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ULTRASTRUCTURAL CHANGES OF THE INTERNAL LIMITING MEMBRANE REMOVED DURING INDOCYANINE GREEN ASSISTED PEELING VERSUS CONVENTIONAL SURGERY FOR IDIOPATHIC MACULAR EPIRETINAL MEMBRANE

THESE

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Modifications ultrastructurales de la limitante interne de la rétine après pelage de membrane épimaculaire avec ou sans l'aide du vert d'indocyanine (ICG)

Introduction

L'objectif de cette étude était d'une part d'évaluer les caractéristiques histologiques des fragments cellulaires rétiniens attachés à la limitante interne après vitrectomie et pelage d'une membrane epirétinienne, et d'autre part de mettre en évidence des différences histologiques entre les cas opérés avec ou sans l'aide d'ICG dilué dans du glucose 5%.

Méthodes

Nous avons examiné rétrospectivement l'histologie de 88 spécimens de membranes épimaculaires contenant la limitante interne de la rétine, qui ont été enlevés chirurgicalement entre 1995 et 2003.
L'analyse histologique a centré principalement l'attention sur la présence et les caractéristiques des fragments cellulaires rétiniens attachés à la limitante interne.
L'analyse statistique a comparé les résultats entre le groupe I (chirurgie conventionnelle sans l'aide de l'ICG) et le groupe II (chirurgie à l'aide de l'ICG).

Résultats

Soixante et onze patients ont eu une vitrectomie sans l'aide de l'ICG (groupe I) et 17 avec l'aide de l'ICG (groupe II).
Le nombre de débris de cellules de Müller à la surface rétinienne de la limitante interne était plus important dans le groupe I (sans ICG) que dans le groupe II (avec ICG) (40.8% versus 11.8% ; $p = 0.024$). Des larges fragments cellulaires rétiniens attachés à la limitante interne ont été plus fréquemment observés dans le groupe I (sans ICG) que dans le groupe II (avec ICG) (63.4% versus 23.5%; $p= 0.003$).
Dans cinq (7%) cas du groupe I, de gros éléments cellulaires rétiniens ont été mis en évidence (des axones neuraux ou des vaisseaux sanguins). De tels éléments n'ont pas été retrouvés dans les spécimens du groupe II (avec ICG).

Conclusions

L'utilisation de l'ICG dilué dans du glucose 5% pour faciliter le pelage d'une membrane épimaculaire et notamment l'ablation de la limitante interne de la rétine semble diminuer de manière significative le nombre et la taille des débris des cellules de Muller adhérents à la face rétinienne de la membrane limitante interne de la rétine. Cette observation suggère que l'utilisation per-opératoire d'ICG dilué dans du glucose 5% facilite l'ablation de la limitante interne pendant la chirurgie de la membrane epirétinienne en diminuant l'adhérence de la limitante interne à la rétine.

Title:

Ultrastructural changes of the internal limiting membrane removed during indocyanine green assisted peeling versus conventional surgery for idiopathic macular epiretinal membrane

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Key Words

Idiopathic epiretinal membrane, indocyanine green, internal limiting membrane, Müller cells, vitrectomy.

Summary Statement

The use of ICG diluted with 5% glucose in the aid of ILM removal during macular epiretinal membrane surgery was associated in the present study with significantly less retinal debris attached to retinal face of the ILM compared to conventional surgery.

Abstract

Purpose: To evaluate the histological features of cellular retinal fragments on the internal limiting membrane (ILM) removed during idiopathic macular epiretinal membrane (MEM) peeling surgery with and without the aid of ICG diluted in 5% glucose

Methods: ILM specimens removed from 88 eyes during idiopathic MEM surgery between 1995 and 2003 were reviewed retrospectively. Histological analysis focused on the presence and characteristics of retinal fragments on the retinal surface of the ILM. Statistical analysis compared the results between group I (conventional surgery) and group II (ICG-assisted peeling).

Results:
Seventy-one eyes underwent MEM surgery without the aid of ICG (group I) and seventeen underwent MEM ICG-assisted surgery (group II). The amount of Müller cell debris on the retinal surface of the ILM was more significant in the group I than in the group II (40.8 versus 11.8; $p = 0.024$). Large fragments of Müller cells were more frequently observed in the group I (no ICG) than in the group II (ICG) (63.4% versus 23.5%; $p= 0.003$).

Conclusions: The use of ICG diluted with 5% glucose in ILM removal during MEM surgery was associated with less retinal debris attached to the retinal face of the ILM compared to surgery in which ICG was not used.

Introduction

Macular epiretinal membrane (MEM) is a disorder of the vitreomacular interface characterized by fibrocellular proliferation on the anterior surface of the internal limiting membrane (ILM) of the macula that may result in distortion of the retinal architecture and can be associated with visual loss and metamorphopsia.[1, 2] Surgery for MEM has been shown to improve visual acuity in 80% to 90% of cases.[3-5]

Peeling of the ILM as an additional step of MEM excision may remove a scaffold for myofibroblasts and other proliferative cells that may be responsible for recurrence or persistent contraction of the macula [6] and has been demonstrated to be associated with better final vision and a lower risk of recurrent MEM.[6-8]

ILM visualization is a challenge during this procedure. In order to improve visualization and to facilitate the surgical technique, the use of indocyanine green (ICG) was introduced in 2000 to selectively stain the ILM.[9-12]

ICG is a tricarbocyanine hydrophilic dye that binds with proteins, and homogeneously and diffusely stains the collagen of the retinal ILM.[9, 10, 12] Consequently its use facilitates the visualization of the ILM and assures a safer and easier removal of the ILM with less risk of retinal damage.[9, 11, 12]

Good anatomical and visual results have been demonstrated for patients who underwent ICG-assisted ILM peeling for idiopathic MEM surgery.[13, 14] However, several reports suggested a possible toxicity of ICG to the retina [15-32] including a possible alteration of the cleavage plane from the ILM to the innermost retinal layers.[19, 33]
These studies have caused controversy regarding the use of ICG in the vitreoretinal surgery[25, 34] and skepticism concerning the safety of this dye. [34]

Our interest for the present study was mostly triggered by studies that reported the presence of considerable amount of retinal fragments adherent to the retinal surface of the ILM when ICG was used for its peeling. [35] [33] [36] These reports were contrary to our observations. For that reason we decided to retrospectively study the histological

features of ILM specimens of all our idiopathic MEM cases treated with and without the aid of ICG in order to evaluate the presence and characteristics of retinal structures on the retinal surface of the ILM. We are furthermore attempting to evaluate the parameters that might influence differences between the present study and previous reports.

Methods

Patient Selection

This study is a retrospective, nonrandomized study and includes patients who underwent vitrectomy, peeling of an idiopathic MEM and ILM excision with or without intraoperative intraocular ICG injection. All patients were operated between 1995 and 2003 at the Jules Gonin University Eye Hospital. Institutional approval was obtained for this study.

Exclusion criteria were the presence of macular hole or lamellar macular hole, previous vitreoretinal surgery and the presence of any other macular pathologic features potentially interfering with histological results (such as diabetic retinopathy or age-related macular degeneration). We also excluded cases where the ILM specimen was not valid for histological examination for various reasons.

Surgical Technique

Vitrectomy was performed in all cases by the same surgeon (E.H.B.) using the same technique. A standard three-port pars plana vitrectomy was performed in each case using a vitreous cutter surrounded by a coaxial optic fiber connected to a xenon light source (Lausanne set, Oertli, Switzerland- Developed at Jules Gonin, Lausanne, by Gonvers and Bovey).
Separation of the posterior hyaloid membrane was performed when necessary. Visualization of the fundus was achieved with a special noncontact wide-angle viewing system [37] during vitrectomy and with a planoconcave contact lens for

macular peeling. The MEM was peeled in the macular area using an end-gripping forceps. In the non-ICG group, after peeling of the MEM the macular retina was carefully inspected with forceps in search of the ILM, which was removed subsequently. In some cases, the MEM and the ILM were removed together as one membrane.

For the patients in ICG group, ICG was diluted in 5% glucose solution (at a concentration of 0.1%) according to our technique described elsewhere.[38] The ICG was slowly injected over the macula so that the dye spread over the retina as far as the superior and inferior temporal vascular arcades. The infusion was not turned off. The dye was left for approximately 30 seconds and then aspirated mechanically.

Histologic Examination

Excised MEMs were placed on a Millipore filter, fixed in a 10% paraformaldehyde solution, and examined by light and transmission electron microscopy. Peeled ILM specimens were placed in a second bottle of paraformaldehyde solution at pH 7.3, post fixed with 2% sodium tetroxyde, dehydrated with acetone and embedded in epoxy resin. Semithin sections were cut with an ultramicrotome, stained with toluidine blue and examined by light microscopy. Ultrathin sections were stained with uranyl acetate-lead citrate and inspected in a Zeiss EM 10 electron microscope. Examination focused on the research of retinal structural elements on the retinal face of ILM. The assessment was done in a blinded, masked fashion so that the pathologist who reviewed the histological specimens was not aware about whether ICG was used or not as an adjuvant to the surgical procedure.

Statistical Analyses

Data collected included: age, gender, symptoms, preoperative and postoperative anatomical status of the macula, surgical and postoperative complications. Cases where the MEM and the ILM were removed together as one membrane were recorded additionally.

The following characteristics of the ILM were analyzed and graded: quantity of debris of Müller cells, (few, many) and size of debris (small, large). Debris size was considered as small if ≤5μm and large if >5μm. Histological specimens were

classified as presenting big-sized debris in case of cellular elements with a diameter measured greater than 5μm at electron microscopy. All surgical specimens were evaluated and graded in terms of quantity of debris of Müller cells by one experienced pathologist of the Jules Gonin Eye Hospital. The presence of other retinal elements (neural axons, vessels etc.) was additionally evaluated.

Non parametric Kruskal-Wallis and Wilcoxon tests were used to compare continuous clinical variables between the two groups. A $\chi 2$ test was used to compare categorical variables. Statistical analysis was performed with the JMP statistical software version 6.03 for Mac. (SAS Institute, Cary, NC, USA)

Results

Eighty-eight patients were included in this study. Seventy-one subjects (no ICG group) underwent MEM surgery without the aid of ICG between 1995 and 1999 and seventeen (ICG group) underwent ICG-assisted MEM surgery between 2001 and 2003. The demographic characteristics of the patients are summarized in table 1.

In 14 (82.4%) eyes of the ICG-group, the MEM and the ILM were removed together as one membrane. In 3 (17.6%) other cases, ILM was removed subsequently to the MEM peeling as a second membrane. In 5 patients (29.4%), posterior hyaloid was attached and had to be detached surgically prior to the MEM peeling.

In the non ICG-group, the MEM and the ILM were removed together as one membrane in 60 (84%) cases. In 11 other cases (16%), ILM was removed subsequently to the MEM peeling as a second membrane. In 16 patients (22.5%), posterior hyaloid was attached and had to be detached surgically prior to the ERM peeling.

ILM specimens in the ICG group presented statistically significantly ($p=0.024$) less debris of Muller cells on their retinal face than specimens in the non-ICG-group (Table 2), (fig. 1).
Additionally, the use of ICG was significantly associated with the presence of smaller retinal fragments on the retinal surface of the peeled ILM (Table 2), (fig. 2).
Large fragments of retinal tissue adherent to the retinal surface of the ILM were discovered more frequently in cases that presented a lot of debris ($p = 0.03$).

In 5 (7%) cases of the non-ICG group, the presence of large retinal elements was detected. More precisely, we observed the presence of neural axons (3 cases) and vessels (2 cases) attached to retinal face of the ILM (fig. 3). On the contrary, such retinal elements were not found in any of the histological ILM specimens of the ICG-group.

Discussion

The use of ICG facilitates the removal of the ILM during MEM surgery. However, several reports have suggested a possible toxicity of the ICG to the retina, [15-32] including a possible anatomical disruption of the retinal layers underneath the ILM. [19, 33 39] ICG toxicity has been attributed to high ICG concentration, prolonged tissue contact time of ICG, [15, 40, 41] the presence of sodium in the solvent, [42, 43] the osmolarity and type of the solvent [8, 28], and the type of light source. The toxicity of ICG was found less pronounced when ICG powder was diluted with 5% glucose rather than with BSS [28, 44]. The concomitant use of xenon light source during vitrectomy was found less harmful than the halogen light one. [45]

In the present study we have focused on the presence and characteristics of retinal remnants on the retinal surface of the ILM as an indirect indication of the disruption of the anatomical cellular structures of the underlying retina after ILM peeling. Müller cells play an active role in retinal function [46] and consequently the loss of their cell footplates during ILM peeling may interfere with retinal function.

To our knowledge, we present the largest case series that investigates ultrastructural changes of the peeled ILM during idiopathic MEM surgery. Furthermore, to our knowledge this is the first non-postmortem study to report histological characteristics of the ILM harvested during idiopathic MEM surgery with the aid of ICG (0.1%) diluted with 5% glucose.

Our interest for the present study was mostly triggered by studies that reported the presence of considerable amount of retinal structures adherent to the retinal surface of the ILM when ICG was used for its peeling as compared to conventional surgery. [35 33 36]

Haritoglou et al. [35] in a study regarding macular pucker surgery reported retinal debris found on the retinal face of the peeled ILM that was more important when ICG was used.

Gandorfer et al. [19], Haritoglou et al. [39] and Schumann et al., [36] respectively, in their studies regarding idiopathic macular holes, found large fragments of retinal tissue to

be adherent to the retinal surface of the ILM after ICG use. Haritoglou et al [39] and Schumann et al. [36] additionally concluded that conventional ILM peeling was not associated to significant disruption of the underlying retinal cellular structures.

These reports were in opposition to our results. More precisely, in our study the use of ICG was significantly related to the presence of few and small retinal fragments on the retinal face of the peeled ILM. On the contrary, when ICG was not used, larger pieces and a greater amount of cellular fragments was found. Furthermore, in these specimens, cellular debris was found throughout the retinal surface of the ILM and was not limited to undulations of the retinal surface of the ILM as previous studies suggested. 33 Additionally, very large retinal elements like neural axons and vessels were only related to conventional surgery.

It is difficult to explain why the results are so different between our study and those mentioned above. One difference seems to be related to the solvent used to dilute ICG. We used 5% glucose instead of the BSS that was used in the other studies. A photosensitizing effect of ICG might be responsible for some of its adverse effects. [18] Light-absorbing properties of ICG depend on the solute and the use of sodium free 5% glucose as a solvent might be advantageous in comparison to the used balanced salt solution. It has been demonstrated that a shift of the absorption band toward longer wavelengths is observed when 5% glucose was used for dilution. [44] As a consequence, the overlap of the light source used during surgery and the absorption band of ICG might be limited by the use of 5% glucose, preventing photochemical adverse reactions on the retinal surface. This phenomenon is more pronounced with the concomitant use of xenon light sources during vitrectomy as in our cases. More precisely, the emission of xenon light sources is different from that of halogen light sources, with a shift of the maximum spectral radiance toward a lower wavelength that may limit furthermore the overlap between the emission spectrum of the light source and the absorption band of ICG. [45]

However Haritoglou et al. [20] in an experimental setting in four postmortem eyes evaluated the effect of indocyanine green (ICG) diluted with 5% glucose on the human retina and reported disorganization of the inner retinal layers similar to that reported with BSS diluted ICG. [39] Nevertheless they stated that the ILM specimens

harvested during vitrectomy using glucose 5% diluted ICG for staining showed cellular elements at the retinal side of the ILM, which were less pronounced than observed in their previous studies with BSS diluted ICG. [39] [20] This is the only study to our knowledge that investigates histological characteristics of the ILM using ICG diluted with 5% glucose. However, some limitations exist regarding experimental, postmortem studies and these results should be interpreted with caution. Furthermore, although not clearly indicated a halogen light source was likely used in this study considering that authors report the use of a light source emitting light between 380 and 760 nm.

In our study, when ICG was not used, a significant amount of large cellular debris was torn away from the neural retina and found to be attached to the ILM. This could be related to the compromised visualization of the ILM and to mechanical damage by the intraocular forceps and manipulation of the surface during surgery. Consequently, a more aggressive removal of the ILM is realized with more traumatic consequences to the underlying retina as might be reflected by the presence of more retinal debris on the retinal surface of the ILM.

One interesting observation made by the surgeon was that the ILM could be removed more easily when ICG was used. The visualization of the ILM was enhanced and the adhesion with the underlying retina appeared decreased. This latter fact could reflect a possible alteration of the cleavage plane between the ILM and the underneath retina. This is in accordance with the study of Wollensak et al. [47], which demonstrated that ICG staining of the ILM facilitated ILM peeling by increasing the biomechanical stiffness. This was explained by a photosensitizing effect of ICG leading to collagen crosslinking.

An interesting question remains to be investigated: does the loss of Müller cell footplates interfere with retinal function? Electrophysiological changes or visual field changes have been reported after peeling of the ILM with [48] [29] and without [49] [50] the use of ICG. Hillenkamp et al. [51] reported less visual field defects with the use of ICG diluted with 5% glucose instead of conventional surgery. It would have been interesting, though, to see in the present study if such changes were correlated with

the type and quantity of retinal remnants on the ILM. Unfortunately, such tests were not performed.

Interestingly, Ducournau et al. [52] suggested that detachment of Müller cell footplates after ILM peeling was not necessarily a negative event but that it could stimulate the Müller cells across the retina to generate a transretinal glial reaction with possible positive effects in retinal function since Muller glial cells seem to exhibit neural stem cell properties. [53 54 55]

In our institution systematic use of ICG was abandoned after the first reports of adverse retinal reactions. Furthermore, postoperative visual acuities measured in a previous series were not better when ICG was used. [13] However we still use ICG diluted in 5% glucose, though infrequently, when ILM presents a very low visibility and when its removal is considered necessary.

In conclusion, the use of ICG diluted with 5% glucose in order to facilitate ILM removal during macular epiretinal membrane surgery was associated with less retinal debris attached to the retinal face of the ILM compared to conventional surgery. More clinicopathological studies are needed to define the significance of the retinal fragments removed during ILM peeling.

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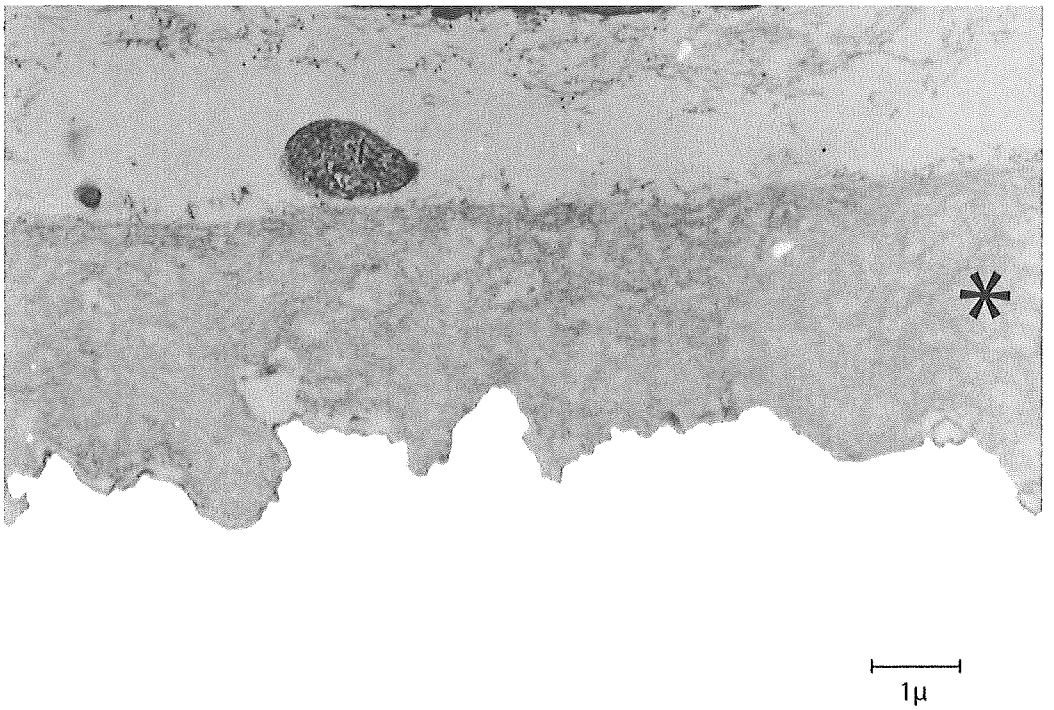
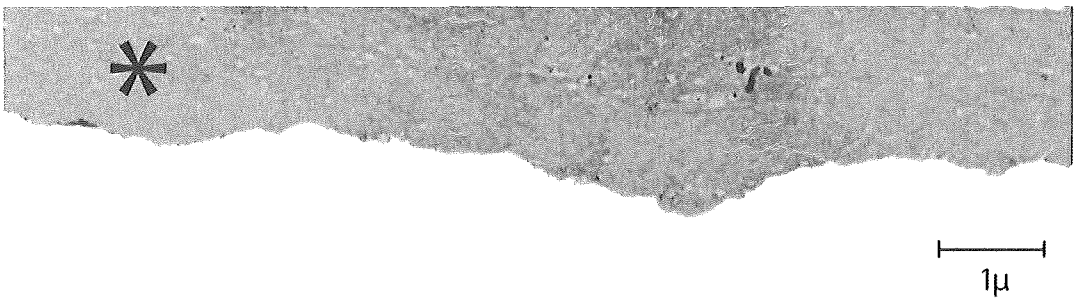
Figure 1A



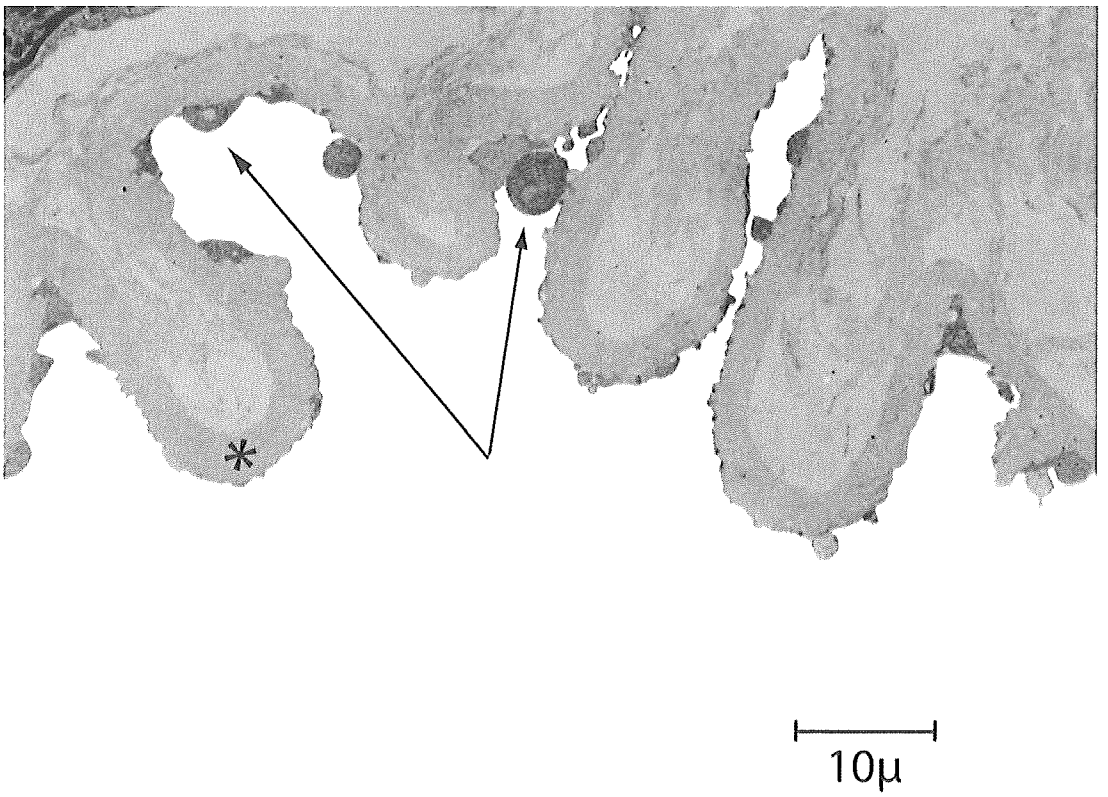
Transmission electron micrographs of internal limiting membranes removed from eyes with idiopathic macular epiretinal membrane.
1A: Specimens removed by ICG-assisted ILM peeling showing very few fragments of retinal debris at the retinal side of ILM (asterisk) (1A: bar:1 μ

Figure 1B



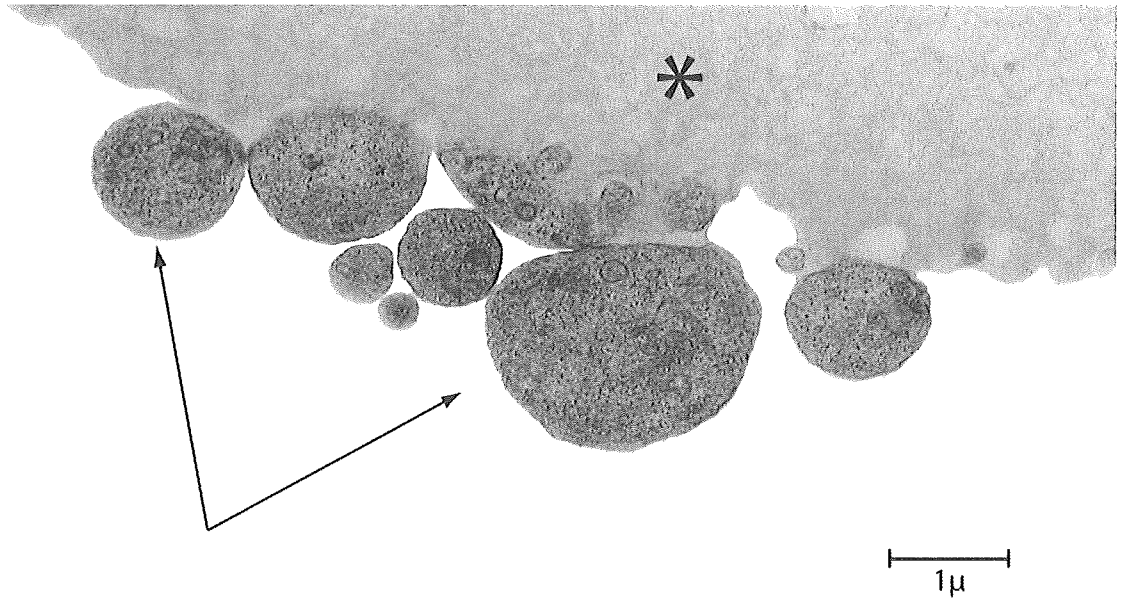
Transmission electron micrographs of internal limiting membranes removed from eyes with idiopathic macular epiretinal membrane.
1B: Specimens removed by ICG-assisted ILM peeling showing very few fragments of retinal debris at the retinal side of ILM (asterisk)
1B: bar: 1μ

Figure 1C



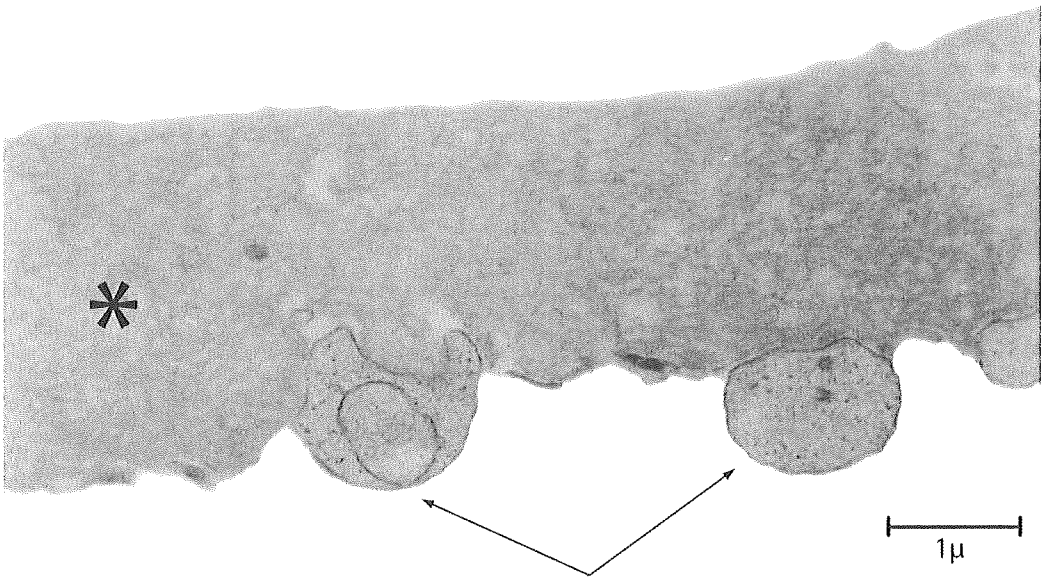
Transmission electron micrographs of internal limiting membranes removed from eyes with idiopathic macular epiretinal membrane.
1C: Specimen removed without dye-assisted ILM peeling showing a lot of retinal debris (arrows) at the retinal side of the ILM (asterisk) (bar: 10µ).

Figure 2A



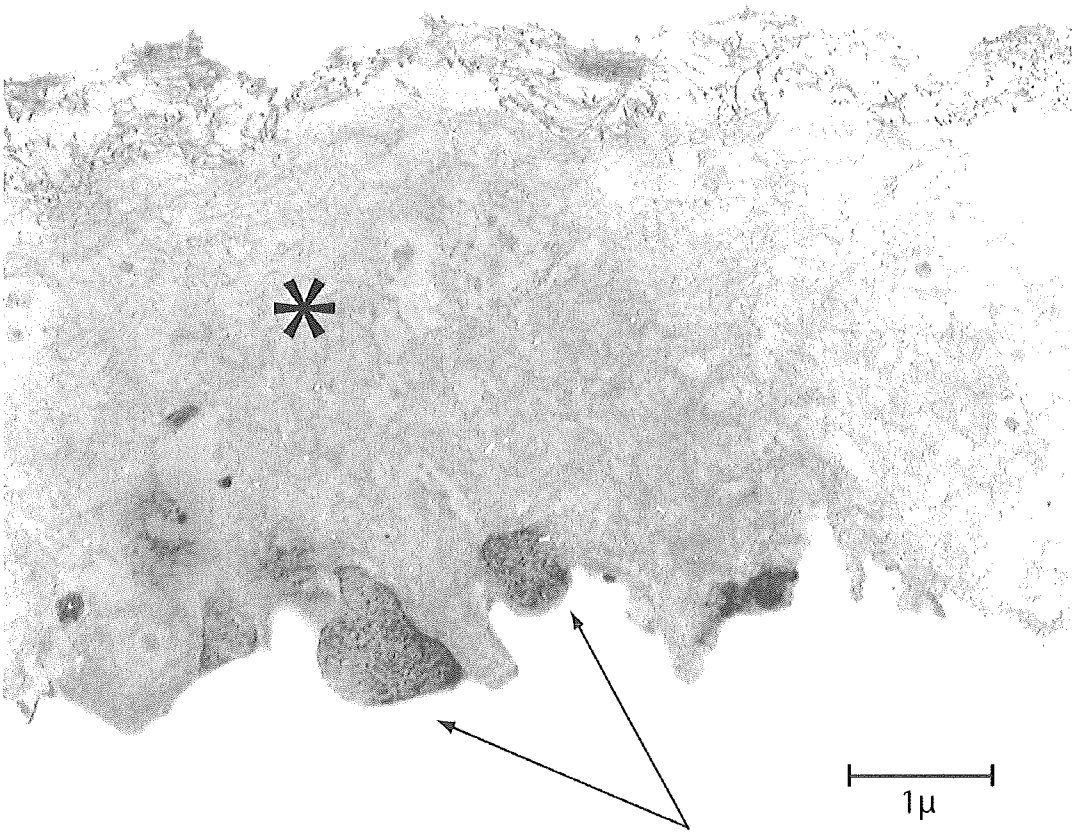
Electron microscopy of peeled ILMs showing cellular fragments attached to the retinal side of ILM.
2A: Specimen removed by ICG-assisted ILM peeling showing small fragments of retinal debris (arrows) at the retinal side of ILM (asterisk). The round structures shown in these micrograph represent inner portions of Müller cells torn away with the ILM.
(2A: bar: 1µ).

Figure 2B



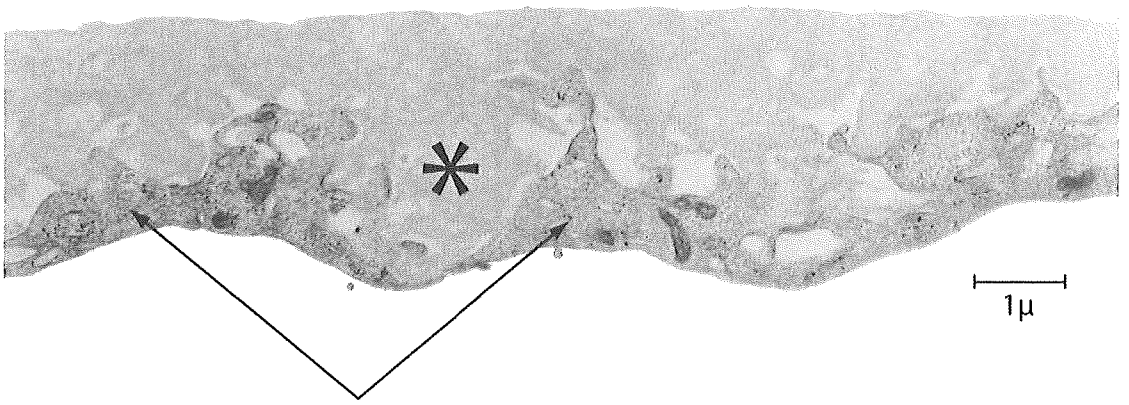
Electron microscopy of peeled ILMs showing cellular fragments attached to the retinal side of ILM.
2A: Specimen removed by ICG-assisted ILM peeling showing small fragments of retinal debris (arrows) at the retinal side of ILM (asterisk). The round structures shown in these micrograph represent inner portions of Müller cells torn away with the ILM.
(2A: bar: 1µ).

Figure 2C



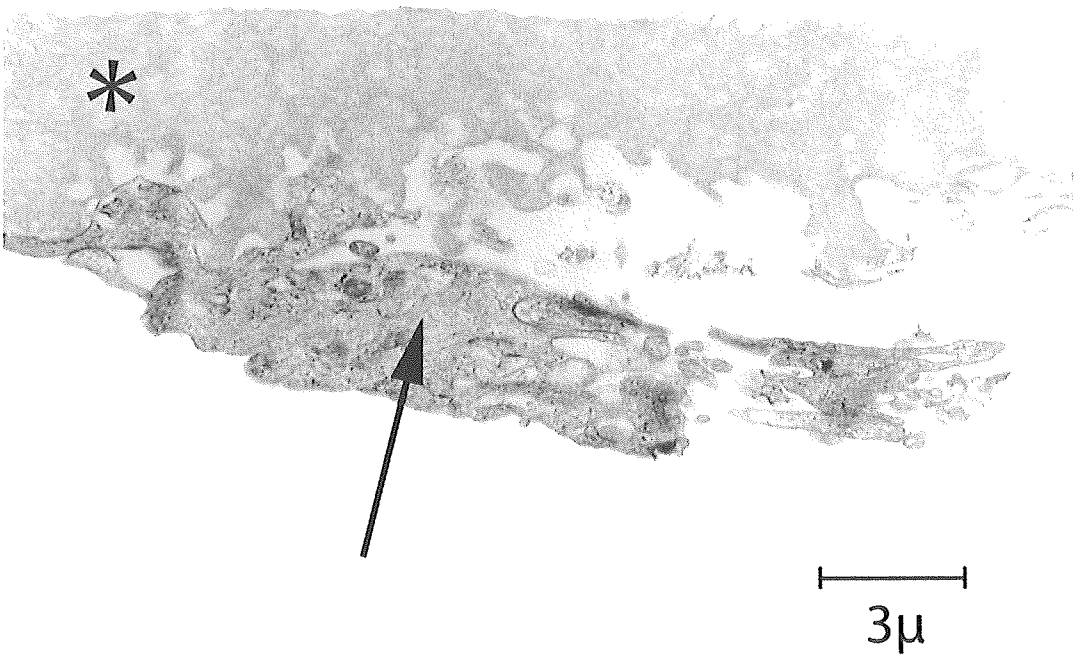
Electron microscopy of peeled ILMs showing cellular fragments attached to the retinal side of ILM.
2C: Specimen removed by ICG-assisted ILM peeling showing small fragments of retinal debris (arrows) at the retinal side of ILM (asterisk). The round structures shown in these micrograph represent inner portions of Müller cells torn away with the ILM.
(2c: bar: 1μ).

Figure 2D



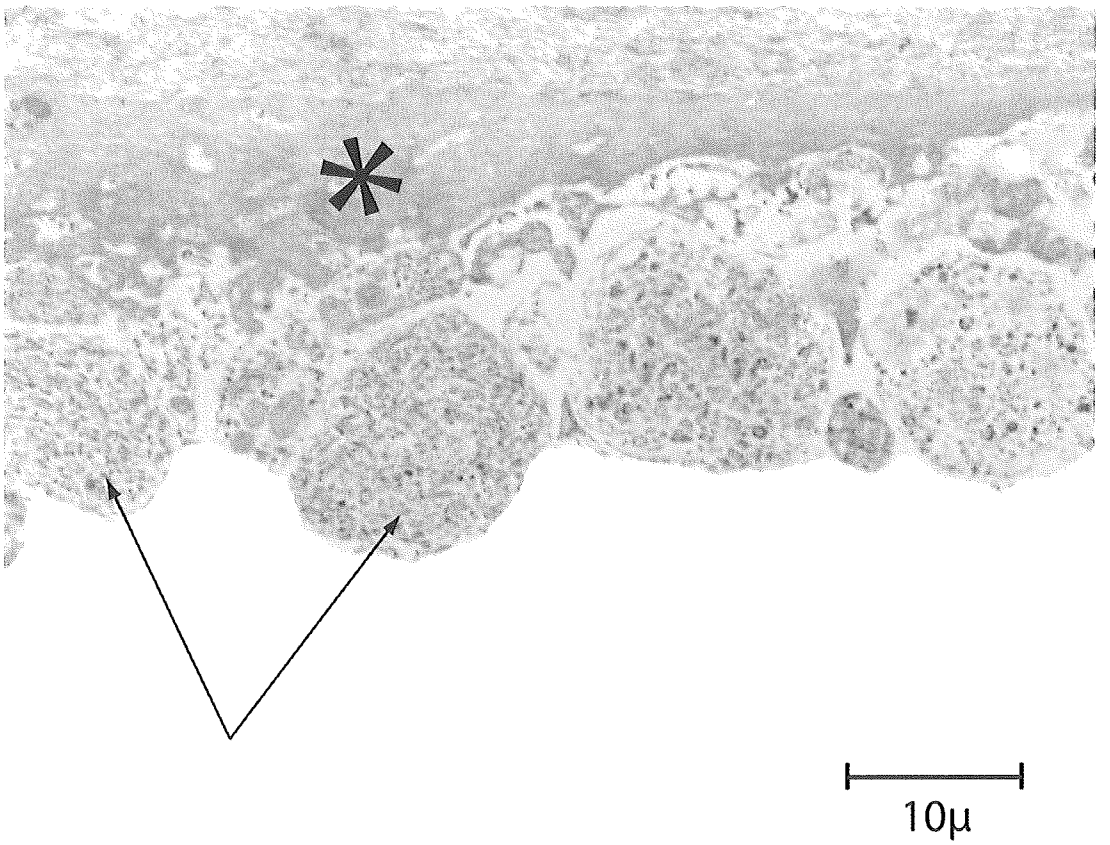
Electron microscopy of peeled ILMs showing cellular fragments attached to the retinal side of ILM.
2D: Specimens removed without dye-assisted ILM peeling showing Müller cell end plates (arrows) at the retinal side of the ILM (asterisk) (2D: bar: 1µ).

Figure 2E



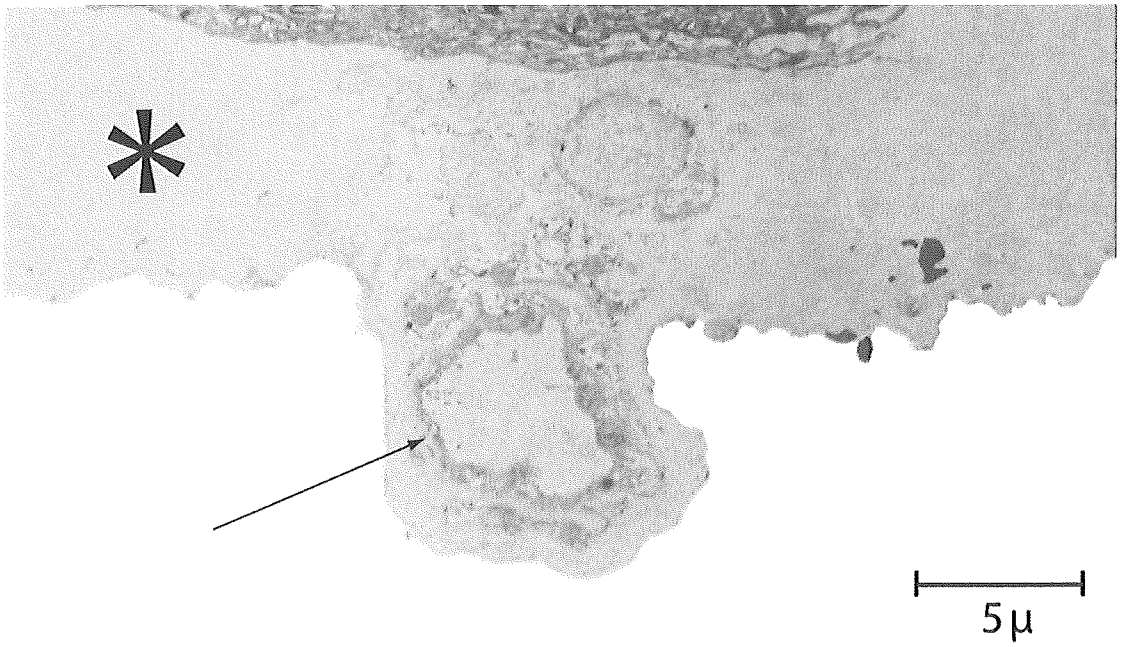
Electron microscopy of peeled ILMs showing cellular fragments attached to the retinal side of ILM.
2E: Specimen removed without dye-assisted ILM peeling showing Müller cell end plates (arrows) at the retinal side of the ILM (asterisk) (2E: bar: 3µ).

Figure 3A



Specimen removed without dye-assisted ILM peeling showing the presence of neural axons (arrows) (3A: bar: 10µ) adherent to the retinal face of the ILM

Figure 3B



Specimen removed without dye-assisted ILM peeling showing the presence of a capillary (arrow) (3B: bar: 5) adherent to the retinal face of the ILM

Table 1

TABLE 1 – Clinical data.

	Group 1 (without ICG)	**Group 2** (with ICG)
Number of cases	71	17
Male	25 (35.2%)	6 (35.3%)
Female	46 (64.8%)	11 (64.7%)
Mean Age (years): (Range)	69.9 (45 to 86)	67.2 (42 to 79)

Table 2

TABLE 2

Quantity and size of debris of Muller cells on the retinal surface of the ILM in the two groups.

	Group 1 (No ICG. 71 eyes)		**Group 2** (ICG. 17 eyes)		**P**
	N	%	N	%	
Quantity of debris					
Many	29	40.8	2	11.8	< 0.05
Few	42	59.2	15	88.2	
Size of debris					
Large	45	63.4	4	23.5	< 0.005
Small	26	36.6	13	76.5	