Brilliant Peel® and other vital dyes for chromovitrectomy

Bastian Mühling
Fluoron GmbH, Biberkopfweg 1, 89231 Neu-Ulm, Germany
Tel.: 49 0731 / 81060 www.fluoron.de
Email: bastian.muehling@fluoron.de
New biocompatible dye for selective ILM staining
Triphenylmethane dyes

Common name: Trityl-dye

Crystal violet

Patent Blue (Blueron®)

Brilliant Blue G (Brilliant Peel®)

Bromophenol blue

The interaction between the molecular structure of the dye and the biological membrane determines the staining ability.

Meinert et al, Production of a dye for colouring cells in the human or animal body, 2004, WO 2004/035091 A1
Triphenylmethane dyes

Many structures possible

Basic chemical structure of Triphenylmethane-dyes

Variation of substituents in the inner and outer spheres

Crystal violet
Patent Blue

Triphenylmethane dyes
Triphenylmethane dyes

Brilliant Blue G
Composition and Properties of Brilliant Peel®

Composition in one 0.5 ml vial

- 0.125 mg Brilliant Blue G
- 0.95 mg Na$_2$HPO$_4$ x 2 H$_2$O
- 0.15 mg NaH$_2$PO$_4$ x 2 H$_2$O
- 4.1 mg NaCl

Concentration: 0.25 g/l
pH-value: 7.52
Osmolarity: 306 mOsm/kg H$_2$O
Maximum absorption: 584.0 nm
Indocyanine Green (ICG)

Chemical group: Cyanine (push-pull-systems)

Color: Dark green
ILM-Affinity: high
ERM-Affinity: low
Retinal Toxicity: moderate
### Indocyanine Green (ICG)

<table>
<thead>
<tr>
<th>Registration:</th>
<th>off-label use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration:</td>
<td>staining solution has to be mixed,</td>
</tr>
<tr>
<td></td>
<td>concentration values fluctuate between 0.05 und 0.5 g/L</td>
</tr>
<tr>
<td>Suppliers:</td>
<td>Pulsion Medical Systems, Munich, 25 mg and 50 mg vials</td>
</tr>
<tr>
<td></td>
<td>Ophthalmos, Saõ Paulo, 10, 25 und 50 mg vials</td>
</tr>
<tr>
<td></td>
<td>Akorn Inc, Buffalo Grove, Illinois, 25 mg vials</td>
</tr>
<tr>
<td></td>
<td>Daiichi Pharmaceutical, Tokyo, 25 mg vials</td>
</tr>
<tr>
<td>Mechanism of coloring:</td>
<td>high affinity to the extra-cellular matrix components of the ILM, increased rigidity and reduced elasticity of the ILM after ICG staining facilitate peeling (photo-induced cross linking of the collagen fiber)</td>
</tr>
<tr>
<td>Contact time:</td>
<td>time fluctuates between 10 to 60 seconds (comparable to Brilliant Peel®)</td>
</tr>
<tr>
<td>Application:</td>
<td>either a little amount of the substance in the air-filled vitreous or injection into the liquid-filled (BSS) vitreous to avoid highly toxic concentrations</td>
</tr>
</tbody>
</table>
Indocanine Green (ICG)

Side effects: relatively many cases with peripheral visual field defects (30%) damage / changes to the retinal pigment epithelium (RPE) optic nerve atrophy ICG exposure may cause retinal cell apoptosis or necrosis upon contact with light histological changes in the upper sphere of the retina

Causes: photosensitive properties of ICG after staining the ILM, ICG is hard to remove from the vitreous and can continue to have effects dependent on the producer prescription there are high fluctuations in the concentrations of the ICG staining solutions

Toxicity: inconsistent literature, but the dye is generally considered unsafe

Clinical use: up to now ICG has been the standard dye for ILM Peeling
Trypan Blau (TB)

Chemical group: Azo-Dye (diazo)

Color: Dark blue
ILM-Affinity: low
ERM-Affinity: high
Retinal Toxicity: moderate
Trypan Blue (TB)

Suppliers: Membrane Blue (Dutch Ophthalmic Research Center, DORC)
Vision Blue (DORC)

Registrations: Membran Blue => Registered for ILM und ERM staining
Vision Blue => Registered for capsulorhexis

Packaging: Membran Blue: Box with 5 sterile ready-to-use syringes (0.5 ml)
Vision Blue: Box with 10 sterile ready-to-use syringes (0.5 ml)

Concentrations: Membran Blue => 0.15 % solution
Vision Blue => 0.06 % solution
Trypan Blue (TB)

In vitro studies: in vitro examinations offer inconsistent results
Lüke et al => TB causes considerable retinal damage
(Contact time between 20 and 120 seconds)
Mennel et al => Application of Trypan blue to RPE-cells
causes no damage or negative changes

Clinical studies: clinical trials demonstrate only slight to no toxic side effects
when using Trypan Blue as vitreous staining solution

Contact time: 3 to 5 minutes for effective ILM staining

Mechanism of coloring: unknown
Living cells or tissues with intact cell membranes will not be stained. Since cells are very selective in the compounds they allow to pass through their membranes, trypan blue is not absorbed by living cells; however, it traverses the membrane of a dead cells. Hence, dead cells are shown in a distinctive blue color under a microscope.
Patent Blue (PB)

Chemical group: Triphenylmethane dye

Color: blue
ILM-Affinity: low
ERM-Affinity: high
Retinal Toxicity: very low
Patent Blue (PB)

Supplier: Fluoron GmbH, Neu-Ulm

Packaging: Box with 5 sterile vials (each with 0.5 ml solution)

Concentration: 2.4 g/L

Registration: for capsulorhexis and as an intraocular staining solution

In vitro studies: examinations offer consistent results
    Lüke et al => PB causes no retinal damage
        (contact time between 20 and 120 seconds)
    Mennel et al => Application of Patent blue to RPE-cells
        causes no damage or negative changes

In vivo studies: the rabbit model proves that PB is safe (subretinal injection)

Clinical trials: up to now there have been no publications reporting toxic side effects
Bromophenol Blue (BrB)

Chemical group: Triphenylmethane dye

Color: Dark blue
ILM-Affinity: moderate
ERM-Affinity: moderate
Retina-Toxicity: unknown
**Bromophenol Blue (BrB)**

In vivo Studies: Harritoglu et al (Munich)

- intensive capsulor staining of pigs’ eyes with a 0.2 % staining solution
- Bromophenol Blue stains the retinal surface and lens capsule at a low concentration of 0.2% with no signs of toxicity (light and electron microscopy, colorimetric test, inhibition measurement of RPE proliferation and cell viability measurements)

In vitro Studies: Harritoglu et al (Munich)

- no cell damage of ARPE- and RPE-cells upon contact with BrB at concentrations between 0.2 and 0.02%

**Coloring:** dye concentrations between 1 and 2% stain the ILM very effectively

**Registration:** up to now there has been no registration of BrB as a medical device for intraocular use or published data of clinical trials
Effects of ICG, TB, PB and BBG on bovine retinal function

Method

Bovine retina preparations were perfused with a standard nutrient solution and electro-retinograms (ERG) was recorded repeatedly.

After recording stable ERG amplitudes, the pure nutrient solution was replaced by a dye/nutrient solution.

Contact time: 10, 15, 30, 60, 120 seconds.

Concentration: 0.25mg/ml BBG dissolved in the nutrient solution.

Reperfusion with the standard nutrient solution for at least 115 minutes.
Experimental setup: isolated bovine retina

- Nutrient solution
- Pump
- Thermostat
- Waste
- Perfusion chamber
Experimental setup: isolated bovine retina

Perfusion of the retina with the nutrient solution
Retinal exposure to a dye solution
Light exposure with a standard light intensity
Measurement of ERG amplitudes

perfusion chamber
The faster the recovery of the B-wave amplitude after dye application, the lower the toxic effect to the retina.

Final results of the BBG measurements are available at the end of July 2007.

First measurements show a non-toxic behavior similar to that of Patent Blue.

**Figure 1** Average of representative test series (n = 6). Black arrow marks dye application for 30 seconds ICG, indocyanine green (dot); TB, trypan blue (dash); PB, patent blue (solid). One representative standard deviation given.
Cytotoxicity in accordance with DIN EN ISO 10993 and ILM staining ability

<table>
<thead>
<tr>
<th>Dye</th>
<th>Significant cytotoxic effect</th>
<th>ILM Staining behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brilliant Blue G</td>
<td>&gt; 0.3 g/L</td>
<td>+++</td>
</tr>
<tr>
<td>Indocyanine Green</td>
<td>&gt; 0.24 g/L</td>
<td>+++</td>
</tr>
<tr>
<td>Patent Blue</td>
<td>&gt; 2.8 – 3.0 g/L</td>
<td>+</td>
</tr>
<tr>
<td>Trypan Blue</td>
<td>&gt; 0.13 g/L</td>
<td>++</td>
</tr>
</tbody>
</table>

Brilliant Peel®: 0.25 g/L

Biocompatibility of Brilliant Peel®
in accordance with DIN EN ISO 10993

Cytotoxicity Test
Cell Growth Analysis via BCA-Staining with Brilliant Peel® (0.25 g/L)

Brilliant Peel® was shown to be non-toxic.

Maximization Test
Test for delayed-type hypersensitivity with Brillaint Peel® (0.25 g/L)

Brilliant Peel (0,25 g/L) was shown to not have any sensitizing properties.

Test for Dermal Irritation
Acute Dermal Irritation/Corrosion with Brilliant Peel® (0.25 g/L)

Brilliant Peel® was shown to not irritate intact skin.
Publications concerning Brilliant Blue G (BBG)

Biocompatibility of BBG in a rat model

Part 1:
Subretinal injection of the dyes. The biocompatibility was evaluated for a period of 10 weeks with ophthalmic examinations (light, fluorescence and transmission electron microscopy, apoptotic cell death counting)

Subretinal injection of BBG (0.25 mg/ml) displayed high biocompatibility.

Literature:
Biocompatibility of BBG in a rat model

Part 2:
BBG (0.25 mg/ml) was injected into the vitreous cavity of 78 rat eyes following gas compression vitrectomy.

Ophthalmic examinations (light, fluorescence and transmission electron microscopy, apoptotic cell death counting and electroretinography) of the enucleated eye after 10 weeks.

The results demonstrate the low potential for toxicity of BBG (0.25 mg/L).

Literature:
Publications concerning Brilliant Blue G (BBG)

Capsular staining ability of BBG in pigs’ eyes

The capsular staining ability of BBG (10, 1, 0.5, 0.25, 0.1, 0.01 mg/ml) was evaluated in enucleated pigs’ eyes.

Ophthalmic examinations (light, fluorescence and transmission electron microscopy and apoptotic cell death counting) of the enucleated eyes after 10 weeks.

BBG was shown to be effective and safe for capsular staining.

Literature:
Publications concerning Brilliant Blue G (BBG)

Clinical trial (20 patients)

20 patients with macular hole (MH) and epiretinal membrane (ERM) surgery underwent BBG–assisted (0.25 mg/ml) ILM and ERM removal.

BBG was shown to selectively stain the ILM without any adverse effects observed during the observation period (mean follow up = 7.3 months ± 1.0 months).

Literature:
Internal limiting membrane (ILM)

Internal limiting membrane of the retina

Thickness between 3.2 and 0.01 µm

Transparent

Because the collagen fibers of the vitreous are connected to the ILM, tractions can be transferred to the retinal surface.

A stained ILM is easier and safer to remove and can be peeled more effectively, minimizing the risk for the patient.
ILM Peeling

Clinical procedure:
- Vitrectomy
- Liquid / gas – exchange
- Application of the staining solution (contact time!!!)
- Removal of the staining solution (residues!!!)
- ILM Peeling

ILM staining administers better clinical results for macular surgery.

Due to the selective staining behavior of the dye, surgeons can better identify insufficiently peeled ILM regions of the retina and can complete the peeling.

Safe and effective differentiation of the ERM, retina and vitreous body.

The risk of mechanical damage to the retina by unskilled surgeons is lowered.

Clinical risks with ILM Peeling:
- Retinal bleeding
- Retinal tears
- Damage to the retinal pigment epithelium
Epiretinal Membrane (ERM)

The epiretinal membrane is a thin, flat piece of skin which slowly grows over the surface of the macula at the center of the retina.

This membrane shrivels with time and the underlying retina wrinkles and swells up, causing vision to slowly worsen.

Without treatment, the membrane continues to grow, and vision increasingly worsens.
ERM Peeling

The epiretinal membrane must be removed operatively.

The surgeon enters the eye with a fine instrument laterally to the cornea over a 1 mm cut.

After removing part of the vitreous, the membrane is pulled with a pair of fine forceps from the retinal surface.

To prevent the membrane from regrowing, a thin lamella of the retinal surface is removed as well.
Results
Numerous studies report positive reports following vitrectomy with removal of the epiretinal membrane. Vision improved by at least two points in 60-87% of the cases.

Vision improved 1 to 3 months following the operation at the earliest and stabilized after 6 to 12 months.

The flattening of the retina caused blurry vision to decrease considerably.

Complications

Apart from cataracts, complications occur intra and postoperatively in approx. 8 to 13% of the cases. The most common intraoperative complication which occurs in 1 to 5% of the cases is the creation of retinal tears centrally or peripherally. Rare intraoperative complications include retinal detachment, vitreous bleeding, and phototoxic damage to the macula as a result of exposure to intense light. The most common postoperative complication is retinal detachment, occurring in 4 to 6% of the cases. Regeneration of the membrane occurs in 2.5 to 7.3% of the cases. Infections within the eye are very rare.
Clinical trial (Cologne)

Results:

The ILM staining ability of Brilliant Peel® (0.25 g/L) is comparable to that of Indocyanine Green.

Brilliant Peel®(0.25 g/L) can be completely removed from the eye.

Fluid/gas exchange is not necessary.

Postoperative follow-up results are comparable to those of Indocyanine Green.

Due to the excellent staining ability and the better biocompatibility when compared to Indocyanine Green, Brilliant Peel® is preferable.

Prof. Dr. Bernd Kirchhof
Uniklinik Cologne
Comparison of the dyes for chromovitrectomy

<table>
<thead>
<tr>
<th>Chemical group</th>
<th>ICG</th>
<th>BBG</th>
<th>TB</th>
<th>PB</th>
<th>BrB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Dark green</td>
<td>Blue</td>
<td>Dark blue</td>
<td>Blue</td>
<td>Dark blue</td>
</tr>
<tr>
<td>Affinity to ILM</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>Affinity to ERM</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Affinity to vitreous</td>
<td>Low</td>
<td>Unknown</td>
<td>Low</td>
<td>Moderate</td>
<td>Unknown</td>
</tr>
<tr>
<td>Toxicity to Retina</td>
<td>Moderate</td>
<td>low</td>
<td>Moderate</td>
<td>low</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
### Properties of Trypan Blue (TB), Indocyanine Green (ICG) and Brilliant Blue G (BBG)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ICG</th>
<th>BBG</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registration</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Toxicity</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Selective Staining of ILM</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Staining intensity of ILM</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Ready-to-use</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Exposure time</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Fluid / gas exchange</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>
Summary

First CE-marked dye for ILM peeling

No adverse side-effects

Staining intensity is comparable to that of Indocyanine Green

Selective staining of the ILM

Fluid/gas exchange not necessary

Product launch: July 2007

Sterile vials with 0.5 ml dye-solution
Publication list


Author / Contact

Dr. Bastian Mühling

Fluoron GmbH, Biberkopfweg 1, 89231 Neu-Ulm, Germany
Tel.: 49 0731 / 81060
Fax: 49 0731 / 9807805

Internet: www.fluoron.de
Email: bastian.muehling@fluoron.de