Natural Dyes for Chromovitrectomy

The efficacy of natural dyes in ocular surgery is under investigation.

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For many years, vitreoretinal surgery has been performed for the treatment of a range of retinal diseases, including symptomatic vitreomacular adhesion, macular hole, diabetic macular edema, and epiretinal membrane (ERM). The complexity of this surgery is attributed to the difficulty in visualization of the internal limiting membrane (ILM)—a semitransparent membrane—and the vitreous. Recently, chromovitrectomy has overcome these issues through the use of vital dyes and crystals, which made an otherwise optically transparent structure much easier to visualize and remove.1,2

Although chromovitrectomy is a useful technique,1,3-9 toxicity to the neurosensory retina and the retinal pigment epithelium (RPE) from the dyes has become a major concern, and, therefore, its safety profile must be assessed. Many different dyes, such as evans blue, light green, fast green, indigo carmine, bromophenol blue, and infracyanine green, have been studied for this purpose.10-16 Different dyes have been used to stain various intraocular structures, such as triamcinolone acetonide for the vitreous and posterior hyaloid, trypan blue for ERM, and indocyanine green (ICG) for ILM identification.17,18 Despite world-wide use of ICG, toxicity from this dye has been reported to be the cause of RPE atrophy during macular hole surgery due to the osmolarity of the ICG solution, decomposition of the ICG molecule, the effect of iodine, carbolinic complex formation, and the oxidative effect due to singlet oxygen release from the ICG molecule after light exposure.19-21 Brilliant blue G has been used in macular hole surgery as an alternative, although migration of the dye into the subretinal space may cause atrophy of the RPE.22

Recent reports on the use of natural dyes in radiology have demonstrated their safety, efficacy, and low cost.23 These dyes are acquired through biochemical or physico-chemical reaction from raw material of invertebrates, minerals, or, primarily, vegetables. The potential advantages of natural dyes ought to be investigated in ocular surgery.

Preparation of Natural Dyes for Chromovitrectomy

Anthocyanins and related flavonoids are natural dyes found in grapes, blueberries, açai berries, and any purple fruit. We recently tested the flavonoid/anthocyanins dye from the açai berry (Euterpe oleracea) and 10 natural vital dyes from 10 sources: pomegranate (Punica granatum), logwood (Haematoxylum campechianum), extract of chlorophyll from alfalfa (Medicago sativa), cochineal (Dactylopius coccus), hibiscus (Hibiscus rosa-sinensis), indigo (Indigofera tinctoria), paprika (Capsicum annuum), curcumin (Curcuma longa), old fustic (Maclura tinctoria), and grape (Vitis vinifera).

The preparation of the dyes involves lyophilizing a commercial natural extract stored in dark vials. The pH was adjusted to 7.00, and osmolarity was corrected to 300 mOsm. HPLC-DAD and HPLC-MS analysis combination of the UV-Vis and mass spectra were performed for constituent identification. Through this means, 1 main flavonoid derivative was identified as luteolin-5,7-O-dixylopyranoside, and two anthocyanins were identified as peonidin-3-O-galactoside and peonidin-3-O-glucoside, previously detected in the açai berry.

Safety of Natural Dyes

The toxicity profile of the açai berry was assessed in rabbit eyes and has been proven to be a safe and less expensive alternative for chromovitrectomy. The toxicity of the extract of açai berry has been tested in rabbit eyes but has not yet been published. In the histopathologic exam, all cases showed retina vacuolization in all layers and destruction of the photoreceptors and ganglion cells (post-mor-
Retinal detachment occurred during preservation of the specimens. The most important finding was that the retina without an ILM could be removed without damage to the ganglion cells (Figure 1). We believe that we will soon be able to use açai berry dye in chromovitrectomy in human eyes to facilitate surgery.

Eighty-six eyes were included in our study. After the procedure, light microscopy was used to confirm the removal of the ILM from the macula area, present in all cases (Figure 2). Figures 3, 4, and 5, respectively, show ILM peeling using extract of açai berry, extract of chlorophyll from alfalfa, and cochineal. Comparing all 11 dyes, the ILM was best stained with extract of açai berry, cochineal.

The toxicity of the other 10 natural vital dyes is yet to be determined, but the osmolarity and pH were adjusted before injection to similar levels to the ones used in human eyes. In future studies, the toxicity of these dyes can be evaluated in animals and cell cultures. Our research team will test the cochineal and extract of chlorophyll from alfalfa toxicity in rabbits and in cell cultures in the near future.

**STAINING PROPERTIES OF NATURAL DYES**

Eighty-six eyes were included in our study. After the procedure, light microscopy was used to confirm the removal of the ILM from the macula area, present in all cases (Figure 2). Figures 3, 4, and 5, respectively, show ILM peeling using extract of açai berry, extract of chlorophyll from alfalfa, and cochineal. Comparing all 11 dyes, the ILM was best stained with extract of açai berry, cochineal.
neal, and extract of chlorophyll from alfalfa. The anthocyanins dye from the açaí berry intensely stained the ILM purple in 100% of cases, creating a contrast between the purple ILM and the unstained retina beneath, facilitating ILM peeling. The extract of chlorophyll from alfalfa intensely stained 25% of the cases and moderately stained 75%. The cochineal intensely stained 50% of eyes, moderately stained 37.5%, and poorly stained 12.5%. These three dyes clearly differentiated between the stained ILM and unstained retina beneath. Our experience with ILM peeling using these dyes was similar to ILM peeling with ICG in post-mortem eyes, tested previously for comparative purposes; it was also comparable to ICG-guided ILM peeling in human eyes during vitrectomy.

Posterior vitreous detachment (PVD) was facilitated in all cases with the following dyes: extract of açaí berry, logwood, cochineal, and old fustic. Figure 6 shows a posterior hyaloid detachment stained with old fustic. Table 1 presents the staining classification used for intraoperative view of the posterior hyaloid and the ILM. The ability of each dye to stain the vitreous and the ILM is presented in Tables 2 and 3, respectively. We concluded that PVD was facilitated in 100% of cases in which the following dyes were used: extract of açaí berry, logwood, cochineal, and old fustic. In comparison with posterior hyaloid detachment assisted by triamcinolone acetonide, performed in a similar model, these 4 natural dyes achieved an equal level of difficulty for the same procedure. In addition, the vitreous was best stained using the extract of logwood and old fustic.

**Table 1. Staining Classification Used for Intraoperative View of the Posterior Hyaloid and ILM Using 10 Natural Vital Dyes**

<table>
<thead>
<tr>
<th>Posterior Hyaloid Identification</th>
<th>No staining</th>
<th>Poor staining</th>
<th>Moderate staining</th>
<th>Intense staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>ILM</td>
<td>No staining</td>
<td>Poor staining</td>
<td>Moderate staining</td>
<td>Intense staining</td>
</tr>
<tr>
<td>Score</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
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**Table 2. Ability of Each Dye to Stain the Posterior Hyaloid and Promote PVD**

<table>
<thead>
<tr>
<th>Staining</th>
<th>Açaí fruit</th>
<th>Pomegranate</th>
<th>Logwood</th>
<th>Extract of chlorophyll from alfalfa</th>
<th>Cochineal</th>
<th>Hibiscus</th>
<th>Indigo</th>
<th>Paprika</th>
<th>Curcumin</th>
<th>Old fustic</th>
<th>Grape</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Poor</td>
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<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>50%</td>
<td>75%</td>
<td></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Intense</td>
<td>100%</td>
<td>100%</td>
<td>25%</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td></td>
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Natural vital dyes allow staining of the vitreous and ILM in human cadaver eyes and may be useful during vitreoretinal surgery. These dyes may be valuable alternatives for chromovitrectomy. Additional toxicity studies followed by clinical studies are necessary in order to consider the clinical use of natural dyes in vitreoretinal surgery.

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