# Education

## How it appears: electron microscopic evaluation of internal limiting membrane specimens obtained during brilliant blue G assisted macular hole surgery

The intravitreal application of the novel dye, brilliant blue G (BBG), has recently been suggested to facilitate macular hole and epiretinal membrane surgery because BBG has been shown to selectively stain the internal limiting membrane (ILM).[1] Several advantages compared with other dyes such as indocyanine green or trypan blue have been reported.[2] In particular, BBG did not show apoptotic death of retinal cells as it was found in laboratory investigations on indocyanine green and trypan blue.[3 4] To determine if there are any pathological changes in the ILM and adherent structures or apparent damage to the retina, we analysed surgical specimens of BBG assisted ILM peeling in macular hole surgery using electron microscopy.

Nine eyes from nine patients (six women, three men) presented with full thickness macular holes and underwent standard three port pars plana vitrectomy with induction of a posterior vitreous detachment by suction with the vitrectomy probe around the optic nerve head. A sterile 0.2 mg/ml BBG solution (Fluoron GmbH, Neu-Ulm, Germany) was injected into the fluid filled vitreous cavity over the macular area and washed out by irrigation immediately. The ILM was then removed using an end gripping forceps. Preoperative and postoperative ophthalmic examinations included slit lamp microscopy, ophthalmoscopy, best corrected visual acuity, intraocular pressure and optical coherence tomography. Excised specimens from all eyes were immediately placed into phosphate buffered 4% glutaraldehyde solution for fixation. Postfixation in osmium tetroxide 2% (Dalton's fixative), dehydration in graded concentrations of ethanol and embedding in Epon 812 followed. Ultrathin sections of 60 nm were contrasted with uranyl acetate and lead citrate for electron microscopy. Analysis and imaging was performed using a Zeiss light microscope and a Zeiss EM 9 S-2 electron microscope (Zeiss, Jena, Germany). All in all, five sections of each specimen were evaluated. Twenty-eight specimens of the nine eyes were analysed.

Transmission electron microscopy confirmed the presence of the ILM in all specimens. On the retinal side of the ILM (determined by the undulated surface), small fragments of Mueller cells were seen in all eyes. Figure 1 demonstrates the minimal (fig 1A) and maximum (fig 1B) amount of debris observed. Cellular proliferation at the vitreal side of the ILM (determined by the smooth surface) was found in specimens of five eyes. In three of these eyes, we found multilayered epiretinal membranes (fig 1C) with interspersed collagen, consisting of native vitreous collagen and newly formed collagen (fig 1D). Myofibroblasts and fibrous astrocytes were the predominating cell type, but fibroblasts were also seen. In the two other eyes, single fibrous astrocytes were loosely distributed at the vitreal side of the ILM. The remaining four eyes did not show such fibrocellular proliferation. Retinal pigment epithelial cells or macrophages were not found in any of the specimens. In contrast with previous reports, where we described disruption of epiretinal cells in indocyanine green assisted macular surgery,[5] all cellular and collagenous elements appeared well preserved after BBG staining. Intracellular components such as cell nuclei, mitochondria or intracytoplasmatic filaments did not show any sign of damage or irregularity. In addition, we did not observe entire foot plates of Mueller cells

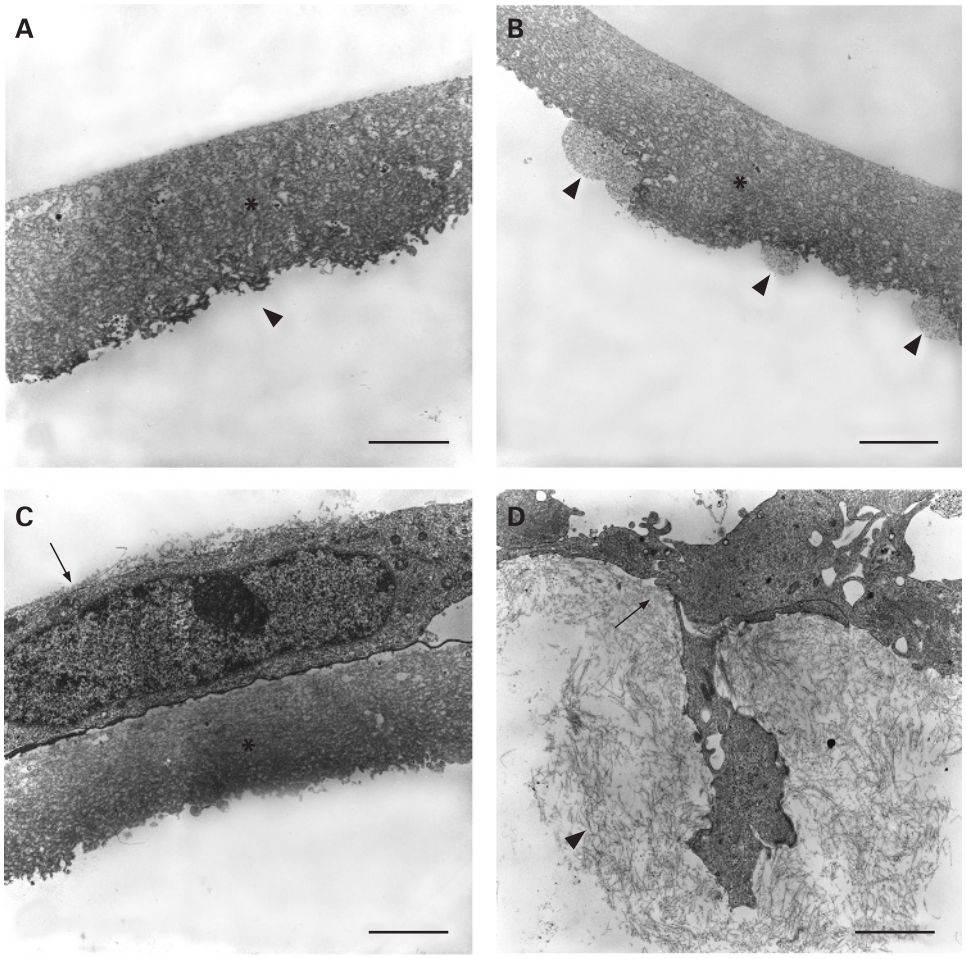


**Figure 1** Transmission electron micrographs of specimens removed by brilliant blue G assisted internal limiting membrane (ILM) peeling in macular hole surgery. (A) The ILM (asterisk) shows an undulated retinal side (arrowhead) without retinal debris or (B) with small fragments of Mueller cells (arrowheads). (C) The vitreal side of the ILM shows cellular proliferation (arrow) in five of nine eyes and (D) newly formed collagen (arrowhead) embedded in between cellular proliferations in one eye. Original magnification 4800×, bar 2.0 μm.

or large amounts of intracytoplasmatic Mueller cell components such as cell nuclei adherent to the retinal surface of the ILM, as noted following indocyanine green assisted peeling of the ILM.[6–8]

In summary, neither the retinal nor the vitreal side of ILM specimens obtained during BBG assisted ILM peeling revealed morphological alterations one might relate to adverse effects of BBG. However, ultrastructural analyses alone may not be sufficient to allow definite conclusions on the safety of a vital dye used for macular surgery. Nevertheless, electron microscopy provides useful additional information, especially in comparison with previous reports on morphological abnormalities seen after the application of other dyes such as indocyanine green. Further safety studies are required in the future.

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