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Novel 'heavy' dyes for retinal membrane staining during macular surgery: multicenter clinical assessment

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ABSTRACT.

Purpose: **To evaluate the feasibility of two novel 'heavy' dye solutions for staining the internal limiting membrane (ILM) and epiretinal membranes (ERMs), without the need for a prior fluid-air exchange, during macular surgery.**

Methods: **In this prospective nonrandomized multicenter cohort study, the high molecular weight dyes ILM-Blue™ [0.025% brilliant blue G, 4% polyethylene glycol (PEG)] and MembraneBlue-Dual™ (0.15% trypan blue, 0.025% brilliant blue G, 4% PEG) were randomly used in vitrectomy surgeries for macular disease in 127 eyes of 127 patients. Dye enhanced membrane visualization of the ILM and ERMs, 'ease of membrane peeling', visually detectable perioperative retinal damage, postoperative best-corrected visual acuity (BCVA), dye remnants and other unexpected clinical events were documented by 21 surgeons.**

Results: **All surgeries were uneventful, and a clear bluish staining, facilitating the identification, delineation and removal of the ILM and ERMs, was reported in all but five cases. None of the surgeries required a fluid-air exchange to assist the dye application. BCVA at 1 month after surgery improved in 83% of the eyes in the MembraneBlue-Dual™ group and in 88% in the ILM-Blue™ group. No dye remnants were detected by ophthalmoscopy, and no retinal adverse effects related to the surgery or use of the dyes were observed.**

Conclusion: **The 'heavy' dye solutions ILM-Blue™ and MembraneBlue-Dual™ can be injected into a fluid-filled vitreous cavity and may facilitate staining and removal of the ILM and/or ERMs in macular surgery without an additional fluid-air exchange.**

Key words: brilliant blue G – epiretinal membrane – internal limiting membrane – macular surgery – polyethylene glycol (PEG) – trypan blue

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Introduction

As epiretinal membranes (ERMs), the internal limiting membrane (ILM) and the vitreous cortex are essentially transparent tissues, nontraumatic removal may be challenging in various types of macular surgery. In 1998, Melles et al. introduced the use of vital dyes for staining (the capsulorhexis and) epiretinal membranes, allowing for better visualization of these tissues during vitrectomy, and selective 'membrane peeling' from the underlying retina (Melles 1999; Melles et al. 1999).

Veckeneer, Feron, Stalmans and Teba et al. pioneered the use of trypan blue (TB) for selective staining of ERMs and the ILM (Feron et al. 2002; Teba et al. 2003; Veckeneer et al. 2001). However, while awaiting FDA approval for trypan blue for use in 'chromovitrectomy', surgeons started using off-label indocyanine green (ICG) in macular hole surgery, indicating the demand for tissue

staining in removal of the ILM (Burk et al. 2000; Da Mata et al. 2001; Gandorfer et al. 2001a; Kadonosono et al. 2000; Kusaka et al. 2001; Kwok et al. 2001; Stalmans et al. 2001). Clinical observation showed that ICG was more effective in staining the ILM than TB, while TB had better affinity for epiretinal membranes (Teba et al. 2003; Enaida et al. 2006a,b). Alternative dyes such as brilliant blue G (BBG) were later preferred over ICG because of the potential toxicity of the latter dye (Engelbrecht et al. 2002; Gandorfer et al. 2001b; Sippy et al. 2001; Weinberger et al. 2001). To further improve the staining effect of TB and BBG, a fluid-air exchange is commonly performed. This limits swirling of the dye within the fluid-filled vitreous cavity and achieves a more concentrated dye near the target tissue. However, a fluid-air exchange may jeopardize subsequent visualization of the macula during peeling due to clouding of the posterior lens capsule and may relate to postoperative visual field defects (Hasumura et al. 2000; Hirata et al. 2000; Yang et al. 2006).

To overcome these problems, sequential 'double' staining (Stalmans et al. 2003) and addition of glucose (Lesnik Oberstein et al. 2007) or deuterium oxide (D_2O) (Gerding et al. 2011) to increase the viscosity and/or density of the dye solution have been evaluated. Preferably, these combined, 'heavy' dyes should come as premixed, 'ready-to-use' dye solutions with acceptable shelf-life, good biocompatibility and CE and/or FDA approval. Recently, extensive laboratory studies have yielded two novel commercially available and CE-approved dye solutions, MembraneBlue-Dual™ and ILM-Blue™, that may have a higher efficacy as a result of the synergenic effect through the use of two dyes within the same sample, combined with polyethylene glycol (PEG) to increase the molecular weight and viscosity, potentially eliminating the need for fluid-air exchange. The purpose of our study was to prospectively evaluate whether these novel, polyethylene glycol (PEG)-enriched, 'heavy' dye solutions (Membrane Blue-Dual™ and ILM-Blue™) effectively stained ERM's as well as ILM without prior fluid-air exchange.

Materials and Methods

A total of 127 eyes of 127 patients enrolled in this prospective study and were randomly assigned into two groups: Group I had macular surgery performed with the intra-operative use of MembraneBlue-Dual™, a solution with 0.15% trypan blue + 0.025% brilliant blue G + 4% polyethylene glycol (D.O.R.C. International, Zuidland, the Netherlands) (63 eyes, 35 male and 28 female, mean patient age 68 ± 1.3 years); and Group II with ILM-Blue™, a solution with 0.025% brilliant blue G + 4% polyethylene glycol (D.O.R.C. International) (64 eyes, 35 male and 29 female, mean age 68 ± 1.3 years) (Table 1). The study protocol was subjected to IRB review and all patients signed an IRB-approved informed consent. Patients consented to prospective data collection, and the study was conducted according to the declaration of Helsinki.

Vitrectomy surgeries were performed by 21 surgeons in 20 centres (Fig. 1). In all cases, a 23- or 25-Gauge valved cannula 3-port pars plana vitrectomy was performed, during which a posterior vitreous detachment was created when needed. Without performing a prior fluid-air exchange, either 0.1 ml of MembraneBlue-Dual™ (Fig. 2A) or of ILM-Blue™ (Fig. 2B) was applied onto the macula (while the vitreous cavity was completely filled with fluid), and all excess dye was immediately aspirated with a blunt back-flush instrument. In all cases, the intention was to completely remove epiretinal membranes as well as ILM in the central macular area. The stained ILM/ERMs were removed using routine surgical techniques, by engaging the tissue with a pick or hooked needle, peeling the tissue from the underlying retina, and removing it from the eye with an intraocular forceps (Fig. 2C,D). In eyes with a macular hole, the surgery was completed by gas tamponade (C3F8 or SF6). Simultaneous phacoemulsification with intraocular lens implantation was performed in eyes that also had a cataract (Group I: $n = 9$, Group II: $n = 20$).

To evaluate the efficacy of both dyes in the visualization of the ILM and/or ERMs, and the 'ease of membrane removal' after staining, these parameters were graded on a scale of 1 (poor) to 10 (excellent), by each surgeon for each individual intervention. Patients were examined before surgery, at the first postoperative day, and 1, 6 and 12 months after surgery. At each visit, the best-corrected visual acuity (BCVA), intraocular pressure (IOP), slit-lamp biomicroscopy and funduscopy details were documented. Particular attention was given to postoperative dye remnants, and/or unexpected clinical events possibly related to the use of dye. Surgeons were requested to report any signs of mechanical trauma such as 'pinch'

Table 1. Patient data.

	MembraneBlue-Dual™	ILM-Blue™	p
Number of patients included	63	64	
Age (mean ± SD) (years)	68 ± 1.3	68 ± 1.3	0.83
Male/female	35/28	35/29	0.92
OD/OS	29/34	37/27	
Lens status before surgery			
Phakic	34	27	
Pseudophakic	18	15	
Cataract	9	20	
Unknown	2	2	
Indication			0.26
Macular hole	9	25	
Macular pucker	45	28	
Macular oedema	3	3	
Retinal detachment	2	4	
Proliferative vitreoretinopathy	1	0	
Vitreomacular traction syndrome	0	4	
ERM with central vein occlusion	3	0	

Fig. 1. World map of participating surgeons and their location. (1) Marc Veckeneer, M.D., Oog ziekenhuis Rotterdam, the Netherlands; (2) Andreas Mohr, M.D., St. Joseph Stift, Bremen, Germany; (3) Essam Alharthi, M.D., Alhokama Eye Specialist Centre, Riyadh, Saudi Arabia; (4) Rajvardhan Azad, M.D., FRCSed., Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India; (5) Ziad F. Bashshur, M.D., American University of Beirut, Lebanon; (6) Enrico Bertelli, M.D., Div. Oculistica, Azienda Sanitaria dell'Alto Adige-Südtirol, Bolzano, Italy; (7) Riad A. Bejjani, M.D., Lebanese American University, Lebanon and Saint Joseph University, Beirut, Lebanon; (8) Brahim Bouassida, M.D., Clinique Ophthalmologique et O.R.L. De Tunis, Tunesia; (9) Dan Bourla, M.D., Rabin Medical Centre, Petah Tikva, Israel; (10) Iñigo Corcóstegui Crespo, M.D., Instituto Clínico Quirúrgico de Oftalmología, Bilbao, Spain; (11) Charbel Fahed, M.D., Lebanese American University of Beirut, Lebanon; (12) Faisal Fayyad, M.D., Jordan Hospital, Amman, Jordan; (13) Marco Mura M.D., Academic Medical Centre, University of Amsterdam, the Netherlands and Oogziekenhuis Zonnestraal, Hilversum, the Netherlands; (14) Jerzy Nawrocki, M.D., Ph.D., Klinika Okulistyczna, Okulistyczna 'Jasne Blonia', Lodz, Poland; (15) Kelvin Rivett, M.D., Medivision, Beacon Bay, South Africa; (16) Gabor B. Scharioth, M.D., Ph.D., Aurelios Augenzentrum, Recklinghausen, Germany; (17) Dmitry O. Shkvorchenko, M.D., S. Fyodorov Eye Microsurgery State Institution, Moscow, Russia; (18) Peter Szurman, M.D., Ph.D., Knappschafts krankenhaus Sulzbach, Sulzbach, Germany; (19) Hein Van Wijck, M.D., Keravision, Johannesburg, South Africa; (20) Ian Y. Wong, FHKAM and David S.H. Wong, FRCOphth., University of Hong Kong, China; (21) Johannes Frank, Ph.D., Delft University of Technology, the Netherlands; (22) Silke Oellerich, Ph.D., Marieke Bruinsma, Ph.D. and Gerrit R.J. Melles, M.D. Ph.D., Netherlands Institute for Innovative Ocular Surgery (NIIOS), Rotterdam, the Netherlands.

haemorrhages and local retinal tears. The clinical outcome at 1 month was used for comparison with the pre-operative data, because the use of gas tamponade in macular hole cases did not allow for reliable visual acuity measurements on the first postoperative day.

Statistical analysis

A total of 21 patients with incomplete follow-up or data collection were excluded from the study.

For statistical analysis, a *t*-test was conducted to detect differences regarding age, gender, indication for surgery, and pre-operative and postoperative BCVA, between Group I and II. Pearson correlation analysis was performed to assess the correlation between the efficacy of tissue staining and the 'ease of membrane peeling', the indication for surgery and efficacy of staining, and the indication for surgery and the 'ease of membrane peeling'. Linear regression analysis was conducted to evaluate whether the dye-choice or the indication for surgery related to the postoperative visual acuity. $p < 0.05$ was considered significant.

Results

Efficacy of staining and 'ease of membrane peeling'

In Group I (MembraneBlue-Dual™), efficacy of tissue staining was graded 8 (±2), and membrane removal 7 (±2), and in Group II, (ILM-Blue™) were graded 6 (±3) for both parameters (Table 2). Hence, the 'ease of membrane peeling' correlated with the density of staining achieved (Pearson correlation analysis; $p < 0.001$).

In both Group I and II, no correlation was found between the indication for surgery (Table 1) and the efficacy of staining ($p = 0.35$), or between the indication for surgery (Table 1) and 'ease of peeling' ($p = 0.72$). A second dye application was required in 25 (40%) cases in Group I and 21 (33%) cases in Group II (Table 2). In one centre, a secondary ICG application was performed after unsatisfactory staining with MembraneBlue-Dual™ (two cases) or ILM-Blue™ (three cases). After surgery, none of the eyes showed residual staining or dye remnants. No side-effects related to the intra-operative use of the dyes were observed. No signs of mechanical trauma related to membrane peeling were reported.

BCVA and IOP

In Group I, BCVA averaged 0.2 (±0.4) before, and 0.4 (±0.5) at 1 month after surgery (Table 3): BCVA improved in 52 eyes (83%) was stable in nine eyes (14%) and worsened one line in two eyes (3%). In Group II, BCVA averaged 0.15 (±0.5) pre-operatively, and 0.3 (±0.5) at 1 month (Table 3): BCVA improved in 55 eyes (88%) was stable in six eyes (9%), and worsened one line in three eyes (3%).

All patients had a normal IOP (Table 3), except for two eyes in Group I and one eye in Group II (Table 3), which showed a transient rise in IOP that returned to normal after topical treatment.

Between Group I and II, no differences were found in pre-operative BCVA ($p = 0.94$), postoperative BCVA ($p = 0.4$), pre-operative IOP ($p = 0.64$), postoperative IOP ($p = 0.32$), age ($p = 0.83$), gender ($p = 0.92$) or indication for surgery ($p = 0.26$). Postoperative BCVA did not correlate with the dye-choice or the indication for surgery.

Complete data files were available of 35 eyes reaching 6 months of follow-up and 28 eyes with 12 months

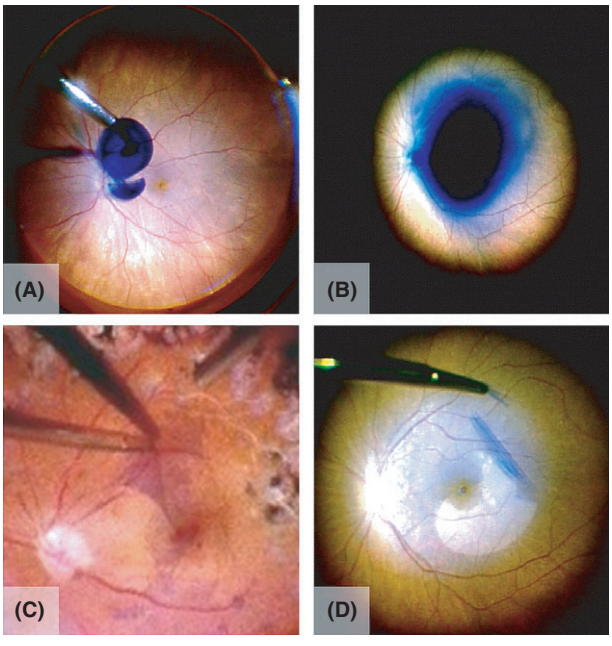

Fig. 2. Intra-operative images of (A) MembraneBlue-Dual™ and (B) ILM-Blue™ staining of the ERM and/or ILM. Before staining, the epiretinal tissue can hardly be visualized. After a core vitrectomy, a posterior vitreous detachment was induced. With the vitreous cavity filled with fluid, MembraneBlue-Dual™ (A) or ILM-Blue™ (B) was gently injected into the fluid-filled vitreous cavity, over the macular area. Note that these dyes immediately sink to the posterior pole without dispersing throughout the vitreous cavity. After the excess dye is aspirated, the size and extent of the ERM and ILM are clearly visible, following staining with MembraneBlue-Dual™ (C) or ILM-Blue™ (D).

Table 2. Staining and injection characteristics.

	MembraneBlue-Dual™ (n = 63)	ILM-Blue™ (n = 64)
Scoring 1–10: poor-excellent		
Staining result		
Mean ± SD	8 ± 2	6 ± 3
Range	4–10	1–10
Ease of membrane peeling		
Mean ± SD	7 ± 2	6 ± 3
Range	3–10	1–10
Second dye application	25	21

follow-up. No complications or side-effects related to the intra-operative use of the dye solutions were found.

Discussion

In the past decade, the use of vital dyes during vitrectomy has become standard practice for most surgeons. The currently available dye solutions, however, require application 'under air', show variable staining capacities and may sometimes have a questionable safety record. We therefore conducted extensive laboratory studies to determine which dyes can be combined to broaden the staining profile without the risk of chemical interaction between the dyes (NIIOS, unpublished). Furthermore, we investigated which high molecular weight additives would be most suitable for use in such a combined dye solution, to facilitate the application of the dye in balanced salt solution, that is, without performing a fluid-air exchange. Finally, it would be preferable that a solution is subjected to CE and/or FDA approval, to avoid variation in shelf-life and/or contamination of the dye solution, as has been reported with off-label use products (Centres for Disease Control and Prevention 2012).

In the current study, two 'novel' compound 'heavy' dye solutions, MembraneBlue-Dual™ and ILM-Blue™, were evaluated by a panel of experienced vitreo-retinal surgeons (Fig. 1). Our study showed that these solutions met a first objective, as none of the surgeries required a fluid-air exchange to obtain effective staining of the targeted area. If so, these 'heavy' dyes may be easier to use during vitrectomy than earlier solutions, while fluid-air exchange-related complications are avoided.

So far, the dyes commercially available were dissolved in balanced salt solution resulting in a density of the dye solutions similar to that in the infusion system. To facilitate the application, and to obtain sufficient intensity of staining, 'swirling' of the dye throughout the vitreous cavity was commonly avoided by performing a fluid-air exchange before injecting the dye (Engelbrecht et al. 2002; Gandorfer et al. 2001a; Sippy et al. 2001; Weinberger et al. 2001). However, because a fluid-air exchange may interfere with media clarity and may hold the risk of postoperative visual field defects, surgeons would want to avoid this manoeuvre (Hasumura et al. 2000; Hirata et al. 2000; Yang et al. 2006).

To increase viscosity and density of the dye solution, glucose (Lesnik Oberstein et al. 2007) or deuterium oxide (D_2O) (Gerding et al. 2011) has been added to the dye solution, to promote immediate settling of the dye onto the macula and to minimize dispersion throughout the vitreous cavity. With D_2O, the density of the dye solution is increased, but not its viscosity, so that the dye is dispersed throughout the vitreous cavity when the infusion line is opened. Alternatively, the addition of glucose to the dye solution improves both the viscosity and the density of the solution but the osmolarity may be increased to toxic levels (Costa et al. 2009). Also, most 'short-chain sugars' (glucose, maltose, etc.) show poor stability of the solution, rendering them less suitable for 'ready-to-use' dye solutions (Frank et al., unpublished).

From a panel of high molecular weight compounds, PEG was chosen as an additive to the dye solution to obtain effective tissue staining, but without the need for a prior fluid-air

Table 3. Pre-operative and 1 month postoperative evaluation results.

	MembraneBlue-Dual™ (n = 63)	ILM-Blue™ (n = 64)	p
BCVA pre-op			
Mean ± SD	20/100 (0.2 ± 0.4)	20/100 (0.15 ± 0.5)	0.94
Range	HM -20/25 (HM-0.7)	HM -20/40 (HM-0.5)	—
BCVA 1 month postop			
Mean ± SD	20/50 (0.4 ± 0.5)	20/60 (0.3 ± 0.5)	0.40
Range	CF -20/25 (CF-0.8)	20/200-20/20 (0.1–1)	—
AT pre-op			
Mean ± SD	15 ± 3.5	15 ± 3.4	0.64
Range	9–24	8–22	—
AT 1 month postop			
Mean ± SD	15 ± 3.7	14 ± 2.8	0.32
Range	8–24	11–24	—
Changes in BCVA			
Improved	52	55	
Stable	9	6	
Worsened	2	3	

CF = Counting fingers, HM = Hand movements.

exchange. A good biocompatibility and chemical stability (for acceptable shelf-life) of the PEG enriched dyes should make them suitable as 'ready-to-use' product, as PEG is an additive commonly used in human pharmacology products. PEG 3350 (as used in MembraneBlue-Dual™ and ILM-Blue™) has a molecular weight of 3350 Dalton resulting in an osmolarity of 0.012Osm for a 4% solution, 18 times lower than the osmolarity of a 4% glucose solution (0.22Osm). Thus, adding PEG 3350 increases the viscosity and density of the solution without significant impact on its osmolarity (Money 1989).

PEG also has several other advantages. First, PEG reduces the potential toxicity of a dye solution (Awad et al. 2011). A recent study on cytotoxicity in retinal pigment epithelial cell culture reported that dye solutions containing 4% PEG 3350 showed lower toxicity than those without PEG. Furthermore, using electrophysiological evaluation, good biocompatibility of Membrane-Blue-Dual™ and ILM-Blue™ has been found with application times up to 5 min (Januschowski et al. 2012).

Our study showed that both dyes investigated effectively stained (epi)retinal membranes during surgery in all eyes, except for five all operated on by the same surgeon, who used ICG as a back-up stain in these cases. Most surgeons reported that both dyes showed effective staining within approximately 15 seconds (although some cases required repeated injections) and that they could be injected into and aspirated from the fluid-filled vitreous cavity while the infusion line remained open. Swirling of the dye may also have been limited by the use of the valved cannula system.

As the tissue composition of various ocular structures such as the vitreous cortex, epiretinal membranes or the ILM may differ greatly, it may be expected that staining patterns also differ among these tissues, and between disease entities, severity and stage. With regard to PVR, the cellularity of the membrane as compared to the fibrous extracellular matrix components depends on the phase of the wound healing and with the progression of the disease, the affinity of a specific dye will also vary. Oberstein et al. (2011) reported that cellularity and active proliferation is particularly pronounced in 'fresh' PVR, whereas 'older' membranes are much less active. Intra-operative application of trypan blue selectively stains degenerating cells which are usually present in PVR membranes but particularly common in 'older' membranes. This agrees with the fact that TB usually stains these membranes more intensely at the time of oil removal (Feron et al. 2002). However, the efficacy of TB may vary with the fibrotic component of ERM, showing less intense staining with less cellular or reactive tissue elements. Hence, the 'naked' ILM, essentially an a-cellular matrix, is better visualized with ICG or BBG. In most macular diseases, however, ILM contains some degree of ERM as was demonstrated by Kenawy et al. (2010), and Gandorfer et al. (2005, 2012). These observations have added to the debate about whether a 'naked' ILM that does not contain any ERM (and would stain poorly with TB) should be removed to begin with.

To improve the overall staining effect, a combination of several dyes has been suggested. Stalmans et al. (2003) reported the technique of sequential 'double' staining using TB for ERM peeling, followed by ICG to visualize ILM. More recently, the combination of TB with BBG has been investigated (Awad et al. 2011). The aim of the current study was to expand on a mixed dye solution that would provide a broader range of tissue staining, while allowing discrimination between the various tissues. Although overlapping to some extent, both dye solutions investigated may have a different use in application. ILM-Blue™ may be primarily indicated for retinal disease with a minor fibrotic component, such as ILM removal in macular hole surgery. MembraneBlue-Dual™ was found to have a broader scope of application, rendering it suitable for more complex retinal disease, with either variable disease stages, different types of (fibrotic) membranes or a more extensive surface area of pathology. However, our study also showed that Membrane-Blue-Dual™ may better facilitate macular membrane peeling. Owing to the addition of trypan blue, the area of ILM covered with ERM does not demonstrate the 'negative' staining as is usually seen with the use of ICG or BBG alone (Park et al. 2008; Schumann et al. 2010).

Although the long-term studies are warranted to determine the safety of the dyes investigated, no intra- or postoperative complications or side-effects related to the use of the dyes were reported up to 12 months after surgery. With an improved BCVA at 1 month after surgery in 80–90% of cases, both novel dyes solutions appeared effective and user-friendly in vitreo-retinal macular surgery.

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Conflict of interest

Dr Melles is a consultant for D.O.R.C. International/Dutch Ophthalmic USA; Dr Mohr has obtained reimbursement for travel expenses connected to products of D.O.R.C. No conflicting relationship exists for any other author.

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