

# Soluble lutein in combination with brilliant blue as a new dye for chromovitrectomy

Emmerson Badaro · Bruno Furlani · Juliana Prazeres · Mauricio Maia ·  
Acácio Alves Souza Lima · Diogo Souza-Martins · Cristina Muccioli ·  
Luiz Filipe Adami Lucatto · Rubens Belfort Jr

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## Abstract

**Summary** A new dye for vitreoretinal surgery comprised of soluble lutein/zeaxanthin 1 % and brilliant blue 0.025 % is advantageous compared with other dyes currently used for chromovitrectomy, and showed no signs of toxicity at 1 month of follow-up.

**Purpose** To evaluate the feasibility and safety of a dye [soluble lutein/zeaxanthin (LZ) 1 % and brilliant blue (BB) 0.025 %] for improving removal of vitreous, epiretinal membranes (ERM), and internal limiting membranes (ILM) in humans.

**Methods** We prospectively evaluated 18 eyes treated surgically for a macular hole or ERM. Eighteen surgeons performed chromovitrectomy using the dye, and completed a questionnaire to evaluate the efficacy and safety of the dye. Examinations included best-corrected visual acuity and intraocular pressure measurements and optical coherence tomography, fluorescein angiography, and autofluorescence performed at baseline and days 1, 7, and 30 postoperatively.

**Results** The green dye was deposited on the posterior pole; vigorous dye flushing into the vitreous cavity was unnecessary. All surgeons reported that the ILM stained greenish-blue; 94.4 % reported ILM peeling adequate; the ERM stained poorly. No evidence of toxicity was observed.

**Conclusion** The new dye deposited on the posterior pole due to its higher density. The ability to stain the ILM was similar to BB. The new dye has ability to stain the vitreous, hyaloid, and especially the ILM satisfactorily. The new dye may be useful during chromovitrectomy.

**Keywords** Chromovitrectomy · Internal limiting membrane · Lutein · Macular hole · Vitreoretinal surgery · Zeaxanthin

## Introduction

Vitreoretinal surgery involves complex and delicate vitreoretinal techniques to manage many vitreoretinal diseases, macular hole (MH), epiretinal membrane (ERM), and vitreomacular traction syndrome. Chromovitrectomy [1] includes the use of vital dyes or crystals to improve visualization of the intraocular tissues during vitrectomy.

The ideal vital dye should be safe for intraocular use, selectively stain the intraocular membranes, and be eliminated rapidly from the eye. Many dyes are available for chromovitrectomy [1–7], i.e., trypan blue (TB), brilliant blue (BB), indocyanine green (ICG), and infracyanine green. ICG is currently used to stain the internal limiting membrane (ILM) to aid peeling, even though there are concerns about retinal toxicity [8]. TB is thought to be the ideal dye for ERM identification, ICG and BB are ideal for ILM peeling, and triamcinolone acetonide (TA) is useful for vitreous identification [1–7]. Despite this, the United States Food and Drug Administration has not approved any dyes for use during chromovitrectomy. Currently, BB is not approved for human use in the US, and other agents such as ICG, TB, and TA are considered off-label drugs to be used during vitreoretinal procedures. However, BB is approved for chromovitrectomy in Europe.

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E. Badaro · B. Furlani · J. Prazeres · M. Maia · A. A. S. Lima ·  
D. Souza-Martins · C. Muccioli · L. F. A. Lucatto · R. Belfort Jr  
Department of Ophthalmology, Federal University of São Paulo, São  
Paulo, Brazil

M. Maia (✉)

Ophthalmology Department, Vitreoretinal Surgery Unit,  
Universidade Federal de São Paulo, Rua Botucatu 822, São  
Paulo 06023-062, Brazil  
e-mail: maia mauricio@terra.com.br

Lutein and zeaxanthin are lipophilic pigments belonging to the group of carotenoids traditionally found in fruits and vegetables [9, 10]. These carotenoids are also physiologically present in the macula lutea, an integral retinal structure in humans [11]. The carotenoids are structural isomers that have a hydroxyl group at the terminal part of the molecule, which partly explains their different polarities and tropism for certain biologic structures such as the macula [12–15]. Both are considered to be dyes, and are associated with prevention of age-related maculopathies due to their antioxidant effects and exclusive distribution in the macula [11, 16, 17].

Lutein- and zeaxanthin-based dyes alone or combined with BB also might be used effectively as intraocular dyes in human cadaveric eyes to stain different intraocular microstructures, including the ILM [18].

The current study evaluated the feasibility, advantages, and safety of a novel dye (soluble lutein/zeaxanthin [LZ] 1 % with BB 0.025 %) for improving identification and removal of the vitreous, hyaloid, ERM, and ILM performed by different surgeons.

## Patients and methods

We prospectively studied 18 eyes of 18 consecutive patients (16 women, two men; mean age,  $67.2 \pm 7.7$  years; range, 50–85 years); 50 % of patients were Caucasian, 44 % were African-American, and 5.6 % were Hispanic. Patients had a diagnosis of macular hole or ERM, and underwent pars plana vitrectomy (PPV) using the combined soluble formulation of LZ 1 % with BB 0.025 %. Inclusion criteria allowed inclusion of eyes with chronic macular hole and/or chronic ERM. Tables 1 and 2 show the patient preoperative and postoperative data. The Ethics Committee of the Federal University of São Paulo approved the study, which was conducted according to the research guidelines of the Association of Research in Vision and Ophthalmology and the tenets of the Declaration of Helsinki. The protocol was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (number NCT01627977). All patients provided informed consent regarding the benefits and risks of the surgical procedure and the new dye tested.

### Dye preparation

The preparatory technique described below is valid for lutein and zeaxanthin regardless of the chemical form, purity, crystallization, or degree of esterification. The primary steps in the current study were development of a water-soluble solution, characterization of the absorbance of the LZ solution with the Aquamate device™ (Thermo Spectronic, Cambridge, UK), and identification of the resultant color of the chromophore groups. The physicochemical parameters were tested for the optimum pH, osmolarity/osmolality, and concentration. We

performed solubility tests in water and polyvinyl alcohol, with ultraviolet spectrophotometry, and analyzed blends comprised of different colors within the visible spectrum.

### Preparation

For the final formulation, the active component BB G-B0770™ (Sigma-Aldrich, St. Gallen, Switzerland) and lutein and zeaxanthin were weighed using the H33AR™ balance (Mettler Toledo, Barueri, Brazil), and when stirred with the Q298-1™ device (Quimis, Diadema, Brazil), dissolved in a buffer solution (pH, 7.0–7.4); the pH was checked using the Q600M device™ (Quimis), and balanced saline solution (BSS) served as a control for the osmolarities. The syringes were filled in a sterile area with one 0.5-ml dose of the dye solution, and each syringe was connected to a rubber stopper and then crimped. The final solution was sterilized by autoclaving for 20 min at 121 °C in the Controller Odt-74 version 2.0™ (Wilson Autoclaves, São Paulo, Brazil).

The final dye solution contained LZ 1 % and BB 0.025 % (osmolarity, 280 mOsm; density, 1.1; pH, 7.00). The new 0.5-ml syringes were bottled and stored at 4–8 °C, and used within 30 days of production.

### Inclusion and exclusion criteria

Patients of both genders were included if they were older than 18 years, not pregnant, and required an elective vitrectomy to treat a macular hole of any size and any BCVA level or an ERM with a BCVA below 20/25. Patients were excluded if they had any ocular condition that could limit or affect the postoperative results.

### Physical evaluation and ocular examinations

All patients underwent a physical evaluation preoperatively that included measurement of the systemic blood pressure and heart rate. An ocular examination was performed preoperatively and 1, 7, and 30 days postoperatively, which included spectral-domain optical coherence tomography (SD-OCT) and fluorescein angiography (FA) using the Spectralis device (Heidelberg Engineering, Heidelberg, Germany), unless media opacity was present.

The BCVA was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, and the intraocular pressure (IOP) was measured using a Goldmann tonometer; a slit-lamp evaluation also was performed.

### Surgical procedures

Eighteen surgeons experienced in current chromovitrectomy performed the standardized surgical procedures. Phakic patients underwent standard combined phacoemulsification with

**Table 1** Patient demographic data

Patient	Preop BCVA	Postop BCVA	Preop IOP	Postop IOP	Preop biomicroscopy finding	Postop biomicroscopy	Preop funduscopy finding	Postop funduscopy finding
1	03	23	10	10	Cataract	Pseudophakic	MH	None
2	20	20	12	18	Cataract	Pseudophakic	MH	None
3	35	35	10	14	Cataract	Pseudophakic	ERM	None
4	15	15	12	13	Cataract	Pseudophakic	MH	None
5	37	38	14	14	Cataract	Pseudophakic	MH	None
6	12	13	10	11	Cataract	Pseudophakic	MH	MH
7	12	20	18	16	RK scars, Cataract	RK scars, Pseudophakic	MH	None
8	1	2	13	16	Cataract	Pseudophakic	MH	None
9	2	3	10	15	Cataract	Pseudophakic	MH	MH
10	3	13	20	12	Cataract	Pseudophakic	MH	MH
11	3	8	18	15	Cataract	Pseudophakic	MH	None
12	2	9	19	17	Cataract	Pseudophakic	MH	None
13	8	8	12	14	Cataract	Pseudophakic	MH	MH
14	5	5	13	14	Cataract	Pseudophakic	MH	MH
15	1	6	15	15	Cataract	Pseudophakic	MH	None
16	13	15	13	10	Cataract	Pseudophakic	MH	MH
17	23	31	18	12	Cataract	Pseudophakic	MH	None
18	2	3	12	12	Pseudophakic	Pseudophakic	ERM	None

*Preop* preoperatively, *postop* postoperatively, *RK* radial keratotomy, *MH* macular hole

IOL and then 3-port pars plana vitrectomy using 23-gauge instruments. After this, 0.3 ml of the new dye was delivered gently through the posterior pole using a 23-gauge soft-tip cannula (Synergetics, O'Fallon, MO, USA). Flushing of the

**Table 2** Preoperative and postoperative OCT and AF findings

Patient	OCT findings preoperatively			OCT findings postoperatively			Preop AF findings	Postop AF findings
	Base diameter	Minimal diameter	Height	Base diameter	Minimal diameter	Height		
1	MH 473	273	407	None			Window defect	Normal
2	MH 1,218	958	452	None			Window defect	Normal
3	ERM			None			Normal	Normal
4	MH 830	625	344	None			Window defect	Normal
5	MH 836	619	392	None			Window defect	Normal
6	MH 1,135	791	351	MH 801	526	410	Window defect	Window defect
7	MH 633	467	399	None			Window defect	Normal
8	MH 996	558	392	None			Window defect	Normal
9	MH 2,071	726	516	MH 533	647	228	Window defect	Window defect
10	MH 1,239	689	367	MH 718	520	357	Window defect	Window defect
11	MH 588	471	387	None			Window defect	Normal
12	MH 1,172	749	450	None			Window defect	Normal
13	MH 912	629	342	MH 632	425	298	Window defect	Window defect
14	MH 852	563	379	MH 458	419	268	Window defect	Window defect
15	MH 821	534	347	None			Window defect	Normal
16	MH 1,032	618	366	MH 613	481	257	Window defect	Window defect
17	MH 461	214	398	None			Window defect	Normal
18	ERM			None				Normal

*Preop* preoperatively, *postop* postoperatively, *AF* autofluorescence, *MH* macular hole

dye was not needed because it was heavier than BSS (density, 1.1). After the vitreous was removed, a second flush of 0.3 ml of the combination dye was delivered through the posterior pole using the same technique as described previously. Octafluoropropane 15 % was injected into the vitreous cavity. The patients were instructed to remain prone for 3 days postoperatively.

#### Questionnaire

After each procedure, the surgeons completed a questionnaire that compared the ability of the combination dye to stain the intraocular structures. The findings were compared with the surgeons' experience with the dyes currently used in chromovitrectomy (Table 3).

#### Follow-up

The patients underwent a complete ophthalmologic examination on days 1, 7, and 30 postoperatively that included measurement of the BCVA, IOP measurement, and a slit-lamp evaluation. SD-OCT of the macular area was performed when the media was transparent preoperatively, and on days 1, 7, and 30 postoperatively. On postoperative day 30, fundus imaging, FA, and autofluorescence were performed. A parametric tailed Student's *t*-test was used for statistical analyses of the BCVA improvement and IOP variations.  $P < 0.05$  was considered statistically significant.

### Results

The final solution of the combination dye contained soluble LZ 1 % and BB 0.025 % (osmolarity, 280 mOsm; density, 1.1; pH, 7.00) and resulted in a green solution.

#### Clinical and intraoperative findings

Each of the 18 surgeons performed surgery on only one patient in this series, and no systemic effects were noted during the follow-up period. With regard to the causes leading to surgery, MH was diagnosed in 16 patients and ERM was observed in two eyes. The mean baseline number of ETDRS letters was  $10.6 \pm 11.6$  (range, 0–37) and the mean number of ETDRS letters on day 30 postoperatively was  $12.4 \pm 10.6$  (range, 0–35). All eyes were followed for 30 days.

#### Intraoperative findings

Phaco-vitreotomy was performed in 17 eyes, and vitrectomy alone in one pseudophakic eye. All procedures were performed uneventfully. Five intraoperative characteristics of the new dye were observed: first, the solution consisted of

soluble orange LZ 1 % previously mixed with blue BB 0.025 %, which resulted in a green solution (Fig. 1). Second, the solution was deposited on the posterior pole due its higher density than BSS, and there was no need to flush vigorously (Fig. 2). Third, the dye indirectly showed the posterior hyaloid and the vitreous base by deposition of soluble lutein in the posterior vitreous. Fourth, the solution stained the ILM a marked greenish-blue (Fig. 3). Fifth, the solution had poor affinity for the ERM.

#### Follow-up

On postoperative day 30, no dye-related abnormalities were observed by FA, autofluorescence, or OCT. MH persisted in six cases, and IOP did not change significantly ( $P = 0.6061$ ) (Table 1). No dye-related changes were observed by biomicroscopy or funduscopy, and no adverse event occurred during the follow-up period.

#### Questionnaire

The responses to the questionnaire that compared the ability of the soluble LZ-based dye to stain the distinct intraocular structures with the experience of the 18 surgeons who had been using the “gold-standard dyes” during chromovitrectomy indicated that all surgeons considered that the ILMs were stained similarly to their experience using BB; All surgeons considered satisfactory the staining of the dye; 44.4 % considered the stain to be a weak greenish-blue and 55.6 % considered it an intense greenish-blue ( $P < 0.001$ ) (Table 3). Moreover, 94.4 % identified dye deposition in the vitreous ( $P < 0.001$ ), although this was less effective than their experience with TA (Table 3); no lutein crystals were present due to the soluble solution. At the end of the procedure, one surgeon reported that the dye was in the vitreous base in one patient (5.6 %;  $P < 0.02$ ). Other surgeons considered the wash-out satisfactory, with no evidence of the dye at the end of surgery (Table 3).

### Discussion

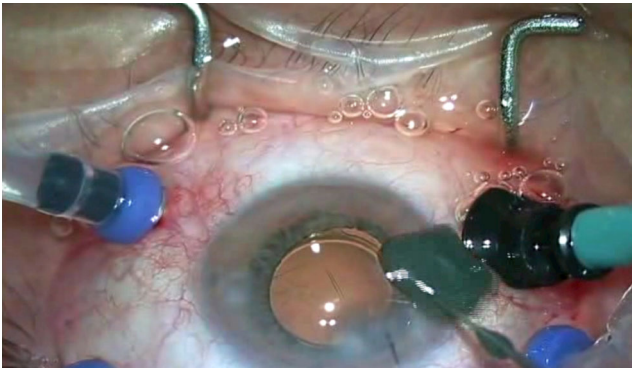
A previous study reported that a solution containing LZ 1 % crystals and BB 0.025 % efficiently stained the vitreous, ILM, and posterior hyaloid in cadaver eyes [18]. Clinical, histologic, and electroretinographic evaluations after intravitreal injections of these dye concentrations in rabbit eyes showed no signs of toxicity (unpublished data). In addition, lutein crystals 1 % in combination with BB 0.025 % also were used safely in 12 eyes and were advantageous intraoperatively because only one dye was used to remove both the posterior hyaloid/vitreous base and the ILM; however, only one surgeon performed all the procedures [19]. All studies supported the

**Table 3** Surgeons' general impressions and the usefulness of the dye

Patient	ILM visibility with dye injection	Degree of dye impregnation in ERM	Degree of dye impregnation in ILM	Staining of other structures	Surgeon impression of dye usefulness	Dye coloration satisfactory for dyeing ERM	Dye color satisfactory for staining the ILM	Residual dye at end of surgery
1	Good	NA	Weak (light greenish-blue)	No	Good	NA	Yes	No
2	Good	NA	Weak (light greenish-blue)	No	Good	NA	Yes	No
3	Good	Intense (dark green)	Intense (dark greenish-blue)	No	Good	Similar	Yes	No
4	Good	NA	Intense (dark greenish-blue)	No	Good	NA	Yes	No
5	Good	NA	Intense (dark greenish-blue)	No	Good	NA	Yes	No
6	Good	NA	Weak (light greenish-blue)	No	Good	NA	Yes	No
7	Good	NA	Intense (dark greenish-blue)	No	Good	NA	Yes	Yes, vitreous base
8	Good	NA	Weak (light greenish-blue)	No	Good	NA	Yes	No
9	Good	NA	Weak (light greenish-blue)	No	Good	NA	Yes	No
10	Good	NA	Intense (dark greenish-blue)	No	Good	NA	Yes	No
11	Good	NA	Intense (dark greenish-blue)	No	Good	NA	Yes	No
12	Bad	NA	Weak (light greenish-blue)	No	Good	NA	Yes	No
13	Good	NA	Intense (dark greenish-blue)	No	Good	NA	Yes	No
14	Good	NA	Weak (light greenish-blue)	No	Good	NA	Yes	No
15	Good	NA	Weak (light greenish-blue)	No	Good	NA	Yes	No
16	Good	NA	Intense (dark greenish-blue)	No	Good	NA	Yes	No
17	Good	NA	Intense (dark greenish-blue)	No	Good	NA	Yes	No
18	Poor	Not satisfactory	Intense (dark greenish-blue)	No	Bad	No	Yes	No

NA not applicable



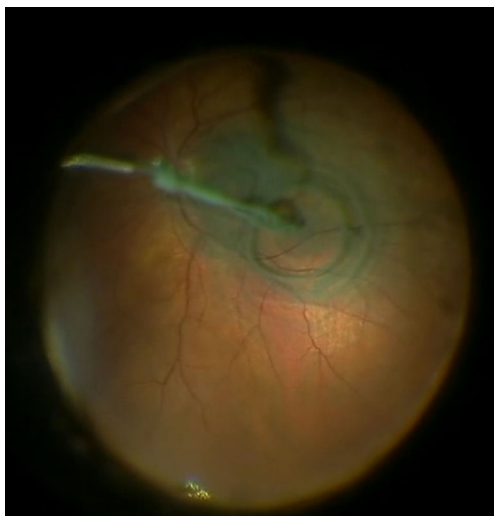


**Fig. 1** Solution consisting in the mixture of the soluble orange LZ 1 % BB 0.025 % that resulted in a green solution

decision to test the soluble formulation of LZ 1 % and BB 0.025 % during chromovitrectomy in human eyes, resulting a final green solution (Fig. 1) that was denser than BSS and did not require flushing of the posterior pole (Fig. 2).

In the current study, the posterior hyaloid and the vitreous base were identified easily after deposition of the LZ dye in 18 eyes. The ILM was identified easily and peeled promptly using an intraocular forceps (Fig. 3). The possibility of performing all the chromovitrectomy steps using only one dye for different structures is convenient, because currently two different substances are used for these distinct surgical maneuvers, i.e., TA is used to identify the posterior hyaloid/vitreous base and BB/ICG is used to identify the ILM [20].

In addition, because the LZ molecule has a physiologic antioxidant effect, the theoretical safety profile of the current dye may increase because the singlet oxygen generated by the BB molecule exposed to the endoillumination light may be quenched by the lutein molecule. Further basic and clinical studies are needed to validate this hypothesis [18, 21].



**Fig. 2** Intraoperative view of the injection. The solution is deposited on the posterior pole due to its higher density than BSS with no need for vigorous flushing

The BCVA improved compared with baseline in almost three-quarters (72 %) of patients, and the BCVA did not change in 28 % compared to the initial BCVA. No signs of toxicity [including retinal pigment epithelial (RPE) abnormalities at the subfoveal area] were seen during the 30-day follow-up period during ocular examinations, OCT, FA, and autofluorescence. The limited BCVA improvement should be correlated with the short follow-up period of 30 days and the inclusion criteria that allowed inclusion of eyes with chronic macular holes and/or a chronic ERM, which may limit visual improvement (Table 2).

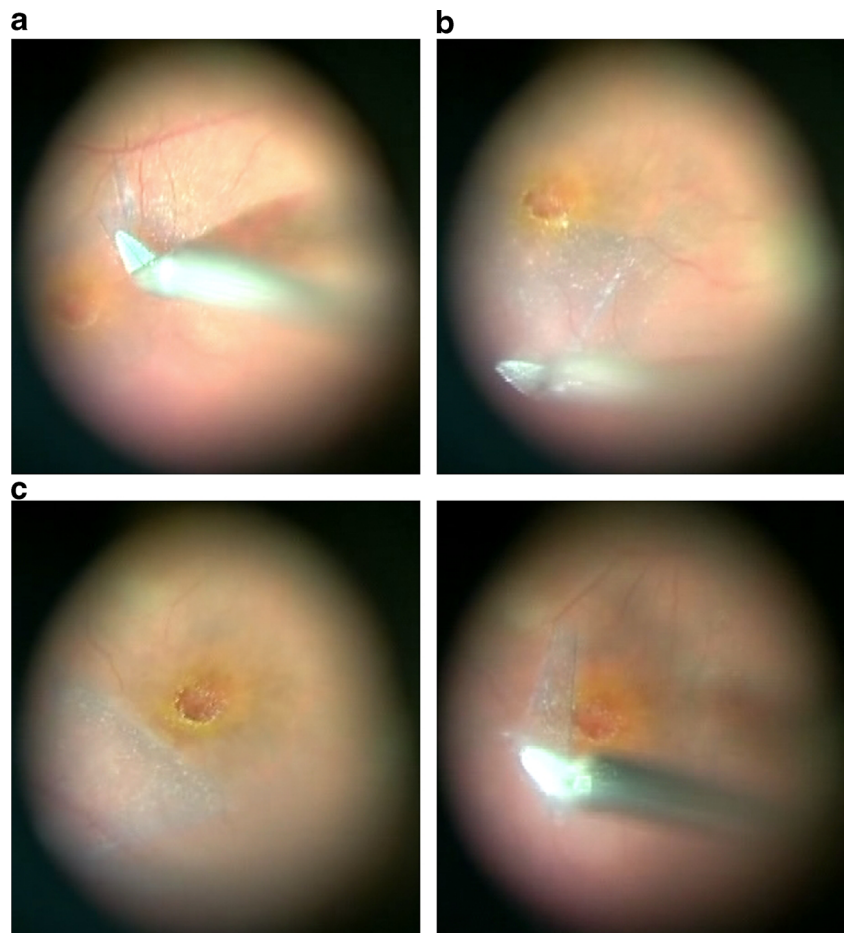
The observation that six eyes had an unsealed macular hole may be related to the lack of postoperative positioning, size, and chronicity of the holes. The preoperative BCVA, mid-hole diameter, and base-hole diameter are correlated with anatomic success in MH surgery. The surgical prognosis is good for MHs with a mid-hole diameter from below 400 to 500  $\mu\text{m}$  on OCT and base-hole less than 1,000  $\mu\text{m}$ . In the current study, the macular holes persisted in six cases after vitrectomy. All patients had a macular hole larger than those values and a baseline BCVA worse than 20/400 (Tables 1 and 2). The BCVA of these patients remained unchanged despite a reduction in the diameter of macular hole.

The short follow-up period and the small number of eyes did not allow definitive conclusions about the dye. However, possible toxic effects of the dye in this series were unlikely based on the absence of deterioration in VA compared to baseline, absence of an inflammatory reaction by ophthalmic examinations, and favorable results of autofluorescence and FA.

In addition to the theoretical advantages of the use of a LZ-based dye for chromovitrectomy, three major differences were evident when the new dye was compared with the dyes currently used during chromovitrectomy. First, because the combination dye is heavier than BSS, it was deposited gently onto the posterior pole by turning off the infusion and positioning the soft-tip cannula at the center of the vitreous cavity; this avoided unnecessary aggressive maneuvers such as dye flushing into the posterior pole or an fluid–air exchange. Interestingly, the injection of the green dye into the vitreous cavity resulted in rapid deposition onto the posterior pole (Fig. 2). All surgeons except one considered that the dye was useful, and the ILM was stained greenish-blue in all eyes.

Second, the dye administration resulted in the deposit of the soluble LZ over the posterior hyaloid and within the peripheral vitreous fibers, leading to a green appearance of these structures. This resulted in easier manipulation of the posterior vitreous and the vitreous base; however, the surgeons reported that hyaloidal detachment and shaving of the vitreous base were more difficult than when using TA alone, suggesting that soluble LZ is also useful for this surgical maneuver, although less efficient than TA alone [20]. All surgeons identified the deposition of the dye in the vitreous, and 55.6 % reported intense deposition.

**Fig. 3** Dye deposition at the ILM in an eye with a macular hole. **a** An intraoperative image shows the initial removal of the ILM stained greenish-blue. **b, c** The intermediate phase of ILM peeling shows the tip of the forceps grasping the greenish-blue-stained ILM. **c** The final stage of ILM peeling



Third, the ability of the dye to stain the ILM resulted in easier ILM peeling in eyes with macular holes (Fig. 3). There was no evidence that intraocular lutein and zeaxanthin affected the IOP or induced an inflammatory reaction.

The lutein and zeaxanthin molecules absorb light in the visible spectrum (around 446 nm), which coincides with the blue region of the electromagnetic spectrum. For this reason, lutein and zeaxanthin may be natural absorbers of blue light, which may decrease harmful effects on the lens and retina [22]. However, this possible protective effect may be better elucidated by additional basic and clinical studies. ILM peeling guided by ICG staining currently is performed worldwide to treat macular holes, especially in the United States [20]; however, RPE atrophy may occur after ILM peeling guided by ICG staining during macular hole surgery, because of the high osmolarity of the ICG solution, decomposition of ICG, iodine effect, carbolic complex formation, and the oxidative effect due to singlet-oxygen release from ICG after light exposure from the light pipe during PPV [23–25]. We hypothesized that the combination of lutein, zeaxanthin, and BB in one dye solution may have a good safety profile, and provides antioxidant and light-scavenging properties to protect the retina and RPE.

The study limitations were the small number of eyes, the absence of a control group, and the fact that multifocal electroretinography and microperimetry were not performed.

A more extensive evaluation of potential toxicity would probably have limited value, since alterations secondary to ILM peeling (macular manipulation) could lead to inconclusive results. In addition, the main objective of this study was to evaluate peeling of the ILM and ERM in humans during chromovitrectomy performed by different surgeons; because 18 surgeons performed the procedures, the potential for erroneous personal impressions about the staining capacity of the dyes was minimized.

In summary, the current study showed that a new dye comprised of soluble LZ 1 % and BB 0.025 % is useful for chromovitrectomy. The dye improved the intraoperative identification of the ILM similar to BB and the posterior hyaloid/vitreous base identification. Although less effective than TA, the combination solution facilitates injection of only one heavy solution into the posterior pole, and has a theoretically good safety profile. The different surgeons found that the dye might be useful for vitreoretinal surgery, with improved ILM peeling in 100 % of eyes and improved removal of the posterior hyaloid/vitreous base in

94.4 % of eyes. Additional multicenter studies are needed to confirm these findings.

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**Proprietary interest** The use of the combination of lutein/zeaxanthin and brilliant blue in human eyes is protected by international patent law (international patent number 61/468,838). The study was sponsored by Kemin Inc., Des Moines, IA, USA.

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