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Sequential epiretinal membrane removal with internal limiting membrane peeling in brilliant blue G-assisted macular surgery

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**Purpose.** To assess the selectivity of brilliant blue G (BBG) staining by analysing the morphologic components of unstained and stained tissue obtained during epiretinal membrane (ERM) removal with internal limiting membrane (ILM) peeling in BBG-assisted macular surgery.

**Methods.** Twenty-six surgical specimens were removed from 13 eyes with epiretinal gliosis during vitrectomy using BBG for ERM and ILM peeling. We included eyes with idiopathic macular pucker, idiopathic macular hole, and vitreomacular traction syndrome. The dye was injected into the fluid filled globe. Unstained and stained epiretinal tissue was harvested consecutively and placed into separate containers. All specimens were processed for conventional transmission electron microscopy.

**Results.** The first surgical specimen of all eyes showed no intraoperative staining with BBG and corresponded to masses of cells and collagen. The second surgical specimen demonstrated good staining characteristics and corresponded to the ILM in all patients included. In seven eyes, the ILM specimens were seen with minor cell proliferations such as single cells or a monolayer of cells. Myofibroblasts, fibroblasts and astrocytes were present. In five cases, native vitreous collagen fibrils were found at the ILM. In six of all eyes, ILM specimens were blank.

**Conclusion.** Our clinicopathologic correlation underlines the selective staining properties of BBG. The residual ILM is selectively stained by BBG even when a small amount of cells and collagen adheres to its vitreal side. To reduce the retinal exposure to the dye, the surgeon might choose to remove the ERM without using the dye, followed by a BBG injection to identify residual ILM.
Introduction

In macular surgery, the intravitreal administration of brilliant blue G (BBG) became a favorable tool to visualize the otherwise translucent 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.[1-8] Brilliant blue G is now considered the dye of choice to stain the ILM by many vitreoretinal surgeons with respect to its selective staining properties.

In order to perform macular surgery safely and obtain the best possible functional results for the patient, it is of great importance that the surgeon can rely on the staining pattern of BBG. However, in the presence of epiretinal tissue, the dye has no access to the ILM. Therefore, ERM formation may impair sufficient staining of the ILM. Poor staining may result in an incomplete removal of the ILM associated with residual ILM fragments with an indefinite extent of cells and collagen remnants at the vitreal side of the ILM.[9] ILM remnants may contribute to ERM recurrences or reopening of a macular hole by serving as a scaffold for cellular proliferation.

Although vital dyes such as BBG facilitate macular surgery, we need to consider tissue-dye interactions and potential adverse effects associated with these adjuncts.

The differentiation of ultrastructural components of unstained and stained tissue removed by BBG-assisted peeling might have an impact on the intraoperative procedure in macular surgery. Knowing about the staining pattern and the reliability of staining characteristics will help the vitreoretinal surgeon to adjust the use of BBG to situations, in which the dye provides relevant information. We conducted this laboratory investigation to elucidate the morphologic correlate of sequentially peeled ERM and ILM specimens after BBG staining.
**Patients and methods**

Thirteen eyes from thirteen patients underwent vitrectomy with removal of both the ERM and the ILM as a sequential peeling procedure with double BBG staining during January 2008 and April 2009 at the University Eye Hospital Munich. We included nine patients with idiopathic macular pucker (IMP), two patients with idiopathic macular holes (IMH), and two patients with vitreomacular traction syndrome (VMTS). The study was approved by the local Institutional review board (IRB) and ethics committee.

The surgical technique consisted of a 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 0.2mg/ml Brilliant blue G solution (0.5 ml, 0.25%, Brilliant Peel®; Fluoron® GmbH, Neu-Ulm, Germany) was injected into the fluid-filled vitreous cavity over the macular area and washed out immediately. The staining characteristics were assessed by the surgeon. Then, unstained tissue was removed in a first step. This was followed by a reinjection of a few drops of BBG. In a second step, tissue stained with BBG was removed and harvested respectively. ERM and ILM removal was conducted using an end-gripping forceps. Surgery was completed by gas tamponade in IMH patients (15% C2F6 gas mixture) after fluid-air exchange, and patients were encouraged to keep face-down positioning for four days.

The ERM and ILM specimens harvested during vitrectomy were immediately placed into phosphate-buffered 4% glutaraldehyde solution for fixation. Specimens were processed for light and electron microscopy as reported previously.[10] Analysis and imaging of about 50 ultrathin sections per specimen were performed using a Zeiss light microscope and a Zeiss EM 9 S-2 electron microscope (Zeiss, Jena, Germany). The morphological analysis was blinded and focused on both the
vitreal and the retinal side of the ILM if present which included the documentation of
distribution and type of cells and collagen. The ultrastructural features of cells and
extracellular matrix components were characterised as previously listed, and retinal
debris at the ILM was rated using a four-point grading scale as previously
reported.[11,12]

**Results**

Twenty-six surgical specimens from eighteen women and eight men were included in
this study, corresponding to 16 right eyes and 10 left eyes. The average age at time
of surgery was 70 years (range 62-80 years). In all eyes, two specimens per eye
were analysed. Table 1 demonstrates the ultrastructural findings of all patients:

<table>
<thead>
<tr>
<th>no. of patients</th>
<th>diagnosis</th>
<th>ILM</th>
<th>cell proliferation*</th>
<th>collagen</th>
<th>second surgical specimen, good staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IMH</td>
<td>-</td>
<td>multilayer</td>
<td>++</td>
<td>present</td>
</tr>
<tr>
<td>2</td>
<td>IMH</td>
<td>-</td>
<td>multilayer</td>
<td>+</td>
<td>present</td>
</tr>
<tr>
<td>3</td>
<td>IMP</td>
<td>present</td>
<td>multilayer</td>
<td>++</td>
<td>present</td>
</tr>
<tr>
<td>4</td>
<td>IMP</td>
<td>present</td>
<td>multilayer</td>
<td>-</td>
<td>present</td>
</tr>
<tr>
<td>5</td>
<td>IMP</td>
<td>-</td>
<td>monolayer</td>
<td>+++</td>
<td>present</td>
</tr>
<tr>
<td>6</td>
<td>IMP</td>
<td>-</td>
<td>multilayer</td>
<td>-</td>
<td>present</td>
</tr>
<tr>
<td>7</td>
<td>IMP</td>
<td>-</td>
<td>multilayer</td>
<td>+++</td>
<td>present</td>
</tr>
<tr>
<td>8</td>
<td>IMP</td>
<td>-</td>
<td>multilayer</td>
<td>++</td>
<td>present</td>
</tr>
<tr>
<td>9</td>
<td>IMP</td>
<td>-</td>
<td>multilayer</td>
<td>++</td>
<td>present</td>
</tr>
<tr>
<td>10</td>
<td>IMP</td>
<td>present</td>
<td>multilayer</td>
<td>+++</td>
<td>present</td>
</tr>
<tr>
<td>11</td>
<td>IMP</td>
<td>-</td>
<td>multilayer</td>
<td>++</td>
<td>present</td>
</tr>
<tr>
<td>12</td>
<td>VMTS</td>
<td>-</td>
<td>multilayer</td>
<td>+++</td>
<td>present</td>
</tr>
<tr>
<td>13</td>
<td>VMTS</td>
<td>-</td>
<td>multilayer</td>
<td>+++</td>
<td>present</td>
</tr>
</tbody>
</table>

Table 1. Ultrastructural features of both the first and the second surgical specimens
removed consecutively in macular surgery with brilliant blue G staining.

*cell proliferation at the vitreal side of the inner limiting membrane if present

IMH = idiopathic macular hole
IMP = idiopathic macular pucker
VMTS = vitreomacular traction syndrome
The first surgical specimen of all eyes, which showed no staining with BBG (fig. 1), revealed masses of cells and collagen (fig. 2A). In three specimens from eyes with IMP we found fragments of the ILM, which were removed along with the first - unstained - surgical specimen (fig. 2A). Cell proliferations were distributed as cell multilayers on irregularly folded collagen strands (fig. 3A). Myofibroblasts were the predominating cell type. They were characterised by a lobulated cell nucleus and aggregates of 5-7 nm subplasmalemmal cytoplasmatic filaments with fusiform densities. Cell proliferations often showed polarization with prominent microvillous processes and interposition of native vitreous collagen as well as newly formed collagen. Fibrils of native vitreous collagen were regularly arranged with a diameter of 10 nm to 16 nm, whereas fibrils of newly formed collagen were irregularly distributed with a diameter of more than 16 nm. In three specimens, fibrous long spacing collagen was seen that presented with a symmetrical cross-striation of periodicity. In about half of all eyes with IMP, collagen fibrils were densely packed similar to a basal lamina like strand (fig. 3B).

The second surgical specimen was removed sequentially after a second administration of BBG and demonstrated good staining characteristics intraoperatively. The histological evaluation of these specimens confirmed that all these membranes corresponded to the ILM (fig. 2B). In five cases, some remaining collagen fibrils were found at the vitreal side of the ILM, mainly described as native vitreous collagen (fig. 3C). When collagen was found at the ILM, cell proliferation was always present. In seven eyes, the ILM specimens were seen with minor cell proliferations such as single cells or cell monolayers (fig. 3D). Myofibroblasts, fibroblasts and fibrous astrocytes were present. Fibroblasts were characterised by
abundant rough endoplasmatic reticulum, prominent golgi complexes and the absence of intracytoplasmatic filaments. Fibrous astrocytes were characterised by masses of intracytoplasmatic intermediate-type filaments and polarization with basement membrane production. Of note, cell or collagen remnants at the vitreal side of the ILM did not interfere with the positive staining during surgery. In six of all eyes, ILM specimens were devoid of cells and collagen. Retinal debris was solitarily distributed as small round cell fragments directly adjacent to the retinal side of the ILM.

**Conclusion**

Our findings indicate that epiretinal membrane formation reduces sufficient visualization of the ILM during BBG-assisted macular surgery, and that BBG does provide a selective staining of the ILM. In this study, ERM specimens showed intraoperatively no staining with BBG, despite the presence of ILM fragments in some of the ERM specimens, because massive epiretinal cell and collagen proliferation prevented a positive staining with BBG independent from the diagnosis. After having peeled the unstained ERM following the initial administration of BBG, residual collagen fibrils and some epiretinal cells were seen to remain adherent at the ILM. However, ILM remnants could be visualized reliably using BBG, even if a small amount of cells and collagen fibrils adhered to the vitreal side of the ILM.

From previous investigations we have learned that the ILM is often only partly removed along with an ERM.[13] Remaining ILM fragments have been associated with recurrent ERM formation.[14-16] Therefore, a thorough removal of the ILM is crucial for anatomic and functional long term success in order to remove the scaffold for further cell proliferation and contraction.

We believe that our findings have implications for the surgical technique of tractional maculopathies. In this context, firstly, we question whether it is necessary
to apply vital dyes to stain and remove epiretinal tissue. Secondly, in epiretinal
membrane surgery, when the surgeon intents to additionally remove the ILM, it has
to be questioned whether double staining procedures are indeed mandatory as
described previously.[17] We conclude from the present clinicopathological
correlation with BBG that it may be sufficient to remove epiretinal tissue without dye
assistance. The use of the dye could be limited to visualize ILM remnants, to peel
them off safely, thereby removing cells and collagen adherent to the vitreal side of
the ILM that otherwise being left behind at the macula. In other words, BBG helps to
identify the ILM and facilitates its complete removal.

In general, the application of vital dyes should be limited to situations when
they are really helpful and necessary. The results provided in this study represent a
step in that direction.

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Competing Interest
None declared.
References


Figure legends

Fig. 1: Intraoperative observation of unstained tissue (fst) that was removed first, and stained tissue (scd) that was sequentially removed presenting with a blue-coloured appearance.

Fig. 2: Light micrographs of unstained (A) and stained (B) tissue removed consecutively by brilliant blue G-assisted peeling in macular surgery for macular pucker. (A) Cell proliferations (arrows) were irregularly distributed as cell multilayers on folded collagen strands (arrowheads). (B) The internal limiting membrane (asterisk) was seen devoid of cells and collagen with a characteristic undulated retinal side (arrow) and a smooth vitreal side (arrowhead). (Original magnification: x 1000)

Fig. 3: Transmission electron micrographs of unstained (A, B) and stained (C, D) tissue removed consecutively by brilliant blue G-assisted peeling in macular surgery for macular pucker. (A) Myofibroblasts that were characterised by a lobulated cell nucleus and aggregates of 5-7 nm subplasmalemmal cytoplasmatic filaments dominated in cell proliferations on masses of newly formed collagen (arrow). Newly formed collagen was irregularly packed with a fibril diameter of more than 16 nm. (B) Collagen fibrils were often found densely packed similar to a basal lamina like strand (arrow). (C) Single collagen fibrils (arrow) were distributed at the vitreal side of the ILM (asterisk). (D) Native vitreous collagen fibrils (arrow) with a diameter of 10 nm to 16 nm were interposed between single epiretinal cells and the ILM (asterisk). Retinal debris was mostly seen as small round cell fragments (arrowhead) at the undulated side of the ILM. (Original magnification: (A) x 1800; (B, C, D) x 4800)