

Brilliant Blue G for ILM Staining and Peeling

This potent stain is easy to handle and safe, with minimal side effects.

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The internal limiting membrane (ILM) is a basement membrane for the Mueller cells of the retina. Cellular proliferation on the ILM causes distortion of the membrane, which can lead to the formation of epiretinal membranes (ERM) and macular holes. The removal of the ILM can cure both ERM and macular holes; however, the transparent nature of the ILM makes it difficult to peel.

The use of surgical adjuvants is now widely accepted to improve the poor visibility of the ILM and ERM.¹⁻⁴ The introduction of indocyanine green (ICG) and trypan blue initially helped to facilitate the peeling of the ILM during surgery, and these dyes are still widely used in clinical practice;⁵⁻⁷ however, numerous reports, both clinical and preclinical, have noted the potential retinal toxicity of both staining agents.⁸⁻²⁵ Additionally, the poor stain ability of trypan blue requires a complex air-fluid exchange technique that would not be necessary with a dye with higher staining ability. Although other dyes have been introduced, to date none have provided viable alternatives to the potentially toxic dyes in use. Therefore, a dye with both satisfactory staining ability and minimal toxicity is required for safe membrane staining and peeling. We have screened various dyes focusing on safety and stain ability of the ILM. From the results of our extensive preliminary analysis, we selected brilliant blue G 250 (BBG) as a potent candidate for ILM staining.²⁶⁻²⁹

CHARACTERISTICS OF BBG

BBG is a blue stain that is also known as acid blue 90 and Coomassie brilliant blue G 250. BBG has been used for protein staining in biological testing because it binds to proteins nonspecifically. However, there had been no

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reports on the medical use of this dye with the exception of our previous study.²⁹ One of the key factors to consider when selecting a suitable dye is osmolarity for cell survival.^{10,25} We tested the osmolarity of various concentrations of BBG, ICG, trypan blue, and other potential dyes. After these screenings, we selected BBG as the main candidate and applied various safety tests including light microscopy, scanning electron microscopy, and electroretinography.²⁶⁻²⁸

Light microscopic observation revealed no pathologic changes at 2 months with a high dose of BBG. In contrast, clinical doses of ICG and trypan blue showed degeneration of the corneal endothelial cells and apoptotic cell death of the endothelial cells.

Transmission and scanning electron microscopy revealed that a clinical dose of BBG induced no vacuolization in the inner retinal cells, and no apoptosis was detected after 2 weeks or 2 months. ICG caused retinal degeneration and retinal pigment epithelial (RPE) cell atrophy at 2 weeks after subretinal injection. Apoptotic cell death was detected in the inner and outer nuclear layers and the RPE layer, especially among the photoreceptors. Trypan blue caused less retinal degeneration, but it was detected mainly in the area detached by the subretinal injection. BBG showed no reduction in the amplitude of ERG waves.



Figure 1. ILM peeling with BBG. Macular hole ILM was stained bright blue, even with an intraocular (IOL) implanted in the eye. The edge between the peeled and nonpeeled area was also clearly visible. BBG did not stain the IOL.

A dye tends to show stronger toxicity once it is injected into the subretinal space. We observed no clear cell death with a clinical dose of BBG. In contrast, we observed clear cell death with ICG and trypan blue at a clinical dose. Thus, we would expect that even if BBG were injected into the subretinal space, the side effects would be relatively minimal.

PILOT STUDY

We investigated the staining patterns of membranes and the clinical outcomes using BBG in surgery for various vitreoretinal diseases. BBG was dissolved in intraocular irrigating solution and sterilized through a syringe filter to a final concentration of 0.25 mg/mL (pH 7.4). The prepared BBG solution was then injected gently into the vitreous cavity and washed out immediately with balanced salt solution.²⁹ In cases of macular holes, the ILM was stained a light-blue color instantly. Removal of the ILM was performed using forceps (Figure 1). Following the removal of the ILM, a difference in the retinal surface color between the area from which the ILM had been removed and the surrounding area was clearly visible. In cases of diabetic macular edema, BBG solution was injected and washed out immediately after creating the posterior vitreous detachment, and the removal of the stained ILM was performed as easily as in the macular hole cases (Figure 1). In the ERM cases, however, staining

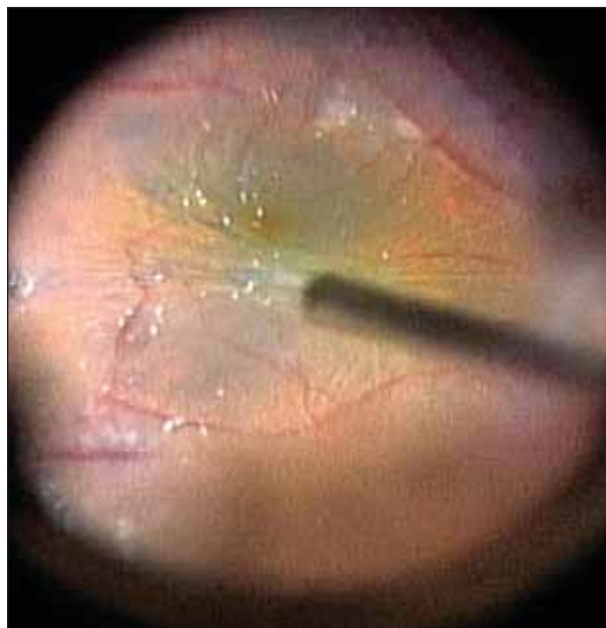


Figure 2. ERM peeling with BBG. The remaining ERM (arrowheads) was not clearly visible, but the edge between the ILM and the remaining ERM could be clearly observed.

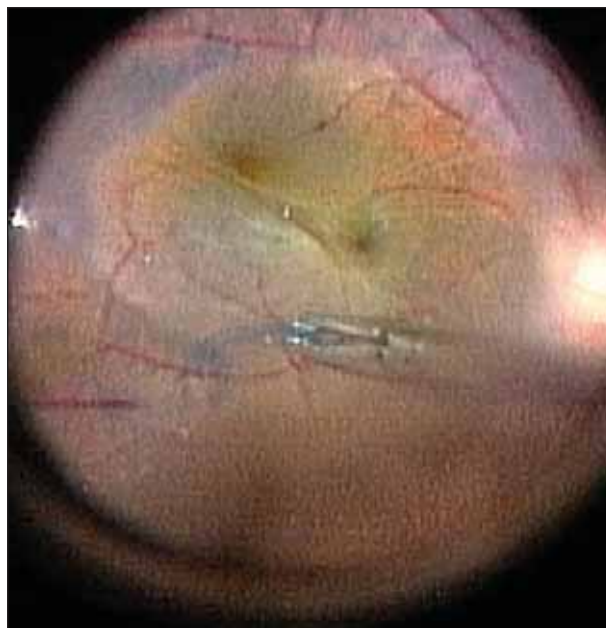


Figure 3. Double staining and peeling. After removing the ERM, BBG was injected to visualize the ILM followed by peeling. The edge between the ILM and the peeled area was clearly visible (arrowhead).

of the ERM could not be confirmed at this concentration (Figure 2). After ERM peeling, BBG solution was injected again, followed by immediate irrigation of the vitreous

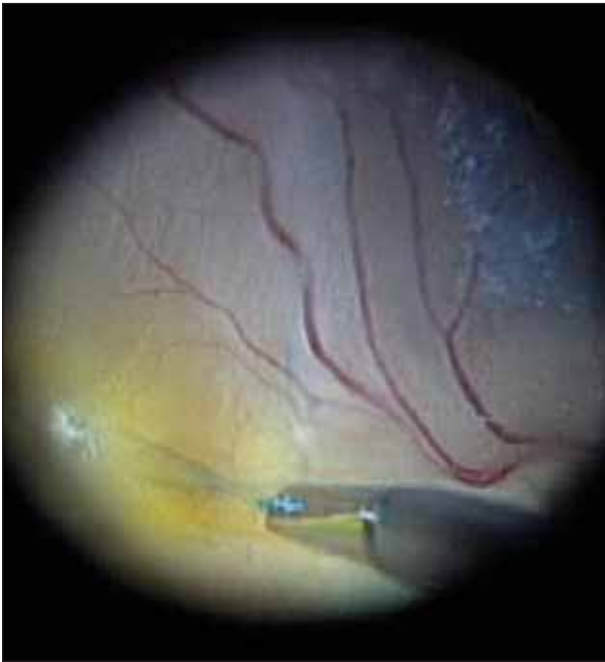


Figure 4. ERM peeling with retinal detachment caused by a macular hole in high myopia. BBG clearly aids the visualization of the ILM, facilitating the peeling process. BBG was applied after ERM peeling.

cavity. The ILM in the area where the ERM had been removed was strongly stained with BBG. The well-stained ILM could be easily removed (Figure 3).

We confirmed the benefit of using 0.25 mg/mL of BBG for ILM peeling with complicated cases such as retinal detachment due to high myopic macular hole (Figure 4). We have conducted clinical studies at Kyushu University, Japan, of BBG for macular hole, ERM, and high myopic macular hole; all patients gained satisfactory outcomes.

ADVANTAGES OVER OTHER DYES

BBG has a number of advantages over both ICG and trypan blue in terms of handling. ICG is packaged as lyophilized powder and will not dissolve in intraocular irrigating solution alone. BBG granules can be easily dissolved in intraocular irrigating solution alone, and can be subsequently sterilized with a 0.22- μ m syringe filter. The osmolality and pH of the BBG solution are also stable.²⁶⁻²⁹ Furthermore, the staining process requires no additional techniques such as fluid–air exchange, which is necessary for trypan blue application.

The ILM staining pattern enhanced by the BBG solution was similar to that of the ICG solution, and, as BBG is not a fluorescence dye, there is little possibility of light toxicity as there is with ICG. In addition, the BBG concentration required for staining the ILM is approximately one-tenth to



Figure 5. ILM Blue, manufactured by DORC and licensed from Aqumen Biopharmaceutical KK (Fukuoka, Japan).

one-twentieth that of ICG. We have performed vitreous surgery using BBG for more than 300 cases of various vitreoretinal diseases. Visual acuity was preserved or improved in more than 92% of these cases, and no adverse effects were noted during the postoperative observation period.

Recently, Shimada et al³⁰ have conducted a prospective, interventional study to evaluate the usefulness of BBG double staining. The ERM recurrence rate was reduced to 0% in 142 eyes with double ERM and ILM peeling compared with 16.3% (17 eyes), and the reoperation rate was 5.8% (6 eyes) among the 104 eyes that underwent single ERM peeling. The ERM peeling methods differed in the rate and extent of residual ILM, and the lowest rate (21/54 eyes; 39%) was achieved with BBG staining ($P < .0001$).³⁰ From these data, we conclude that BBG is the best dye for ILM staining when compared with ICG, trypan blue, and other dyes.

AVAILABILITY

BBG is now marketed in the European Union by DORC (Zuidland, Netherlands) under the brand name of ILM Blue (Highly purified BBG 250, 0.25 mg/mL; polyethylene glycol 3350 4%; density, 1.01 kg/L; pH, 7.3–7.6; osmolality, 334 mOsm/kg, 0.5 mg in prefilled syringe) in ready-to-use prefilled syringes (Figure 5). ILM Blue is expected to be approved in the United States in 2012. We recommend the use of approved and highly purified BBG product because other marketed BBG products may include traces of the heavy metal used during the synthesis process.

CONCLUSION

BBG, which has high staining ability, high safety, and is easy to handle, has a high affinity to the ILM and low affinity to the ERM, enabling successful peeling in vari-

ous vitreoretinal diseases. Our pilot study shows an improvement of postoperative visual acuity in the majority of cases, with no known adverse effects. In addition to our study, BBG is currently being widely researched for its clinical benefits, in particular for the double staining technique. ■

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