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 worldwide 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 though biochemical or physicochemical 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, açaí berries, and any purple fruit. We recently tested the flavonoid/anthocyanins dye from the açaí 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 (*Capiscum annuum*), curcumin (*Curcuma longa*), old fustic (*Maclura tinctória*), 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 açaí berry.

SAFETY OF NATURAL DYES

The toxicity profile of the açaí 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 açaí 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-

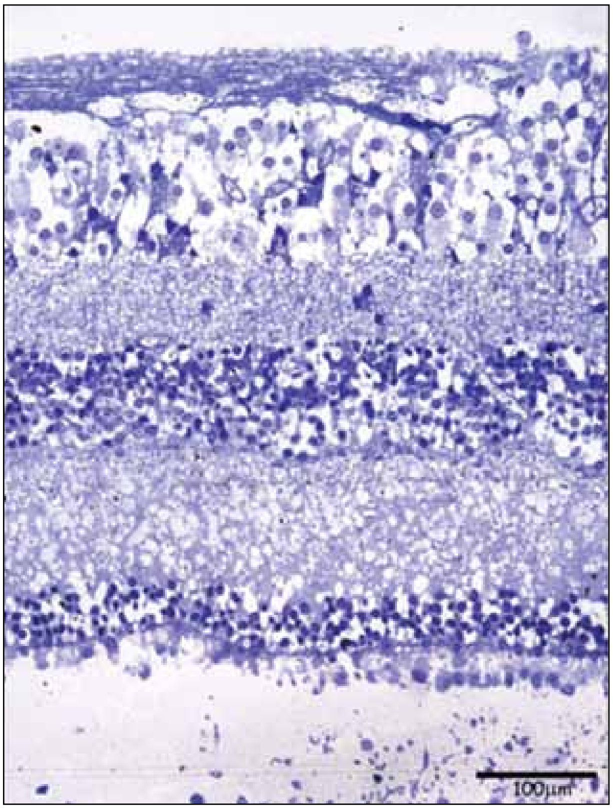

Figure 1. Light microscopy of the retina submitted for ILM peeling with extract of chlorophyll from alfalfa. The image shows the internal retina without the ILM, which indicates that peeling was performed, with preserved ganglion cells and severe vacuolization; this occurs in all layers and is related to changes associated with enucleation and preservation (toluidine blue, x20).

tem and preservative effect). 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.

Figure 2. A light microscopic section of a peeled ILM stained by natural flavonoid/anthocyanin dye from the açai berry (original magnification 40x).

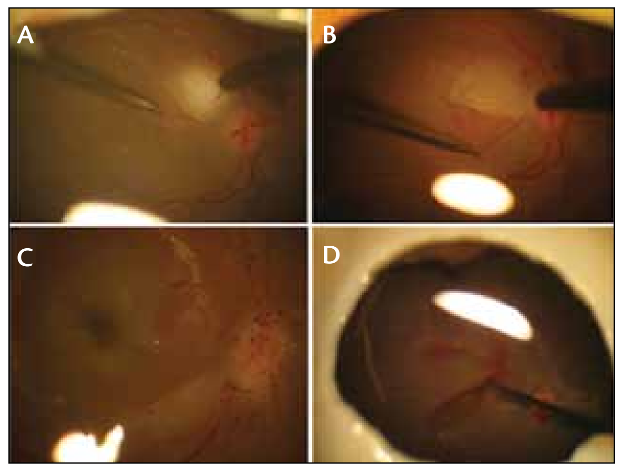

Figure 3. Intraoperative findings during ILM peeling in cadaver eyes, guided by staining with anthocyanins from the açai berry. The initial surgical procedure (A) and progression of the ILM peeling procedure (B) are shown. The ILM peeling is completed for 360° (C), and the ILM is removed completely (D).

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 açaí berry, extract of chlorophyll from alfalfa, and cochineal. Comparing all 11 dyes, the ILM was best stained with extract of açaí berry, cochi-

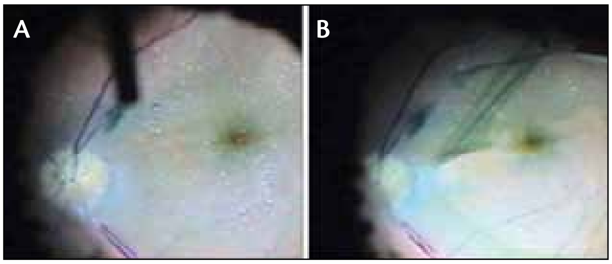

Figure 4. ILM peeling using extract of chlorophyll from alfalfa. The ILM is green similar to staining with ICG. The ILM is stained green with extract of chlorophyll from alfalfa (A), and the ILM peeling is completed (B).

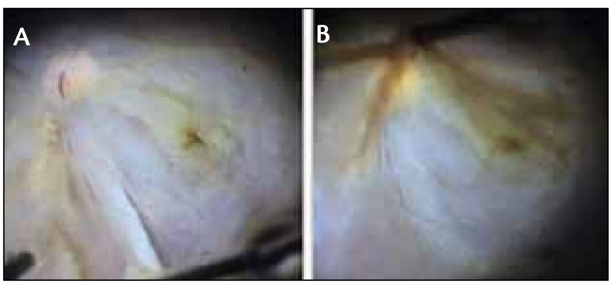

Figure 6. The posterior hyaloid is orange in a cadaver eye stained with old fustic. The posterior hyaloid is removed in the area close to the inferior temporal arcade (A), and the posterior hyaloid is removed from the optic disc and posterior pole (B).

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,

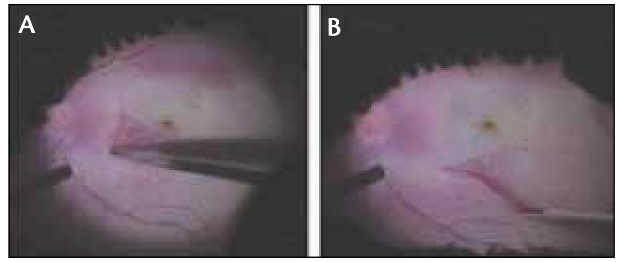

Figure 5. ILM peeling using cochineal. The ILM is stained reddish with cochineal (A), and ILM peeling is completed (B).

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,[7] 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

Posterior Hyaloid Identification	No staining	Poor staining	Moderate staining	Intense staining
Score	0	1	2	3
ILM	No staining	Poor staining	Moderate staining	Intense staining
Score	0	1	2	3

TABLE 2. ABILITY OF EACH DYE TO STAIN THE POSTERIOR HYALOID AND PROMOTE PVD

Staining	Açaí fruit	Pomegranate	Logwood	Extract of chlorophyll from alfalfa	Cochineal	Hibiscus	Indigo	Paprika	Curcumin	Old fustic	Grape
None											
Poor											
Moderate		50%		75%		100%	100%	100%	100%		100%
Intense	100%	50%	100%	25%	100%					100%	

TABLE 3. ABILITY OF EACH DYE TO STAIN THE ILM

Staining	Açaí fruit	Pomegranate	Logwood	Extract of chlorophyll from alfalfa	Cochineal	Hibiscus	Indigo	Paprika	Curcumin	Old fustic	Grape
None		50%								12.5%	
Poor		50%	75%		12.5%	50%		100%	62.5%	12.5%	
Moderate			25%	75%	37.5%	50%	100%		25%	37.5%	100%
Intense	100%			25%	50%				12.5%	37.5%	

CONCLUSION

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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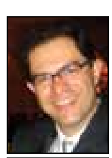

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1. Rodrigues EB, Meyer CH, Kroll P. Chromovitrectomy: a new field in vitreoretinal surgery. *Graefes Arch Clin Exp Ophthalmol*. 2005;243(4):291-293.
2. Rodrigues EB, Penha FM, Furlani B, Meyer CH, Maia M, Farah ME. Historical aspects and evolution of the application of vital dyes in vitreoretinal surgery and chromovitrectomy. *Dev Ophthalmol*. 2008;42:29-34.
3. Burk SE, Da Mata AP, Snyder ME, Rosa RH Jr, Foster RE. Indocyanine green-assisted peeling of the retinal internal limiting membrane. *Ophthalmology*. 2000;107(11):2010-2014.
4. Feron EJ, Veckeneer M, Parys-Van Ginderdeuren R, Van Lommel A, Melles GR, Stalmans P. Trypan blue staining of epiretinal membranes in proliferative vitreoretinopathy. *Arch Ophthalmol*. 2002;120(2):141-144.
5. Kumar A, Prakash G. Differential staining with indocyanine green and trypan blue dye. *Indian J Ophthalmol*. 2004;52(4):339.
6. Perrier M, Sebag M. Epiretinal membrane surgery assisted by trypan blue. *Am J Ophthalmol*. 2003;135(6):909-911.
7. Peyman GA, Cheema R, Conway MD, Fang T. Triamcinolone acetonide as an aid to visualization of the vitreous and the posterior hyaloid during pars plana vitrectomy. *Retina*. 2000;20(5):554-555.
8. Rodrigues EB, Meyer CH, Farah ME, Kroll P. Intravitreal staining of the internal limiting membrane using indocyanine green in the treatment of macular holes. *Ophthalmologica*. 2005;219(5):251-262.
9. Teba FA, Mohr A, Eckardt C, et al. Trypan blue staining in vitreoretinal surgery. *Ophthalmology*. 2003;110(12):2409-2412.
10. Enaida H, Hisatomi T, Goto Y, et al. Preclinical investigation of internal limiting membrane staining and peeling using intravitreal brilliant blue G. *Retina*. 2006;26(6):623-630.
11. Haritoglou C, Yu A, Freyer W, et al. An evaluation of novel vital dyes for intraocular surgery. *Invest Ophthalmol Vis Sci*. 2005;46(9):3315-33122.
12. Jackson TL, Griffin L, Vote B, Hillenkamp J, Marshall J. An experimental method for testing novel retinal vital stains. *Exp Eye Res*. 2005;81(4):446-454.
13. Mennel S, Meyer CH, Tietjen A, Rodrigues EB, Schmidt JC. Patent blue: a novel vital dye in vitreoretinal surgery. *Ophthalmologica*. 2006;220(3):190-193.
14. Schuettauf F, Haritoglou C, May CA, et al. Administration of novel dyes for intraocular surgery: an in vivo toxicity animal study. *Invest Ophthalmol Vis Sci*. 2006;47(8):3573-3578.
15. Enaida H, Hisatomi T, Hata Y, et al. Brilliant blue G selectively stains the internal limiting membrane/brilliant blue G-assisted membrane peeling. *Retina*. 2006;26(6):631-636.
16. Rodrigues EB, Penha FM, Farah ME, et al. Preclinical investigation of the retinal biocompatibility of six novel vital dyes for chromovitrectomy. *Retina*. 2009;29(4):497-510.
17. Farah ME, Maia M, Rodrigues EB. Dyes in ocular surgery: principles for use in chromovitrectomy. *Am J Ophthalmol*. 2009;148(3):332-40.
18. Rodrigues EB, Penha FM, de Paula Fiod Costa E, et al. Ability of new vital dyes to stain intraocular membranes and tissues in ocular surgery. *Am J Ophthalmol*. 2010;149(2):265-277.
19. Gandorfer A, Haritoglou C, Gandorfer A, Kampik A. Retinal damage from indocyanine green in experimental macular surgery. *Invest Ophthalmol Vis Sci*. 2003;44(1):316-323.
20. Maia M, Kellner L, de Juan E Jr, et al. Effects of indocyanine green injection on the retinal surface and into the subretinal space in rabbits. *Retina*. 2004;24(1):80-91.
21. Penha FM, Maia M, Farah ME, et al. Morphologic and clinical effects of subretinal injection of indocyanine green and infracyanine green in rabbits. *J Ocul Pharmacol Ther*. 2008;24(1):52-61.
22. Malerbi FK, Maia M, Farah ME, Rodrigues EB. Subretinal brilliant blue G migration during internal limiting membrane peeling. *Br J Ophthalmol*. 2009;93(12):1687.
23. Sanchez TA, Elias J Jr, Colnago LA, et al. Clinical feasibility of Acai (Euterpe oleracea) pulp as an oral contrast agent for magnetic resonance cholangiopancreatography. *J Comput Assist Tomogr*. 2009;33(5):666-671.