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REVIEW

Trypan blue dye for anterior segment surgeries

V Jhanji[1,2], E Chan[2,3], S Das[4], H Zhang[5] and RB Vajpayee[2,3]

Abstract

Use of vital dyes in ophthalmic surgery has gained increased importance in the past few years. Trypan blue (TB) has been a popular choice among anterior segment surgeons mainly due to its safety, ease of availability, and remarkable ability to enable an easy surgery in difficult situations mostly related to visibility of the targeted tissue. It is being used in cataract surgery since nearly a decade and its utilization has been extended to other anterior segment surgeries like trabeculectomy and corneal transplantation. This review will discuss the techniques and outcome of TB dye-assisted anterior segment surgeries.
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Introduction

In ophthalmology, vital dyes have become very effective and useful surgical tools for ocular tissue identification. They have been used in cataract, vitreoretinal, corneal, glaucoma, orbital, strabismus, and conjunctival surgery. Dye-enhanced surgery is a preferred method in cases of cataract surgery, especially with compromised red fundus reflex. Several types of dyes, including fluorescein, indocyanine green (ICG), autologous blood, and gention violet have been proposed and tested.[1–3] By far, trypan blue (TB) dye has been the most frequently used agent in cataract, as well as other anterior segment, surgeries. This review will discuss the evolution of use of TB dye in various types of anterior segment ocular surgery. Applications of TB dye and safety of its use would be presented.

History of trypan blue (TB) dye staining in anterior segment surgery

Use of TB in ophthalmology dates back to the 1970s, when it was used to stain the corneal endothelium preoperatively.[4] It emerged in the late 1990s as one of the known staining material used intraoperatively to aid in visualization of the anterior lens capsule.[5] Melles *et al*[5] reported the first experience with intraoperative application of TB to stain the anterior capsule and to facilitate capsulorhexis for surgery for mature cataract.

Concentration of TB

Chang *et al*[6] assessed the concentration of TB required to effectively stain rabbit anterior capsule sufficient for capsulorhexis. After staining at concentrations of 0.001, 0.01, 0.1, and 0.4% for 1 min, a minimum of 0.1% concentration was thought to be necessary for staining. In contrast, Yetik *et al*[7] found that a concentration as low as 0.0125% was adequate for staining the capsule. Overall, in the published literature, concentrations used for anterior capsule staining have varied, and have included 0.6,[8] 0.4,[9] 0.1,[2] 0.06,[10] and 0.0125%.[11] The choice of concentration in these instances appears to be related to the availability of various formulations of TB. In terms of the duration of staining, *in vitro* testing using human anterior capsules suggests an exposure time of 60 s is adequate for visualization,[12] whereas clinically, the duration of staining is usually 'a few seconds'.[5,10]

In corneal surgery, TB 0.02% has been used to aid deep lamellar keratoplasty dissection[13] and penetrating keratoplasty.[14] A concentration of 0.1% was used by Sinha *et al*[15] to stain retained Descemet's membranes after penetrating keratoplasty.

The most commonly used commercially available TB was available as Vision Blue (TB 0.1%, DORC International BV, Zuidland,

[1]Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China

[2]Centre for Eye Research Australia, University of Melbourne, Melbourne, Victoria, Australia

[3]The Royal Victorian Eye and Ear Hospital, Melbourne, Victoria, Australia

[4]LV Prasad Eye Institute, Bhubaneswar, Orissa, India

[5]The First Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang Province, China

Correspondence: RB Vajpayee, Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, 32 Gisborne Street, East Melbourne, Victoria 3002, Australia
Tel: +61 3 9929 8368; Fax: +61 3 9662 3959.
E-mail: rasikv@unimelb.edu.au

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The Netherlands) for use in anterior segment surgery. It is now manufactured at the lower concentration of 0.06%. Vision Blue is widely used in an undiluted form by most surgeons. It is also noteworthy that as there is a reported difference in the purity of TB used in different commercial products, a different percentage of TB may be required for the same efficacy and may account for the differences noted in the literature.

Dye-assisted cataract surgery

Phacoemulsification has become the standard surgical technique of management of cataract and creation of a continuous curvilinear capsulorrhexis (CCC) is a critical step of this procedure. The visibility required to create an optimal capsulorrhexis depends on red reflex coming from the posterior segment of the eye. This red reflex and view of anterior capsule can be compromised in eyes with white or dense cataract, corneal haze, and vitreous hemorrhage. Poor visualization of the capsule may be associated with a challenging CCC that is increasing the risk for radial tears toward or beyond the lens equator and other complications such as capsule rupture, vitreous loss, nuclear drop, and intraocular lens displacement.

TB provides several advantages in cataract surgery. The rate of conversion to an extracapsular cataract extraction in white cataract as the result of an incomplete CCC has been as low as 3.85% when TB is used compared with 28.3%, when no dye has been used.[8,16,17] TB use has also been described to stain the leading edge of a lost capsulorhexis.[10]

Techniques for capsule staining

Different methodologies for injecting the dye have been described, including beneath air,[5,18] beneath ophthalmic surgical device (OVD),[19,20] beneath OVD and BSS,[21,22] and mixing with an OVD.[9,23,16] Wong *et al*[24] found both methods equivalent.

Air bubble injection

Melles *et al*[5] initiated the use of TB to stain the anterior capsule under air. This allowed the formation of a dye lake and prevented the dilution of dye by aqueous. An additional benefit of this technique is better staining of the peripheral anterior capsule rim and lack of contact of dye with endothelium. However, this technique has been criticized as time consuming, and it may be difficult to reform the anterior chamber with a single-air bubble.[7] Any instrument entering the eye will cause small air to escape, raising the lens–iris plane. A small amount of high-density viscoelastic material placed near the incision site minimizes the risk of sudden collapse.

Intracameral subcapsular injection

In this technique, aqueous is replaced with viscoelastic before injecting dye beneath the anterior capsule. There might be a slight leakage of dye from subcapsular space during this step. The stained viscoelastic is replaced with clear viscoelastic before starting CCC. This technique was originally proposed for fluorescein injection.[1,25] When the capsular flap is inverted, the stained posterior surface of the anterior capsule enhances visualization and thus facilitates tearing during CCC. It has the advantage of trapping the dye in subcapsular space, mostly in the centre and midperiphery. It gives the surgeon sufficient time for any maneuver until the CCC releases it. It is technically more invasive and anterior capsule may tear if excessive dye is injected.

Injection under viscoelastic agent

An alternative staining technique without the use of air bubble was suggested by Yetik *et al*[7] and involves injection of dispersive viscoelastics. TB solution is painted onto the anterior capsule. The anterior chamber is irrigated with balanced salt solution (BSS) and excessive TB-viscoelastic mixture is aspirated out. After capsular staining, the anterior chamber is refilled with viscoelastic before starting CCC. The advantages of the procedure include a well-maintained anterior chamber depth and minimal contact of dye with corneal endothelium. The main drawback of staining under viscoelastic is the potential for the TB dye at the viscoelastic–anterior capsule interface to obscure the visibility of CCC. Also, this can potentially increase the cost of surgery. Arshinoff[19] has described the ultimate soft-shell technique, in which the anterior chamber is compartmentalized, and TB with Healon 5 or sodium hyaluronate 1% is used when performing the capsulorhexis in eyes with white, mature, or hypermature cataract. In another variation, Khokar *et al*[20] has used a special painting cannula under a viscoelastic agent.

Mixing with viscoelastic injection

Kayikicioglu *et al*[9] mixed TB with sodium hyaluronate and injected onto the anterior capsule under an air bubble. Thereafter, the colored solution is completely washed out, and the anterior chamber is refilled with clear viscoelastic. Dada *et al*[23] recommended mixing TB with sodium chondriotin sulfate-sodium hyaluronate, suggesting that a more dispersive OVD may enhance endothelial protection.

Use of viscoelastic device and balanced salt solution

Marques *et al*[21] have described a three-step technique for TB capsular staining. The anterior chamber is filled with high molecular weight ophthalmic viscoelastic device. Using a 27-gauge cannula, balanced salt solution is injected directly onto the anterior capsule. Thereafter, the dye is slowly introduced through a 27-gauge cannula, which has a curved shaft with a single hole on the posterior surface, onto the lens surface through a tuberculin syringe. As the dye mixes with the BSS, the anterior capsule can be painted approximately according to the size of the intended CCC. This technique allows for better control without excessive staining of other intraocular structures. Arshinoff[22] has recommended the use of TB and BSS in reverse order.

Intracameral one-step injection

In this technique, the dye is instilled via a paracentesis at the beginning of cataract surgery.[26] Aqueous humor is allowed to exit the anterior chamber, which becomes shallow, and the resulting pupil block confines the coloring agent to the anterior chamber. An OVD is used to flush dye-stained aqueous from the anterior chamber. This method requires no additional instruments and materials and is faster.

Types of cataract and TB dye

White cataract

Intumescent, mature, and hypermature cataracts constitute a significant proportion of cases undergoing cataract surgery in developing countries. Creating a CCC may pose a challenge to the surgeon in these eyes. TB dye provides predictable and uniform staining of the anterior capsule and thus allows good visualization of the peripheral anterior rim of the CCC.

The reported success rates of performing CCC in mature cataracts with TB dye staining are as high as 100%.[5] Jacob *et al*[8] could complete CCC uneventfully in 96.15% eyes with white cataract using TB. Kothari *et al*[27] had 100% success in performing CCC in 25 cases of mature or hypermature cataract, including one traumatic cataract. One case had peripheral extension of the capsulorhexis margin but the stained edge of the anterior capsule helped in identification and redirection of the CCC.

Traumatic cataract

Trauma to the crystalline lens may cause cataractous change irrespective of the integrity of the capsule. Selective staining of the anterior capsule enables surgeon to perform a safe and successful cataract surgery. TB dye helps to identify the presence, extent, and direction of any preexisting capsular tear. Kazem *et al*[28] has described three cases of traumatic cataract surgery with use of TB as an adjuvant.

Pediatric cataract

A complete anterior continuous curvilinear capsulorhexis and posterior CCC facilitates intraocular lens (IOL) placement in the bag with or without posterior optic capture during pediatric cataract surgery. The anterior and posterior CCC can be difficult to perform because of elasticity and tension of the capsule in children. Capsular staining dyes have been used to improve the visibility and increase the rate of complete anterior and posterior CCC.[29] Saini *et al*[30] conducted a prospective randomized trial to create anterior and posterior CCC in pediatric cataract surgery with and without using TB dye. The majority (91.3%) of the eyes had complete anterior CCC and 82.6% eyes had complete posterior CCC in the eyes where TB was used to stain the capsule, in comparison with 73.6 and 52.6% of anterior and posterior CCC, respectively, in eyes without TB. The difference was significant between both groups. Sharma *et al*[31] had evaluated the efficacy of TB in posterior capsulorhexis with optic capture in pediatric cataracts in a prospective randomized study. Optic capture was possible in 17 (out of 18) eyes in TB-assisted surgery and 11 (out of 17) eyes where no dye was used ($P = 0.04$).

Cataract with corneal opacity

A combined penetrating keratoplasty with cataract surgery is the method of choice for patients with corneal opacity and cataract. However, factors like risk of graft rejection, graft infection, and lack of frequent and meticulous follow-up decreases the chances of success of penetrating keratoplasty. Patients with nebulomacular corneal opacities and a visually debilitating cataract may become ambulatory following cataract surgery. Bhartiya *et al*[32] have reported phacoemulsification in 11 eyes with nebulomacular corneal opacities. A complete capsulorhexis could be performed in all cases successfully. Titiyal *et al*[33] had described small incision cataract surgery in cases with corneal opacities using TB dye for capsulorhexis.

Wet lab training of phacoemulsification

It is important for surgeons to practice phacoemulsification in a wet lab setting to reduce the learning curve and enhance safety margin before they operate on an actual patient. Visualization during each step can be enhanced by the use of adjuvant dyes. Werner *et al*[34] had used 0.5% ICG and TB 0.1% to enhance visualization for performing critical steps of phacoemulsification in eight human eyes obtained post mortem and compared the results in eight eyes without using dye (control group). Both dyes enhanced visualization during phacoemulsification compared with control group. Dada *et al*[35] evaluated TB dye-assisted CCC in phacoemulsification of immature cataracts by trainee surgeons. Trainee surgeons performed 10 cases each with and without the use of TB dye. A complete CCC was achieved independently in all cases with TB as compared with only 30% cases in the other group.

A report from the American Academy of Ophthalmology in 2006 showed level III evidence that TB dye along with ICG and fluorescein are each effective in staining the lens capsule. There was level II evidence that staining the capsule is helpful in completing capsulorrhexis and that it is helpful for pediatric patients <5 years and in cases of white cataract. There are substantial data indicating that TB 0.1% is not toxic to the cornea. The report concluded that it may be reasonable to use dye when inadequate capsule visualization may compromise the outcome in cataract surgery.[36]

TB in glaucoma surgery

TB has also been used in glaucoma filtration surgery to visualize the treatment area of antimetabolites and to confirm the patency of drainage blebs and tubes. In their observational case series, Healey and Crowston[37] mixed 0.1% TB with mitomycin C (MMC) or 5-fluorouracil (5-FU), for a final concentration of TB between 0.01 and 0.05% with MMC or 0.01% with 5-FU. The mixture of antimetabolite and TB was used as per usual clinical practice with sponges or by subconjunctival injection during trabeculectomy surgery. This helped the authors to identify the antimetabolite treatment area, which is important for both the success of the bleb and risk of antimetabolite toxicity from inadvertent treatment of surrounding tissue and leakage of 5-FU from subconjunctival injection sites. Other useful findings included the observation that there was a relatively poor absorption of antimetabolite in posterior sponges when the sponges were pre-placed dry, but not when pre-soaked sponges were used. Use of TB in this study also resulted in easier visualization of the surgical sponges, which aided in their removal. *In vitro* studies with human Tenon's capsule fibroblasts confirmed that the TB did not affect the antimetabolite function of MMC or 5-FU.

It has also been observed that intracameral injection of TB during cataract surgery leads to diffusion into a functioning, but not a flat bleb.[38,39] Grigg *et al*[40] describe the use of intracameral 0.06% TB injected directly through the opening of a Baerveldt tube with a cannula to confirm its patency. The dye was seen to flow into the tube and onto the plate. They report that they have used this method successfully in six out of six cases. Confirmation of the patency of filtration surgery is important in the management of glaucoma patients.

TB in conjunctival surgery

TB has had some limited usefulness in conjunctival surgery. Kobayashi *et al*[41] describe the successful removal of a conjunctival cyst after initial staining with a 50 : 50 mixture of Healon V (Pfizer Inc., New York, NY, USA) and 0.06% TB. The staining of the capsule of the cyst facilitated its complete removal, and thus reducing the risk of recurrence. The use of sodium hyaluronate helped to prevent cyst collapse during its excision.

TB for keratoplasty

The concept of use of TB during penetrating (PKP), as well as lamellar keratoplasty, mainly stems from its property of staining the Descemet's membrane. TB is commonly used to stain the anterior lens capsule for capsulorhexis in cataract surgery. As both lens capsule and Descemet's membrane are basement membranes, TB highlights the Descemet's membrane and enhances its visibility during the surgery. Sinha *et al*[15] used TB employing this theory for excision of inadvertently retained Descemet's membrane after penetrating keratoplasty. The technique called 'descemetorhexis' was used in two cases with retained Descemet's membrane after undergoing penetrating keratoplasty for congenital hereditary endothelial dystrophy.

Roos *et al*[14] described the use of TB dye during PKP. They injected 0.02% TB to stain the internal and external cut edges of the cornea, as well as the ophthalmic viscosurgical device, enabling the surgeon to improve visualization of the incision and suture depth therefore improving the alignment of host and donor tissues. This technique also helps in visualization of the OVD allowing its complete removal toward the end of the surgery.

TB has been used in lamellar corneal transplantation surgery. Balestrazzi *et al*[13] described a surgical technique of deep lamellar keratoplasty using 0.02% TB to stain the intrastromal bed, allowing good visualization of the dissection depth and decreasing the risk of perforation of Descemet's and endothelial layers. After a two-third trephination of the cornea, 0.02% TB solution is injected intrastromally in four quadrants through a 30-gauge cannula for superficial dissection. Additional dye is injected for deep dissection to stain the stromal fibers.

The authors reported that their technique facilitated a safe and effective dissection of the posterior corneal stroma and a successful deep lamellar keratoplasty. TB disappeared from the corneal stroma in early postoperative period.

More recently, Sharma *et al*[42] described the use of TB during conversion of deep anterior lamellar keratoplasty to penetrating keratoplasty in cases with intraoperative perforation of Descemet's membrane. The presence of extensive corneal emphysema in cases with intra-operative Descemet's membrane perforation results in poor visualization of trephined corneal edges that can make wound apposition difficult during suturing. TB staining of corneal stroma as described in this technique was useful for excising the corneal shelf close to the trephined corneal edge, providing a near-vertical wound profile at the end of surgery. An additional advantage of using the dye is the easy visualization of partially excised Descemet's membrane that can be identified with TB staining.

New techniques for corneal transplantation surgery, such as anterior lamellar keratoplasty, deep lamellar endothelial keratoplasty (DLEK), or Descemet's stripping endothelial keratoplasty (DSEK), are leading to better surgical outcomes, although these techniques require accurate identification of the layers of the cornea. Vital dyes may improve identification of these layers by staining specific structures. TB dye is being utilized by corneal surgeons in different steps of endothelial keratoplasty including identification of diseased corneal endothelial layer.[43] Although TB dye is being used for specific steps in the various keratoplasty techniques, there is as of yet no standardization.

TB in other anterior segment surgeries

Visualization of clear corneal incisions and side ports in anterior segment surgery can often be difficult, particularly in the presence of significant corneal edema, scarring, or arcus senilis. Kayikcioglu[44] described a technique whereby a surgical blade was coated with 0.4% TB 15 min before making a corneal incision. This led to staining of the corneal wound stroma that persisted for only a few days post-operatively. Although such a technique is not routinely used, it has its advantages to reduce unnecessary epithelial trauma when trying to find elusive side ports.

Staining of the anterior vitreous can also be achieved with TB.[45] TB 0.06% injected under air intracamerally has been shown to stain vitreous within the anterior chamber after 60 s, allowing for its complete removal with a vitrector. Although the use of intracameral triamcinolone[46] has become more popular, the use of TB potentially avoids problems with preservatives and post-operative pressure-related complications.

Safety

Since the original description of using TB to facilitate capsulorhexis in cataract surgery in 1999,[5] the dye has been used widely, with relatively few reports of complications in the literature. *In vitro* testing of TB[6] on cultured rabbit corneal endothelial cells did not reveal any significant toxicity at concentrations of up to 0.4% for 1 min. However, van Dooren *et al*[47] found toxicity to cultured human corneal fibroblasts at concentrations of 0.01% or greater, but only after exposure for at least 6 h.

The findings from laboratory studies are supported by clinical studies, where TB has not been reported to cause a significant rise in intraocular pressure, increase in intraocular inflammation, thickening of the cornea or a decrease in endothelial cell counts in a case series report.[2,5,48,49]

The effect of TB on lens epithelial cells has been tested using different techniques.[50,51] Although *in vitro* studies found no effect on the viability of LECs at concentrations ranging from 0.025 to 5.0 mg/ml,[52] Nanavaty *et al*[11] reported a significant decrease in LEC density covering the anterior lens capsule after the use of 0.0125% TB for 30 s compared with that of untreated eyes. Portes *et al*[53] found LEC death with 0.1% TB which supports that staining with TB can help reduce the incidence of posterior capsule opacification.

TB affects the biomechanical properties of the human lens capsule and leads to a significant reduction in elasticity and increase in stiffness.[54] The impairment of elastic properties of the lens capsule could increase the risk for capsular tears and may result in a higher incidence of intraoperative complications. A reduction in the elasticity of the anterior capsule has been reported in studies using human anterior capsule,[54] with a suggestion that this is more significant in diabetic, compared with non-diabetic patients,[55] in a process that appears to be light-exposure dependent.[56]

TB has also been reported to stain hydrophilic acrylic IOLs in digital image analysis, but not poly methyl-methacrylate or silicone lenses[12] after prolonged exposure of 180 min in *in vitro* testing. Clinical reports confirm permanent staining of the Acqua hydrophilic expandable acrylic IOL (Mediphacos, Minas Gerais, Brazil),[57] causing a reduction in glare at a mean of 2.6 months post-operatively.[58] Jhanji *et al*[59] have reported a case, in which the corneal stroma was inadvertently stained with TB during cataract surgery.

Diffusion of TB has been reported to cause inadvertent staining of the posterior capsule, resulting in a poor red reflex intraoperatively. This was thought to be the result of zonular dehiscence and separation of the anterior hyaloid face from the posterior lens surface in a vitrectomised eye from previous retinal detachment surgery. Similarly, there have been several cases of inadvertent vitreous staining following intracameral use of TB, each related to traumatic cataracts and thought again, to be related to zonular dehiscence.[60–62]

The effect of TB on the retina has revealed contrasting results. An *ex vivo* study of porcine retina suggested that exposure of 0.15% TB for 1 min did not cause any histological damage.[63] Using cultured human retinal pigment epithelial cells, no increase in cell death was found in concentrations up to 0.5% for 5 min,[64,65] however, prolonged culture for 6 days at even 0.005% resulted in toxicity.[65] Luke *et al*[66] found that exposure of bovine retina to 0.15% TB for more than 15 s caused a reduction in b-wave amplitude, which was only partially reversible after 115 min. In animal studies, intravitreal injection of 0.2 and 0.06% TB into rabbit eyes that had undergone vitrectomy and gas injection showed sectoral damage to multiple retinal layers histologically, although no full-field ERG changes were detected.[67]

There has been one retrospective study suggesting that the use of TB increases the rate of post-operative cystoid macular edema. The TB group had a worse pre-operative visual acuity, suggesting that these cataracts were denser, and hence an accurate conclusion cannot be made.[68]

Perhaps the most serious reported complications of TB have been a report of two cases of toxic anterior segment syndrome (TASS) leading to irreversible endothelial damage.[69] TB has also been reported to cause a sterile endophthalmitis in 3 out of 17 cases where a single batch of TB 0.6 mg/ml was used.[70] It was commented that the dye was from an unknown commercial manufacturer, with a contaminant suspected to be the causative agent. These cases highlight the importance of recognizing that the use of generic formulations or unfamiliar commercial products for intracameral use should be carried out with caution.

Finally, it is worth noting that TB use in the pediatric population has been less well studied. Saini *et al*[30] found no increase in intraocular inflammation post-operatively when 0.1% TB was used in 42 children with a mean age of 4.13 years, although no other parameters were assessed.

Conclusions

TB is the most commonly used dye in cataract surgery. The prevalence of its use is increasing due to the introduction of wet lab training in almost all the teaching hospitals around the world. Besides, its safety profile allows for expanding indications for its use in corneal, conjunctival, and glaucoma surgeries. The recent ophthalmic technology assessment report from the American Academy of Ophthalmology for the use of TB in cataract surgery provides further evidence of its use and safety profile.[36]

Conflict of interest

The authors declare no conflict of interest.

Method of Literature Search

Pubmed was queried with combinations not limited to the following search terms: trypan blue, ocular surgery, cataract, corneal transplantation, keratoplasty, trabeculectomy, cataract surgery training, and complications. A review of the search results was performed and relevant articles to the topics of clinical manifestations and treatment were included. Relevant articles to the management of corneal in various conditions were also included. Case reports without additional value over another report of the same condition were not included.

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