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## Brilliant Blue G assisted Epiretinal Membrane Surgery

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SUBJECT AREAS:

PHASE IV TRIALS

RANDOMIZED CONTROLLED TRIALS

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We report intensely staining epiretinal membrane (ERM) with Brilliant Blue G (BBG) under air for two minutes. ERM peeling was performed in 21 cases. After removal of posterior hyaloid, 0.2 mL BBG was first applied on the macula, to stain ERM under air conditions for 2 minutes. Internal limiting membrane (ILM) was intensely stained and peeled in all cases following ERM removal. In 4 cases, the ERM was also observed to be intensely stained with BBG and peeled with an ILM forceps. Postoperatively, the ganglion cell layer thickness was lower in three of the cases, however VA improved in all cases and multifocal electroretinogram revealed no toxicity. Light microscopy of ERM revealed masses of cells whereas; the ILM did not. The increased staining characteristics of ERM and ILM may be resulted from longer contact time of BBG under air pressure.

In macular surgeries, the intravitreal application of brilliant blue G (BBG) has gained popularity to visualize the internal limiting membrane (ILM). In contrast to other vital dyes such as indocyanine green (ICG), BBG was shown to have a good safety profile providing satisfactory anatomical and functional postoperative results<sup>1,2</sup>. However, the presence of epiretinal membrane (ERM) formation may impair sufficient staining and complete removal of the ILM, which may result ERM recurrences due to residual ILM fragments including indefinite extent of cells and collagen remnants at the vitreal side of the ILM<sup>3</sup>. In a previous study Shimada et al.<sup>4</sup> found BBG has high affinity to ILM and low affinity to ERM. Additionally, Schumann et al.<sup>5</sup> reported BBG showed no staining with ERM specimens intra-operatively. In this report, we present our observations about BBG's staining features for ERM, and outcomes of the longer exposure time for BBG under air pressure using optical coherence tomography (OCT) and multifocal electroretinogram (mfERG).

## Results

In 4 cases ERM was intensely stained with BBG. Table 1 summarizes best corrected visual acuity (BCVA) measurements and OCT results of the patients preoperatively and postoperatively at 1 month. In all cases the BCVA improved, central macular thickness (CMT) decreased, however, the macular ganglion cell-inner plexiform layer (GC-IPL) thickness decreased postoperatively in three cases. mfERG revealed no toxicity at post-operative first month (Table 2). In all cases P1 amplitude improved and the P1 implicit time reduced at the first month of the surgery (Fig. 1 right, Fig. 1 left).

Histopathologic examination of the ERM revealed fibroblast like cells along with extensive connective tissue. The ILM was absent of cells and a small amount of connective tissue was observed (Fig. 2).

## Discussion

ERM recurrence is observed in approximately %10 of cases after surgery<sup>6</sup>. The reasons for recurrence are the incomplete removal of the ERM and the persisted ILM after ERM peeling, even the ERM seems to have been completely peeled<sup>3</sup>. To enhance the visualization of these transparent or semitransparent structures and to overcome ERM recurrence, various staining methods have been used, including indocyanine green (ICG), trypan blue (TB), triamcinolone acetonide (TA), and brilliant blue G (BBG)<sup>7</sup>. Of these stains, BBG shows no retinal toxicity or adverse effects related to the dye were observed in animal and human studies<sup>2</sup>.

ILM that persists after ERM peeling acts as a scaffold for cell proliferation, leading to ERM recurrence. In a previous study, Shimada et al.<sup>4</sup> found that BBG has low affinity to ERM and recommended the reapplication of BBG after ERM peeling to remove the residual ILM to reduce the risk of ERM recurrence. Soon after Schumann et al.<sup>5</sup> reported that BBG showed no staining with ERM specimens intra-operatively because massive epiretinal



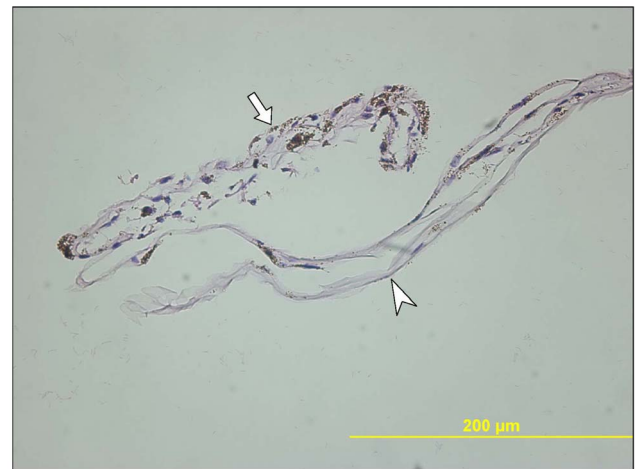
**Table 1 | VA measurements and OCT results of the patients preoperatively and postoperatively at 1 month**

	VA (LogMAR)	CMT ( $\mu\text{m}$ )	GCA ( $\mu\text{m}$ )
<b>Case 1</b>			
Preoperative	0.4	340	72
Postoperative	0.6	285	70
<b>Case 2</b>			
Preoperative	0.6	365	64
Postoperative	0.7	325	53
<b>Case 3</b>			
Preoperative	0.4	410	68
Postoperative	0.5	320	36
<b>Case 4</b>			
Preoperative	0.5	450	28
Postoperative	0.7	300	34

VA: Visual Acuity; CMT: Central macular thickness; GCA: Ganglion cell analyzes.

cell and collagen proliferation prevented a positive staining with BBG. They also noted that after having peeled the unstained ERM, the residual ILM was selectively stained by BBG even when a small amount of cells and collagen adheres to its vitreal side. In this study, BBG has shown low affinity to ERM in the majority of the eyes, whereas intensely stained it in 4 of 21 cases (%19).

In previous reports<sup>4,5</sup> BBG was applied into the fluid filled vitreous cavity and washed out immediately. In the present report the intense staining of ERM with BBG may be due to longer application time of



**Figure 2 | Histopathologic findings of a peeled epiretinal membrane specimen revealed masses of cells and collagen (white arrow) whereas internal limiting membrane seen devoid of cells and collagen (white arrowhead).**

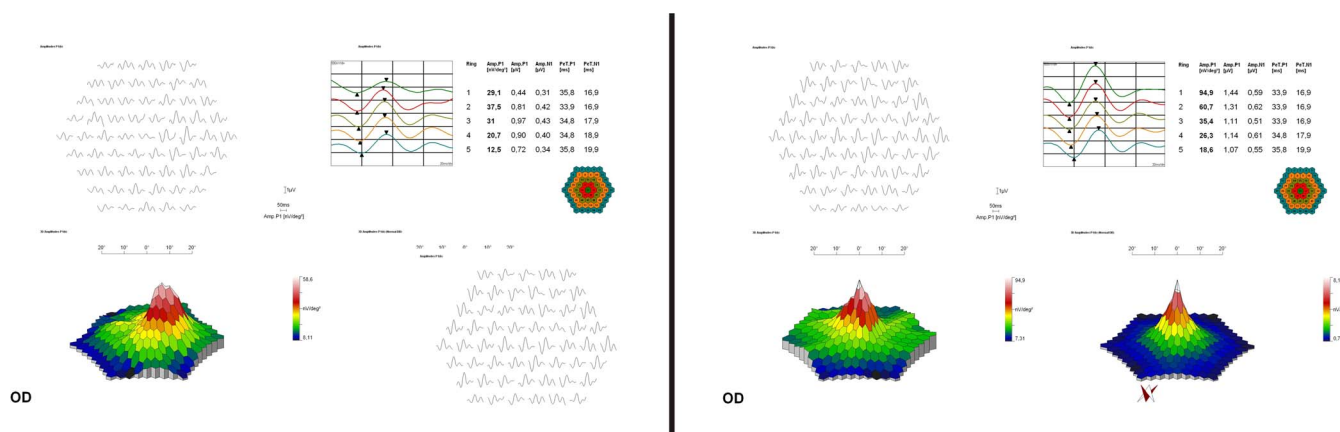
the dye under air pressure, and/or different collagen and other matrix constituents within the ERM<sup>8</sup>.

In a previous report, the ganglion cell (GCC) thickness in the postoperative period was significantly lower than preoperative, in patients underwent macular hole surgery with ICG. This result was

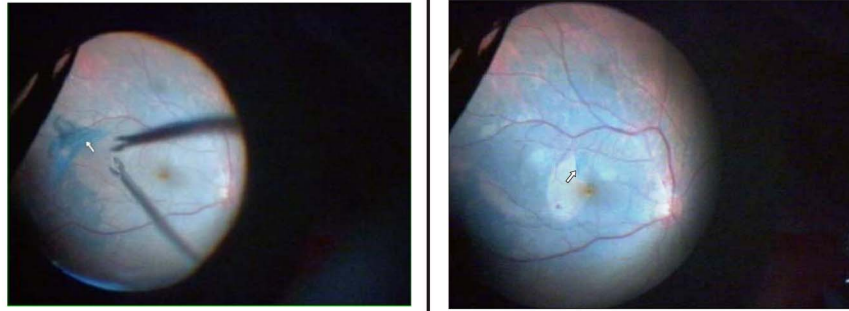
**Table 2 | mfERG values of the patients preoperatively and postoperatively at 1 month**

	Area 1		Area 2	
	Preop mfERG	Postop mfERG	Preop mfERG	Postop mfERG
<b>Case 1</b>				
Amp (nV/deg <sup>2</sup> )	78.7	103.2	63.8	64.7
IT (ms)	34.8	32.9	34.8	33.9
<b>Case 2</b>				
Amp (nV/deg <sup>2</sup> )	87.9	102.7	54.4	72.2
IT (ms)	36.8	35.8	36.8	38.8
<b>Case 3</b>				
Amp (nV/deg <sup>2</sup> )	50.2	108.8	27.4	43.3
IT (ms)	38.8	34.8	34.8	33.9
<b>Case 4</b>				
Amp (nV/deg <sup>2</sup> )	69.9	84.8	25.7	32.7
IT (ms)	35.8	34.8	35.8	34.8

Amp: Amplitude; IT: Implicit Time; mfERG: Multifocal electroretinogram.



**Figure 1 | Preoperative mfERG of a patient (left figure). The same patient's postoperative mfERG. P1 amplitude improved, the P1 implicit time reduced, and the mfERG spikes increased at postoperative first month (right figure).**



**Figure 3** | BBG assisted ERM peeling. White arrow shows well stained ERM (left figure). BBG assisted ILM peeling. White arrow shows the well stained ILM after the ERM had been removed (right figure).

explained with the mechanical damage to the retina by ILM peeling or cytotoxicity of ICG<sup>9</sup>. In three of our cases, we observed a decrease in GC-IPL thickness. This result may be related to two possibilities; first the mechanical damage of the surgery as mentioned in a previous study. Second but the weaker possibility of BBG toxicity on GC-IPL, which still have not affected the mfERG results and BCVA in these cases.

An objective assessment of the visual function can be made by mfERG. Multifocal ERG selects the electrophysiological responses of multiple retinal locations of the macular and perimacular area, which are tested simultaneously, allowing functional mapping of the central retina. In our cases, mfERG functional results also showed an improvement at follow-up visit.

In conclusion it seems that the longer application time of BBG under air pressure may safely increase the ability for ERM and ILM staining. However, larger case series with longer follow-up periods are needed to confirm our findings.

## Methods

21 patients with idiopathic ERMs were examined prospectively before and after vitrectomy. All surgeries were performed at the Tugut Ozal University, Department of Ophthalmology between 2012 and 2013, by one experienced vitreoretinal surgeon (YT). We included eyes with idiopathic ERM with significant loss of visual acuity (20/40). Eyes with other macular pathology, history of ocular inflammation and previous ocular surgery and patients with systematic disorders affecting the eye, such as diabetes, were excluded from the study. Patients with ERM and lamellar hole were also excluded from the study. All patients were informed for the procedures of the study and gave their written consent.

We performed a standard 23 Gauge transconjunctival sutureless vitrectomy (TSV) with EIBOS wide field imaging system under 25 Gauge Torpedo minilight illumination in 21 cases with a diagnosis of ERM based on preoperative OCT. Following core vitrectomy and removal of posterior hyaloid, we injected 0.2 mL BBG with a concentration of 0.25 mg/mL, 0.025% (Brilliant Peel; Geuder, Heidelberg, Germany) on the macula under air conditions for 2 minutes and washed out with irrigation solution. ERM was observed to be intensely stained with BBG in 4 cases and peeled easily with an internal limiting membrane (ILM) forceps (Fig. 3 left). After the ERM was peeled the ILM was again stained with BBG and peeled easily and safely in the same fashion (Fig. 3 right).

Patients were examined preoperatively and at 1 month postoperatively. Examination included slit-lamp examination, fundus examination, the best corrected visual acuity (BCVA-as the logarithm of the minimum angle of resolution (logMAR)), OCT, and mfERG. For the OCT examination the Cirrus HD OCT (Carl Zeiss Meditec Inc., Dublin, California) was used to assess the central macular thickness and ganglion cell layer analyses (GCA). The prototype GCA algorithm, incorporated in Cirrus HD-OCT software Version 6, was used to process and measure the thickness of macular GC-IPL within a 14.13 mm<sup>2</sup> elliptical annulus area centered on the fovea.

For the mfERG, the RETiscan<sup>TM</sup> (Roland Consult system, GmbH, Wiesbaden) was used. Area 1 that corresponds to the 2.8 central degrees of the retina and area 2 that extends from 2.8 to 9 degrees from the center of the fovea were evaluated for the retinal toxicity. The major component P1 amplitudes and implicit times of responses were recorded and assessed.

In a randomly selected case, the stained ERM and ILM were also examined histopathologically. ERM and ILM specimen was fixed in 10% formalin in phosphate buffer (pH 7.4), embedded in paraffin, stained with hematoxylin and eosin, and then examined under a light microscope.

1. Enaida, H. *et al.* Preclinical investigation of internal limiting membrane staining and peeling using intravitreal brilliant blue G. *Retina* **26**, 623–30 (2006).
2. Remy, M. *et al.* An in vivo evaluation of Brilliant Blue G in animals and humans. *Br J Ophthalmol* **92**, 1142–147 (2008).
3. Kifuku, K. *et al.* Residual internal limiting membrane in epiretinal membrane surgery. *Br J Ophthalmol* **93**, 1016–019 (2009).
4. Shimada, H. *et al.* Double staining with brilliant blue G and double peeling for epiretinal membranes. *Ophthalmology* **116**, 1370–376 (2009).
5. Schumann, R. G. *et al.* Sequential epiretinal membrane removal with internal limiting membrane peeling in brilliant blue G-assisted macular surgery. *Br J Ophthalmol* **94**, 1369–372 (2010).
6. Grewing, R. & Mester, U. Results of surgery for epiretinal membranes and their recurrences. *Br J Ophthalmol* **80**, 323–26 (1996).
7. Kwok, A., Lai, T. Y. & Yuen, K. S. Epiretinal membrane surgery with or without internal limiting membrane peeling. *Clin Experiment Ophthalmol* **33**, 379–85 (2005).
8. Kritzenberger, M. *et al.* Different collagen types define two types of idiopathic epiretinal membranes. *Histopathology* **58**, 953–65 (2011).
9. Baba, T. *et al.* Reduction of thickness of ganglion cell complex after internal limiting membrane peeling during vitrectomy for idiopathic macular hole. *Eye (Lond)* **26**, 1173–80 (2012).

## Author contributions

Y.T. performed all ERM surgeries, E.G. helped to write the main manuscript text, and M.S.D. helped to perform examinations.

## Additional information

**Supplementary information** accompanies this paper at <http://www.nature.com/scientificreports>

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