 (89%)	Phakic – 45 (90%)	0.90
Combined phacovitrectomy performed n, (%)	Yes – 6 (15%)	Yes – 8 (16%)	0.99
Preoperative anti–VEGF given n, (%)	Yes – 28 (51%)	Yes – 18 (36%)	0.17
Intraoperative laser applications mean, SD, min–max	1013, 429, 332–1764	887, 368, 398–1985	0.11

ILM: Inner Limiting Membrane, SD: Standard Deviation, VH: Vitreous Haemorrhage, MT: Macular Traction, CTRD: Combined Tractional and Rhegmatogenous Retinal Detachment, PRP: Panretinal photocoagulation, ETDRS: Early Treatment Diabetic Retinopathy Study, VEGF: Vascular endothelial growth factor

Table 2 Comparison of cytokine and growth factor levels measured in undiluted vitreous samples taken prior to vitrectomy for Proliferative Diabetic Retinopathy in valid study eyes (n=106) between patients who underwent additional inner limiting membrane cleaning and those that did not.

Cytokine/ Growth Factor	ILM clean Group (n=56)	Control Group (n=50)	p
MCP-1 (mean, SD)	3619.3, 727.5	3922.0, 819.5	0.11
IL-6 (mean, SD)	921.7, 994.6	1628.4, 2064.6	0.07
IL-8 (mean, SD)	1507.5, 1254.4	1406.8, 1246.9	0.73
VEGF-A (mean, SD)	13957.2, 22973.0	15228.0, 26809.3	0.51
PLGF (mean, SD)	359.9, 628.1	610.6, 1182.1	0.27
CTGF (mean, SD)	529401.2, 433274.3	448099.5, 377463.3	0.38
ICAM-1 (mean, SD)	183.8, 69.7	144.4, 61.5	0.89
MMP-2 (mean, SD)	52.0, 96.2	31.4, 50.1	0.22
MMP-9 (mean, SD)	137.7, 187.7	107.6, 201.5	0.49
TNFα (mean, SD)	21.4, 19.1	26.4, 25.9	0.36
Ang-2 (mean, SD)	18787.7, 29171.8	12658.4, 18899.2	0.27

ILM: Inner Limiting Membrane, MCP-1: Monocyte chemoattractant protein 1, SD: Standard Deviation, IL-6: Interleukin 6, IL-8: Interleukin 8, VEGF-A: Vascular endothelial growth factor A, PLGF: Placental growth factor, CTGF: Connective tissue growth factor, ICAM-1: Intercellular adhesion molecule 1, MMP-2: Matrix metalloproteinase 2, MMP-9: Matrix metalloproteinase 9, TNFα: Tissue necrosis factor α, Ang-2: Angiopoietin 2.

Table 3 Comparison of postoperative outcomes following vitrectomy for Proliferative Diabetic Retinopathy at 3 months postoperatively.

Postoperative Outcome	**ILM clean GROUP (n=56)**	**Control Group (n=50)**	**p**
Foveal ERM (0-2) (n, %)	None – 52 (93%)	None – 39 (78%)	0.03
	1 – 4 (7%)	1 – 9 (18%)	
	2 – 0 (0%)	2 – 2 (4%)	
Eccentric ERM (0-2) (n, %)	0 – 32 (57%)	0 – 12 (24%)	<0.001
	1 – 21 (38%)	1 – 24 (48%)	
	2 – 3 (5%)	2 – 14 (28%)	
Clinically significant ERM (present) (n, %)	2 (4%)	10 (20%)	0.01
Postoperative visual acuity (logMAR) (mean, SD, min-max)	0.35, 0.23 (0.1-1.8)	0.50, 0.40 (0.1-1.3)	0.02
CMT (microns) (mean, SD, min-max)	293, 51 (195-420)	335, 92 (198-593)	0.002
Macular Volume (mm^3) (mean, SD, min-max)	8.0, 1.1 (5.3-10.3)	8.1, 1.3 (5.4-11.2)	0.07
CMT >400 microns (present) n, (%)	3 (5%)	10 (20%)	0.04
Macular cysts (present) n, (%)	7 (13%)	14 (25%)	0.21

ILM: Inner Limiting Membrane, ERM: Epiretinal Membrane, SD: Standard Deviation, CMT: Central Macular Thickness

Table 4 Comparison of epiretinal membrane presence on 3-month imaging broken down by primary indication for vitrectomy.

	Presence of any ERM on SDOCT (i.e. with score ≥1)		
Primary indication for vitrectomy	**ILM clean GROUP (n=56)**	**Control Group (n=50)**	**p**
Vitreous haemorrhage n/N (%)	20/40 (50%)	30/39 (77%)	0.002
Macular TRD n/N (%)	3/14 (21%)	6/8 (75%)	0.22
CTRD n/N (%)	2/2 (100%)	3/3 (100%)	1

ERM: Epiretinal Membrane, SDOCT: Spectral Domain Optical Coherence Tomography, ILM: Inner Limiting Membrane, TRD: Tractional Retinal Detachment, CTRD: Combined Tractional Rhegmatogenous Detachment

Supplementary Table 1 Comparison of postoperative outcomes following vitrectomy due to vitreous haemorrhage secondary to Proliferative Diabetic Retinopathy at 3 months postoperatively.

Postoperative Outcome	**ILM clean GROUP (n=40)**	**Control Group (n=39)**	**p**
Foveal ERM (0-2) (n, %)	None – 38 (95%) 1 – 2 (5%) 2 – 0 (0%)	None – 32 (82%) 1 – 7 (18%) 2 – 0 (0%)	0.09
Eccentric ERM (0-2) (n, %)	0 – 21 (53%) 1 – 18 (45%) 2 – 1 (2%)	0 – 10 (26%) 1 – 21 (54%) 2 – 8 (20%)	0.008
Clinically significant ERM (present) (n, %)	0 (0%)	5 (13%)	0.03
Postoperative visual acuity (logMAR) (mean, SD, min-max)	0.34, 0.23 (0.1-1.3)	0.45, 0.30 (0.1-1.3)	0.07
CMT (microns) (mean, SD, min-max)	294, 51 (223-420)	332, 86 (198-593)	0.02
Macular Volume (mm^3) (mean, SD, min-max)	8.0, 1.3 (5.3-10.3)	8.4, 1.2 (5.4-11.2)	0.19
CMT >400 microns (present) n, (%)	3 (8%)	5 (13%)	0.48

ILM: Inner Limiting Membrane, ERM: Epiretinal Membrane, SD: Standard Deviation, CMT: Central Macular Thickness