

Chromovitrectomy: an Update

No dye in current use is ideal; a number of potential candidates are under investigation.

BY LIHTEH WU, MD

Traction between an incompletely detached vitreous gel and the underlying retina can lead to the development of numerous diseases of the posterior pole. Anomalous posterior vitreous detachment (PVD) can occur with age-related shrinking of the vitreous, when the separation at the vitreoretinal interface is not clean and complete. The location of the remaining adhesion sites between retina and vitreous influences the type of clinical entity that develops. Strong adhesion in the periphery, for example, can lead to retinal tear or detachment. When the strongest adhesions are in the macula, the sequelae can include the development of macular hole (MH), epiretinal membrane (ERM), or vitreomacular traction syndrome (VMTS).¹

One approach to relieving or addressing these conditions is to release the vitreomacular traction. In surgical attempts to effect this release, 3 tissues are of interest: the posterior hyaloid, the ERM, and the internal limiting membrane (ILM). The major difficulty facing surgeons in manipulating these tissues is that they tend to be thin and transparent, and therefore difficult to visualize.

Staining these transparent tissues with vital dyes can facilitate their management during surgery. A number of dyes, including indocyanine green (ICG), trypan blue (TB), and brilliant blue G (BBG), have been proposed and used as staining agents in vitrectomy surgery. The corticosteroid triamcinolone (TA) has also been used for this purpose. The term *chromovitrectomy* has been coined to describe the use of staining agents to facilitate visualization of tissues during these surgical procedures.

While several types of vital dye have proven to be helpful during vitreous surgery, the ideal agent has yet to be found. Staining can help in the identification and removal of tissues, but concerns have been raised about ocular toxicity. This article recounts some of what has been reported regarding the most commonly used dyes and briefly looks ahead at other agents currently under investigation.

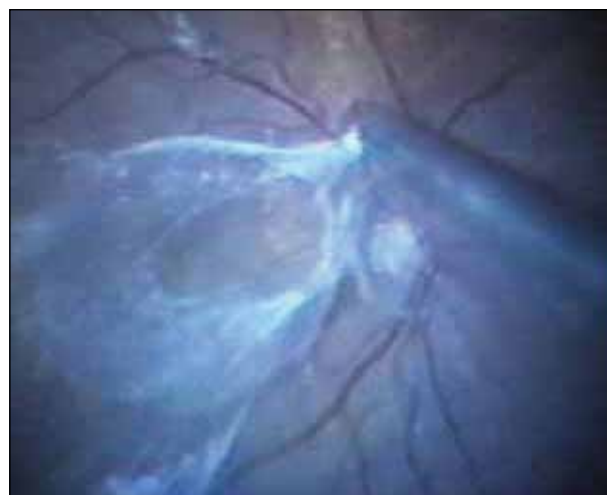


Figure 1. Posterior hyaloid detachment surgery assisted by triamcinolone. The posterior hyaloid is detached from the optic nerve in an eye with diabetic retinopathy.

POSTERIOR HYALOID

Traction on the retina from the posterior hyaloid has been linked to the pathogenesis of MH, proliferative vitreoretinopathy (PVR), proliferative diabetic retinopathy (PDR), and other conditions. Posterior hyaloid separation is therefore a goal in any vitrectomy surgery. Often it is hard to determine whether all of the posterior hyaloid has been removed.

A study in human and animal cadaver eyes comparing fluorescein, TA, ICG, and TB found that TA was best for highlighting the vitreous.² The other agents stained the vitreous but also stained surrounding ocular structures. TA is a well-tolerated corticosteroid, widely used in ophthalmology. Injected into the vitreous cavity, TA particles adhere to the vitreous gel, facilitating visualization and identification (Figure 1). This agent may also reduce breakdown of the blood-aqueous barrier and preretinal fibrosis, further improving outcomes. TA is

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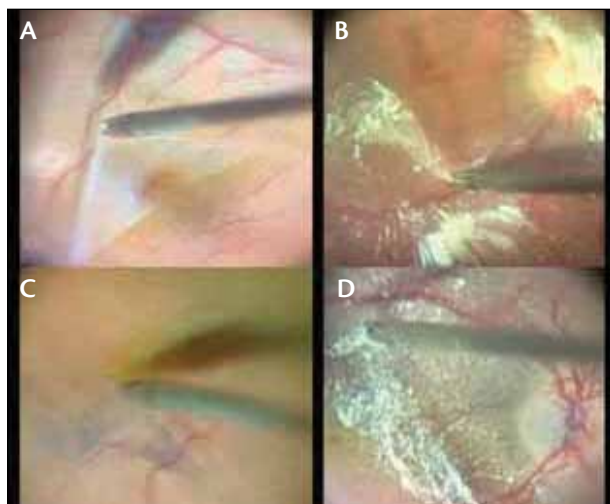


Figure 2. ERM peeling without dyes (A). ERM peeling using triamcinolone acetonide (B). ERM peeling using trypan blue (C). ERM peeling using a double staining technique with triamcinolone acetonide and trypan blue (D).

currently the agent most widely used to visualize the posterior hyaloid.³

EPIRETINAL MEMBRANE

Peeling or removal of the ERM has become a common procedure in macular surgery in recent years. Removal of these membranes, which can range from dense and opaque to fine and transparent, can be a challenge even for experienced surgeons.

TB binds to degenerated cell elements but does not stain live cells or tissues. There is no uptake of the dye by live tissues. TB stains ERM at a concentration of 0.15%, which clinical studies suggest is a relatively safe dose. Experimental studies have shown dose-dependent toxicity at concentrations greater than 0.3%. TB stains the ERM well but the ILM poorly. Currently it is the dye of choice for ERM peeling (Figure 2).^{3,4}

INTERNAL LIMITING MEMBRANE

ILM can serve as a scaffold for cellular proliferation in anomalous PVD. This proliferation can lead to traction and contribute to the formation of ERM, MH, VMTS, and other entities.⁵ Tangential traction has been linked to the pathogenesis of MH, and it is generally recognized that ILM peeling contributes to successful closure of large and chronic holes.⁶

ILM peeling may also reduce the risk of recurrence after ERM removal. Removal of the ILM helps to ensure that ERM has been completely removed.

Unlike TB, ICG binds well to the ILM. It provides good contrast between the ILM and surrounding tis-

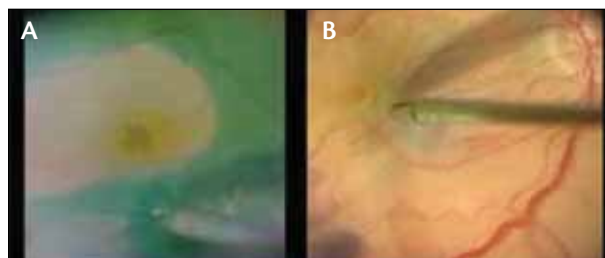


Figure 3. ILM peeling guided by ICG staining in macular hole surgery (A). ILM peeling guided by brilliant blue G staining in macular hole surgery (B).

sues, and in the process of binding it also increases the stiffness of the ILM, making peeling of the membrane easier (Figure 3A).⁷

However, after initial interest in the use of ICG in ILM peeling, numerous reports of toxicity soon dampened enthusiasm.^{3,8} Toxicity to the retina has been reported after in vitro and in vivo studies and after macular surgery.⁸

A meta-analysis of ILM peeling in MH surgery with and without use of ICG, including a total of 837 eyes, found similar anatomic outcomes with both approaches, but statistically worse functional outcomes when ICG was used ($P = .0008$). A higher incidence of alteration of the retinal pigment epithelium (RPE) was seen in eyes with ICG injection.⁹

INVESTIGATING BBG

Another dye with high affinity for the ILM is BBG (Figure 3B). This staining agent appears to be relatively safe in concentrations of up to 0.25 mg/mL.³ It is not fluorescent, so there is little risk of associated phototoxicity. Preclinical investigation in animals showed no signs of toxicity. In a series of 20 eyes with MH or ERM, BBG selectively stained the ILM and assisted in ILM removal. BBG does not stain the ERM.^{10,11}

With this difference in affinity between ILM and ERM, when BBG is used to stain both layers before removing any tissue during surgery the unstained ERM is clearly depicted against the ILM, which is stained blue. After ERM removal, accurate visualization of the ILM during macular surgery is difficult, so it must be restained.

The Pan American Collaborative Retina Study (PACORES) Group conducted a prospective multicenter study to determine the correlation between surgeons' unaided observation of the ILM and the BBG-stained ILM after ERM peeling.¹² The study included 92 eyes of patients undergoing pars plana vitrectomy and membrane peeling for idiopathic ERM.

Core vitrectomy was carried out in each eye, and peeling of the posterior hyaloid was verified by staining with

TA. This was followed by peeling of the ERM and then unaided observation of the underlying ILM. The ILM was then stained with BBG and was again observed.

The conclusion of the investigators was that there is little correlation between the surgeon's observation of the unstained and the BBG-stained ILM. The kappa coefficient of correlation between the 2 observations was 0.377 ($P < .0001$). The study authors concluded that in ERM surgery, if the ILM is to be peeled, it should be stained.

The neuroprotective qualities of BBG have also been assessed. BBG has recently been characterized as an antagonist of the purinergic receptor P2RX7, which is implicated in the pathway of pathologic photoreceptor loss.¹³ Stressed cells release adenosine triphosphate (ATP), which seems to be an initializing event that triggers primary or secondary cell death via a positive feedback loop on P2RX7. In a mouse study, increased extracellular levels of ATP were mediated by BBG. All hallmarks of photoreceptor apoptosis were prevented by premedication or co-application of BBG. The study authors suggested that BBG has a potential application as a neuroprotective agent in retinal diseases or similar neurodegenerative pathologies linked to excess extracellular ATP.

It should be noted, as our colleagues have reported,¹⁴ that unintentional migration of BBG into the subretinal space may cause atrophy of the RPE.

OTHER DYES

The search for the ideal dye continues. The ideal dye would provide excellent contrast, with high biocompatibility for ocular tissues and no toxicity. It would be strongly absorbed at visible wavelengths, bind to tissues of interest, and be physiologically degradable on a practical time scale.¹⁵

Rodrigues et al reported on the abilities of 13 dyes to stain the lens capsule, ERM, ILM, and vitreous.¹⁶ The dyes evaluated in porcine and human cadaver eyes were methyl violet, crystal violet, eosin Y, sudan black B, methylene blue, toluidine blue, light green, indigo carmine, fast green, congo red, evans blue, and bromophenol blue. Most of the dyes bound well to the lens capsule, vitreous, and ERM. BBG demonstrated the best ILM staining of all the dyes evaluated.

Haritoglou and colleagues described the staining and biocompatibility properties of a new cyanine dye for ILM peeling.¹⁵ This dye, called 3,3'-Di-(4-sulfobutyl)-1,1',1'-tetramethyl-di-1H-benz[e]indocarbocyanine (DSS), was evaluated in vitro and in human and animal cadaver tissues and 2 post-mortem eyes. The contrast and staining properties of DSS on the ILM were excel-

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lent, the authors reported, and allowed controlled removal of the ILM during surgery. No penetration into deeper retinal layers was noted.

Last year, Sousa-Martins and colleagues evaluated the use of a natural dye solution based on lutein and zeaxanthin alone and combined with BBG.¹⁷ In 60 post-mortem eyes, they found that lutein and zeaxanthin (20%) crystals precipitate on the vitreous surface, staining it orange. Lutein and zeaxanthin combined with BBG had a high affinity for the ILM and the anterior lens capsule. In eyes in which ILM peeling was performed, no dye solution remained after membrane removal. The authors concluded that this natural dye solution, alone or combined with BBG, efficiently stained the anterior capsule, vitreous, and ILM in human cadaveric eyes and has potential as a tool in intraocular surgery.

In a publication this year, Chen and colleagues evaluated the use of 11 dyes derived from natural products for ILM peeling and posterior hyaloid detachment.¹⁸ In addition to anthocyanin dye from the acai fruit (*Euterpe oleracea*), the dyes evaluated were derived from pomegranate (*Punica granatum*), logwood (*Haematoxylum campechianum*), chlorophyll extract from alfalfa (*Medicago sativa*), cochineal (*Dactylopius coccus*), hibiscus (*Hibiscus rosa-sinensis*), indigo (*Indigofera tinctoria*), paprika (*Capiscum annuum*), turmeric (*Curcuma longa*), old fustic (*Maclura tinctoria*), and grape (*Vitis vinifera*). Although all the dyes facilitated PVD and ILM peeling in cadaveric eyes, the authors reported, the best capability for ILM staining was obtained with acai fruit extract, cochineal, and chlorophyll extract from alfalfa.

CONCLUSIONS

In clinical use, no matter which dye is chosen, common sense rules to avoid toxicity include using the lowest concentration of dye that will achieve staining, and applying it for the shortest period of time. Dilutions with physiologic osmolarity are desirable. To avoid phototoxicity, the light pipe should be kept as far away from the retinal surface as possible.

(Continued on page 64)

(Continued from page 60)

Surgery for MH demands care because the dye may come in contact with the bare RPE through the hole and produce toxicity. Possible precautions include coating the hole with a viscoelastic substance, autologous blood, or a perfluorocarbon liquid.

Transparent tissues such as the posterior hyaloid, ERM and ILM play important roles in multiple diseases of the posterior pole. Surgical removal of these tissues is a principal surgical objective. Staining of these tissues with a variety of vital dyes can facilitate their identification and removal. Several dyes are currently in routine clinical use; however, the ideal staining agent has not yet been identified. Any dye that is injected intravitreally has the potential to become toxic. ■

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