

A histological analysis of lens capsules stained with trypan blue for capsulorrhexis in phacoemulsification cataract surgery

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Abstract

Purpose Staining of anterior lens capsules with dye to facilitate completion of continuous curvilinear capsulorrhexis is now being used more frequently in phacoemulsification of white and mature cataracts with poor red reflexes. This study examined the histological characteristics of anterior lens capsules stained with trypan blue. The layer(s) of the lens capsule that stained with dye and the extent of accumulation of dye in these layers of the lens capsule were determined. To the best of our knowledge this has not been described before. **Methods** A series of 10 stained lens capsules were analysed histologically. The dye used in this study consisted of a standard sterile, noninflammatory, nonpyrogenic, 2 ml solution containing 0.6 mg/ml of trypan blue. Following capsulorrhexis, samples were sent to the laboratory for histological analysis. Frozen sections (8 µm) were prepared and examined with the light microscope. All 10 capsules were cut by frozen section to preserve trypan blue staining (which would be leached by processing) and then subjected to immunohistochemistry for collagen IV. Immunohistochemical analysis using markers for type IV collagen were done on formalin-fixed specimens for morphological comparison with the frozen sections. A counterstain highlighted the epithelium. **Results** Continuous curvilinear capsulorrhexis was successfully and easily completed in all cases without any complications. Frozen section analysis using light microscopy demonstrated accumulation of trypan blue dye in the basement membrane of the lens capsule. Staining was concentrated in the portion of the membrane adjacent to the

lens epithelium. The lens epithelium could not be clearly identified on the frozen sections. Consequently, immunohistochemical analysis with markers for type IV collagen was performed. A counterstain highlighted the epithelium. This confirmed that the layer staining with trypan blue was the basement membrane, a consistent feature on all the specimens.

Conclusions Trypan blue selectively stains the basement membrane of the anterior lens capsule. There is a concentration of dye in the basement membrane adjacent to the lens epithelial cell layer. The lens cortex does not appear clinically to stain with trypan blue. This enables surgeons to distinguish the lens capsule from the cortex and provides sufficient contrast for successful completion of continuous curvilinear capsulorrhexis during cataract surgery.

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Introduction

Phacoemulsification of 'white' and mature cataracts presents a unique challenge for ophthalmic surgeons.¹ The absence of an adequate red reflex makes it difficult to perform continuous curvilinear capsulorrhexis with certainty.² Retroillumination created by coaxial light from the operating microscope is reduced in the presence of a dense cataract and in heavily pigmented fundi. Various methods to overcome this problem have been described and

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recently dye staining of the anterior capsule has been employed.³

Use of an agent containing the dye, trypan blue, has become popular with cataract surgeons.⁴ This product is commercially available as Vision Blue[®] (D.O.R.C. International b.v.), which contains 0.6 mg trypan blue, 1.9 mg sodium monohydrogen orthophosphate, 0.3 mg sodium dihydrogen orthophosphate, 8.2 mg sodium chloride, sodium hydroxide for adjusting pH, and water for injection.

We performed an histological analysis on lens capsules stained with trypan blue using light microscopy on frozen sections. Immunohistochemical analysis was performed on specimens fixed in formalin for morphological comparison with the frozen sections.

Materials and methods

Collection of samples

The lens capsules of 10 patients undergoing cataract surgery by phacoemulsification were collected in this prospective series. Capsulorrhesis and harvesting of specimens were done by the same surgeon (RS) using a standard technique outlined below.

The surgery was performed using a ceiling-mounted Zeiss Opmi Visu 200 operating microscope. The cataract was either white or mature, or had advanced anterior cortical matter, but no hypermature or Morgagnian cataracts were included in this series.

Via a corneal tunnel incision, an air bubble was injected into the anterior chamber to prevent dilution of the trypan blue. The dye was then injected onto the anterior lens capsule using a 27G Rycroft canula mounted on a 2 ml syringe. When sufficient dye was injected to cover the anterior lens capsule, the anterior chamber was then irrigated using the irrigation-aspiration mode of the phacoemulsification machine. The exact amount of dye used for each case was not recorded, but it did not exceed 0.5 ml. Injection of viscoelastic was then followed by capsulorrhesis using a cystitome only. The lens capsule was then removed en bloc and placed in a sterile container without fixative. All lens capsules were oriented with the epithelial side facing upwards. Fresh specimens were transported immediately to the laboratory for histological analysis.

Histological preparation

The lens capsule was laid flat in a small embedding mould. A 2% agar solution was poured into the mould and allowed to set. This provided support for the lens capsule. Using a scalpel blade, the agar surrounding the capsule was cut, the specimen was then turned by 90°

onto its side and then frozen in Tissue Tek OCT Compound mounting medium. Frozen sections (8 μ m) were then cut using a Shandon cryostat and mounted onto glass slides. The section was then coverslipped using either coverslip film or a glass coverslip and XAM mountant.

Immunohistochemical analysis

Immunohistochemical analysis was done on specimens fixed in formalin and paraffin wax embedded. Sections 4 μ m in thickness were pretreated with 0.025% pepsin in 0.01 M hydrochloric acid. These were then incubated overnight at 4°C in the DAKO monoclonal antibody collagen type IV at a dilution of 1 in 10. The following day, specimen staining was completed using the DAKO Strept Ab Complex/HRP Duet Mouse/Rabbit kit and the chromogen diaminobenzidine (DAB).

Results

Using trypan blue, continuous curvilinear capsulorrhesis was successfully and easily completed in all cases. Adequate contrast and visibility was reported by the surgeon (RS) in all cases. There were no complications of radial extension or tearing of the capsule.

A total of 10 suitable specimens were collected and mounted for histological analysis.

Observation of the frozen sections by light microscopy demonstrated accumulation of trypan blue in the basement membrane of the lens capsule. Staining was concentrated in the portion of the basement membrane adjacent to the lens epithelial layer. The epithelial layer was indistinct, but could just be discerned as a refractive layer as it did not stain with trypan blue on the frozen sections (see Figure 1).

This confirms the clinical observation of deeper staining of the internal surface of the lens capsule as the capsule is folded on itself during capsulorrhesis. All samples demonstrated consistently similar staining patterns.

The immunohistochemical specimens with markers for type IV collagen confirmed the layer staining with trypan blue to be the basement membrane. A counterstain highlighted the epithelium. Omission of the primary antisera confirmed the absence of nonspecific staining in the negative controls. The localisation of the reaction confirmed that the accumulation of reaction product in the basement membrane was adjacent to the lens epithelial layer (see Figure 2), the cellular staining is a counterstain, and the brown reaction product stains the basement membrane. Consistent staining patterns were seen in all specimens.

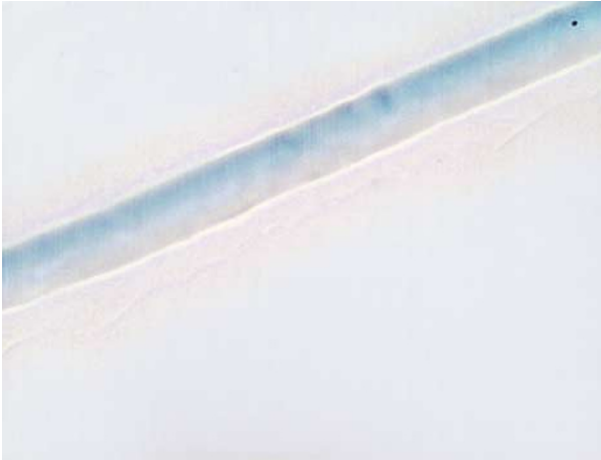


Figure 1 Inner layer of the lens capsule is stained with trypan blue. Frozen section. Original magnification $\times 1000$.



Figure 2 Immunohistochemistry for collagen IV confirming basement membrane-like material adjacent to the epithelium. Original magnification $\times 1000$.

Discussion

Various techniques to successfully complete continuous curvilinear capsulorrhexis of white and mature cataracts have been employed. These include two-stage approach to capsulorrhexis, diathermy, endoillumination, and vitrectome and scissors.⁵⁻⁸

Continuous curvilinear capsulorrhexis is a vital step in phacoemulsification cataract surgery. Successful

completion of this step is associated with low complication rates and good surgical outcomes.² This has been attributed to the inherent strength and elasticity of the continuous curvilinear capsulorrhexis.²

Recently, staining of the anterior capsule for difficult capsulorrhexis because of poor visibility from inadequate retro-illuminated red reflexes has become popular with cataract surgeons. Many staining agents have been tried including autologous blood and a variety of dyes.⁹

Dyes used include indocyanine green 0.5%, gentian violet 0.1%, methylene blue 0.1%, subcapsular fluorescein and trypan blue (Vision Blue).^{3,4,10,11}

Some dyes considered inappropriate for cataract surgery include gentian violet and methylene blue. These have been known to cause corneal oedema postoperatively and this has been attributed to endothelial toxicity of these dyes.^{10,11} They are no longer used intracamerally in cataract surgery.^{10,11}

Fluorescein dye when used in anterior segment surgery migrates into the vitreous cavity and stains the lens cortex, nucleus, and corneal endothelium.³ This provides inadequate contrast between the lens capsule and cortex. These properties limit its usefulness as an aid in performing capsulorrhexis in cataract surgery. This is because contrast between the lens capsule and cortex is necessary for visualisation of these layers during capsulorrhexis when the red reflex is poor.

Indocyanine green has been used successfully to stain lens capsules and appears to be nontoxic to the corneal endothelium based on a few small-scale studies.³ However, trypan blue stains the lens capsule better and has been subject to more rigorous assessment of its safety in intraocular surgery.^{4,12,13}

As early as 1967, trypan blue has been used in ophthalmology to achieve vital staining of the cornea and conjunctiva.¹⁴ It has also been used to examine endothelial cells after cataract surgery and in donor corneal grafts without any documented adverse effects.^{12,13}

Trypan blue is a blue bis-azo dye. It is a symmetrical molecule consisting of three main parts connected by two azo bonds^{14,15,16} (see Figure 3).

The molecular weight is 961 and the empirical formula is: $C_{34}H_{23}N_6O_{14}S_4Na_4$. It is water soluble, contains acid groups, and is pH adjusted for intracameral use.

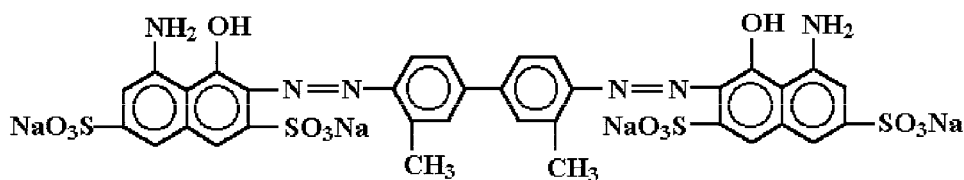


Figure 3 Structural formula of trypan blue.

Intracamerally injected trypan blue is seen to stain selectively the basement membrane of anterior lens capsule and is easily washed out of the anterior chamber. Permanent discolouration of ocular tissue has not been observed.⁴

Clinical observation during capsulorrhexis with trypan blue demonstrates greater staining of the posterior surface of the anterior lens capsule. This is easily seen with the operating microscope during capsulorrhexis when the capsule is often folded on itself. Light microscopy findings demonstrate staining of the basement membrane with accumulation of dye in the deeper layers adjacent to the lens epithelium. This provides histological confirmation of this clinical observation.

Bis-azo dyes, such as trypan blue, stain poorly hydrated tissues better than well-hydrated ones.¹⁷ The lens cortex consisting of lens fibres has high water content compared to the acellular basement membrane.^{18,19} This would explain why the dye stains the basement membrane without clinically significant staining of the lens cortex. This results in marked colour contrast between these two structures. The resulting high visibility of the lens capsule allows the surgeon to precisely identify it from the underlying cortex during capsulorrhexis for phacoemulsification of the lens. The torn edge of the capsulorrhexis is also highly visible, which is especially important for safe completion of this step.

Trypan blue stains mostly in the basement membrane adjacent to the epithelial layer of the lens capsule with minimal lamellar staining in the superficial basement membrane. The lens cortex does not stain. The staining characteristics of trypan blue on the lens capsule allow safe and easy completion of continuous curvilinear capsulorrhexis during cataract surgery. To the best of our knowledge these staining properties have not been described before.

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