Evaluation of mechanical resistance to tearing of the anterior lens capsule after staining with different concentrations of trypan blue

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TITLE PAGE

Evaluation of mechanical resistance to tearing of the anterior lens capsule following staining with different concentrations of trypan blue

Short running head: Capsule edge resistance following staining with trypan blue

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ABSTRACT

Purpose: To evaluate and compare the mechanical resistance to tearing of the anterior lens capsule opening, following staining with different concentrations of trypan blue in ex vivo specimens of porcine.

Setting: Department of Ophthalmology, Faculty of Medicine, Semmelweis University, Budapest, Hungary. Department of Polymer Engineering and Biomechanical Research Centre, Faculty of Mechanical Engineering, Budapest University of Technology and Economics, Budapest, Hungary.

Design: Experimental study.

Methods: In the Control Group (n=25 eyes), the capsule was unstained. In the Stained 1 Group (n=25 eyes) 0.06%, in the Stained 2 Group (n=25 eyes) 0.1% trypan blue was used to stain the capsule. Following the capsulorhexis, the capsule openings were stretched with custom-designed testing equipment until they ruptured. The rupture force (RF), the circumference stretching ratio (CSR), and secant modulus at 10 mN (SM_{10mN}) and 50 mN (SM_{50mN}) were evaluated.

Results: There were no statistically significant differences in RF ($p = .8924$) or CSR ($p = .3876$) among the groups. There were no statistically significant differences in SM_{10mN} ($p = .8215$) or SM_{50mN} ($p = .4184$) among the groups.

Conclusion: In this porcine eye model, we found that, of these trypan blue concentrations that are routinely used in cataract surgery, neither had an effect on capsular rim stability.

SYNOPSIS

Capsular openings created following trypan blue staining are not weaker than those created without application of this dye.

INTRODUCTION

The creation of anterior capsulorhexis is a crucial step of extracapsular cataract surgery. During this process, visualization of the anterior capsule is based on an adequate red fundus reflex, and is therefore more challenging when the red reflex is poor (e.g., due to a mature cataract). Furthermore, especially in such cases, a strong capsular rim is needed to support the possible stress load during the surgical procedure. If the anterior capsule is torn, the position of the intraocular lens may be compromised because of the disrupted integrity of the capsular bag. Moreover, extension of the tear towards the posterior capsule may lead to serious complications (e.g., vitreous loss or dropped nucleus).

Trypan blue is a dye that is commonly used to facilitate visualization of the anterior lens capsule during creation of the anterior capsulorhexis.[1] Several studies have reported that trypan blue affects the mechanical properties of the anterior lens capsule.[2-5] However, only one study evaluated the stained capsular opening; the authors of that study found no difference in tear resistance based on the use of trypan blue.[6] Therefore, to prevent surgical complications, further investigations are warranted to evaluate the biomechanical behaviour of the anterior capsulorhexis edge when stained with trypan blue.

A test method was devised by our study group, based on our prior experiments,[7-9] in order to determine whether the most frequently used concentrations of trypan blue (0.06% and 0.1%) alter the resistance of the capsular opening.

MATERIALS AND METHODS

Fresh porcine eyes were transported from a local abattoir immediately after slaughter, and were randomly divided into three groups. Under an operating microscope, the cornea and iris were removed. In the Control Group (C Group), the anterior lens capsule was unstained. Conversely, in the Stained 1 Group (S1 Group), 0.06% trypan blue was used to stain the capsule for 1 minute; 0.1% trypan blue was used to stain the capsule in a similar manner in the Stained 2 Group (S2 Group). Subsequently, the dye was removed by irrigation with balanced salt solution (BSS). In all groups, continuous curvilinear capsulorhexis (CCC) was performed with a cystotome and forceps. A custom-designed, three-dimensionally printed marker (5.5 mm in diameter) was used to ensure uniform, well-centred, appropriately sized, circular capsular openings. Subsequently, the anterior capsule was cut around the equator, using a pair of micro-scissors to obtain a ring-shaped capsule specimen. All specimens were checked for uniformity via light microscopy (BX 51M, Olympus Co., Tokyo, Japan). Specimens with unacceptable shape, size, or an irregularity at the rim of the opening were excluded. In total, 75 eyes (25 eyes in each group) met the study inclusion criteria.

The mechanical test was performed using custom-designed testing equipment[10] with slight modifications. Briefly, the specimen support comprised four polished metal pins, each with a radius of 0.4 mm. Two pair of holders were placed, perpendicular to each other. The ring-shaped specimen was carefully slipped over the four pins, which were lubricated with methylcellulose to reduce friction. The specimen support was submerged in BSS at room temperature to ensure an appropriate hydration level during the test. The four pins were separated from each other by four stepping motors, which operated at a speed of 10 mm/min until the capsule ring was torn. The force required to stretch and displace the pins was recorded continuously using a computer. The mechanical test arrangement is shown in **Figure 1**. The unstretched circumference (C_{us}) and stretched circumference (C_s) of the opening were calculated based on the relative position of the pins, where the strain reached a small preload of 1.5 mN and the ring was torn. The circumference stretching ratio (CSR) between the stretched and unstretched circumferences was expressed as a percentage based on the following formula:

$$CSR = (C_s/C_{un}) \times 100\%$$

The rupture force (RF), CSR, shape of elasticity curves, and secant modulus at 10 mN (SM_{10mN}) and 50 mN ($SM50_{mN}$) were evaluated.

Data were analyzed using Statistica 8.0 (Statsoft Inc., Tulsa, OK USA). For group comparisons, the one-way analysis of variance (ANOVA) was used. Data were shown as means ± standard deviation. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The RF in each group was as follows: C Group: 108 ± 20 mN, S1 Group: 105 ± 32 mN, and S2 Group: 104 ± 23 mN (**Figure 2A**). The CSR in each group was as follows: C Group: 148 ± 7%, S1 Group: 148 ± 6%, and S2 Group: 150 ± 5% (**Figure 2B**). There were no statistically significant differences in RF (p = .8924) or CSR (p = .3876) among the groups.

Figures 3A, **B,** and **C** show the elasticity curves, which represent the force required for stretching as a function of the displacement of the pins for each group. As illustrated by the figures, the force-displacement relation was similar in all groups. The SM_{10mN} was 13 ± 2 mN/mm for C Group, 13 ± 2 mN/mm for S1 Group, and 14 ± 2 mN/mm for S2 Group. The SM_{50mN} was 38 ± 6 mN/mm for C Group, 38 ± 5 mN/mm for S1 Group, and 39 ± 3 mN/mm for S2 Group. There were no statistically significant differences among the groups (p = .8215 and p = .4184). The shape of the curves was similar in all groups: the increase in force was rapid, and a steep decline occurred at the point indicating tearing of the specimen.

DISCUSSION

The aim of this study was to evaluate and compare the mechanical resistance to tearing of the anterior capsule opening, following staining with different concentrations of trypan blue in ex vivo specimens of porcine lens capsule. In this porcine eye model, we found that, of these trypan blue concentrations that are routinely used in cataract surgery, neither had an effect on capsular rim stability.

An earlier study subjectively noted that the anterior capsule was more fragile and friable in the trypan blue eyes than in the control eyes.[11] Wollensak et al. analysed porcine anterior lens capsules after trypan blue staining for various time intervals, with or without exposure to light. They found that trypan blue staining combined with at least 1 minute of light irradiation led to increased elastic stiffness at 25% strain, as well as reduction in ultimate extensibility. The authors of that study speculated that the effect might have been a result of the photosensitizing action of trypan blue, which led to light-induced crosslinking of capsule collagen.[2] Additionally, Dick et al. reported that trypan blue staining affected the biomechanical properties of the human lens capsule, leading to significant reduction in elasticity and increase in stiffness. They also suggested that these changes may be due to the induction of collagen crosslinking by a photosensitizing action of trypan blue.[3]

Furthermore, Jardleza et al. confirmed that the use of trypan blue led to significantly stiffer anterior lens capsules in human eyes, and that this effect was most pronounced in diabetic anterior lens capsules. The authors of that study concluded that the photooxidizing effects of trypan blue are likely to compound existing glucose mediated collagen crosslinking in the diabetic anterior lens capsule.[4] Haritoglou et al.

evaluated human anterior lens capsules and found an increase in tissue stiffness after trypan blue staining. However, illumination using a standard surgical light source had no significant effect on the analysed mechanical properties.[5] Finally, Simsek et al. assessed human specimens following intracameral trypan blue application; they reported no effect on capsule elasticity and stiffness.[12]

Despite differences in the applied methods (e.g., human vs. porcine samples, uniaxial vs. biaxial test), our results are in accordance with the findings reported by Jaber et al.[6] To the best of our knowledge, their work constitutes the sole existing study regarding the stability of the stained capsular opening. Notably, they found no difference in CCC strength between trypan blue–stained capsules and control capsules; moreover, staining with trypan blue did not reduce CCC tear resistance.

We aimed to simulate real circumstances that occur during dye-enhanced cataract surgery. Therefore, we used the most frequently applied concentrations of the dye. Furthermore, we performed a biaxial mechanical test, because the capsular rim could bear a similar load during surgery. However, we worked with light exposure; we concluded that trypan blue dye does not influence mechanical resistance to tearing of the anterior lens capsule. A possible explanation for this is that staining-induced alteration of the ultrastructure[13] may modify the mechanical properties of the capsule surface; however, this may have no mechanical effect on the whole rim. Our explanation is in accordance with the findings of Haritoglou et al.[5] This theory is also augmented by the elasticity curves in the present study (**Figure 3A, 3B,** and **3C**). The force versus displacement diagrams were similar for all groups, showing a rapid rise in force with abrupt termination of the curve. This characteristic may be clinically useful, as it may assist surgeons in determining the stretching limit of the opening during surgical manoeuvres.

The mechanical behaviour of the porcine capsule is similar to that of the human capsule during infancy;[14] however, it changes during adulthood.[15] Thus, the current results may not be directly applicable to the clinical treatment of adult patients. Nonetheless, our results suggest that, of the trypan blue concentrations routinely used in cataract surgery, neither has an effect on capsular rim stability.

WHAT WAS KNOWN

Several studies have reported that trypan blue affects the mechanical properties of the anterior lens capsule.

WHAT THIS PAPER ADD

In this porcine eye model, we found that, of these trypan blue concentrations that are routinely used in cataract surgery, neither had an effect on capsular rim stability.

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No author has any financial or proprietary interest in any material or method mentioned.

FIGURE LEGENDS

Figure 1. The mechanical test arrangement. (SM=stepping motor, H=holder, SG=strain gauge)

Figure 2. Rupture force (A) and circumference stretching ratio (B) in the study groups.

Figure 3. Elasticity curves in the Control Group (A), Stained 1 Group (B) and Stained 2 Group (C).

Figure 1

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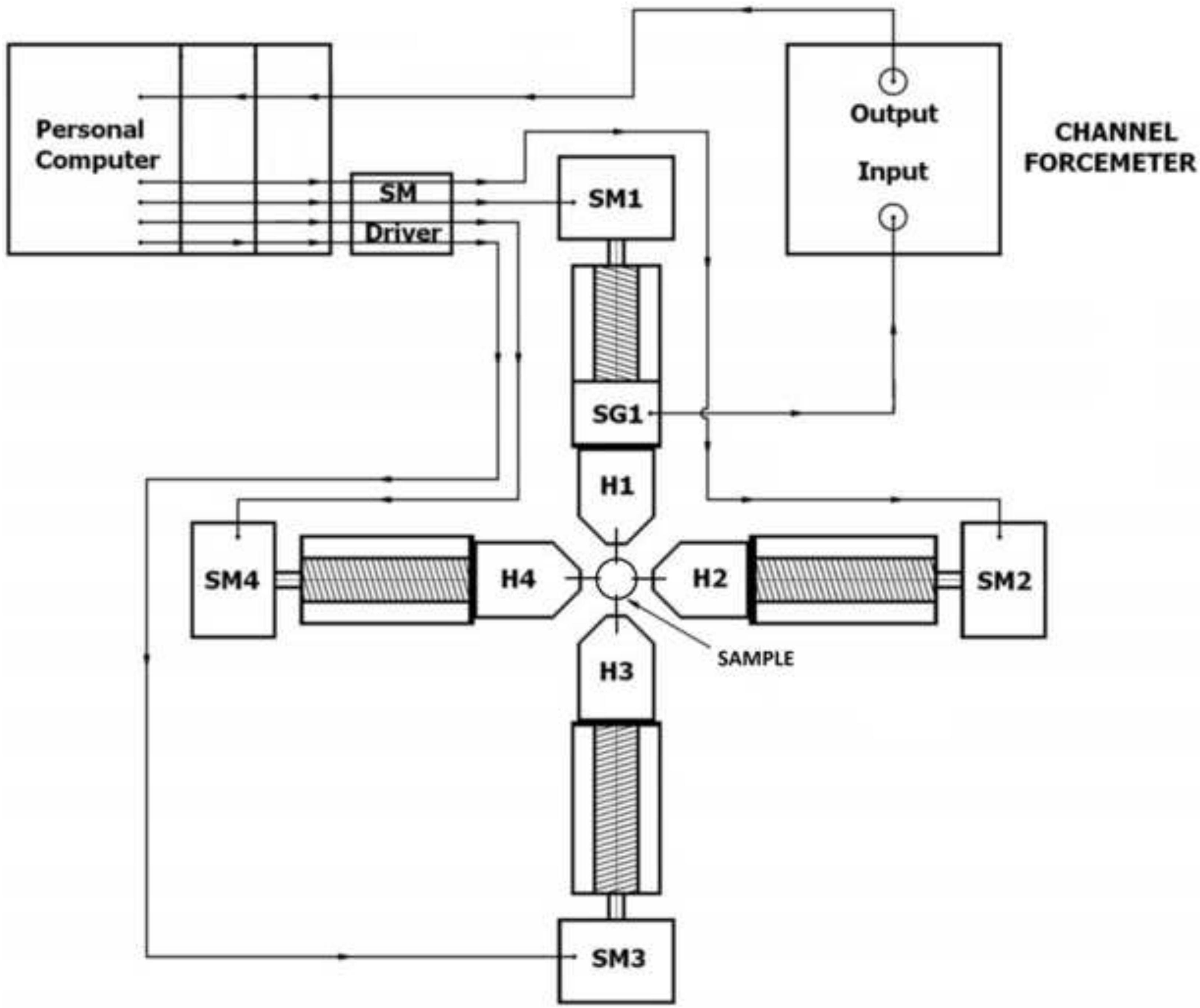

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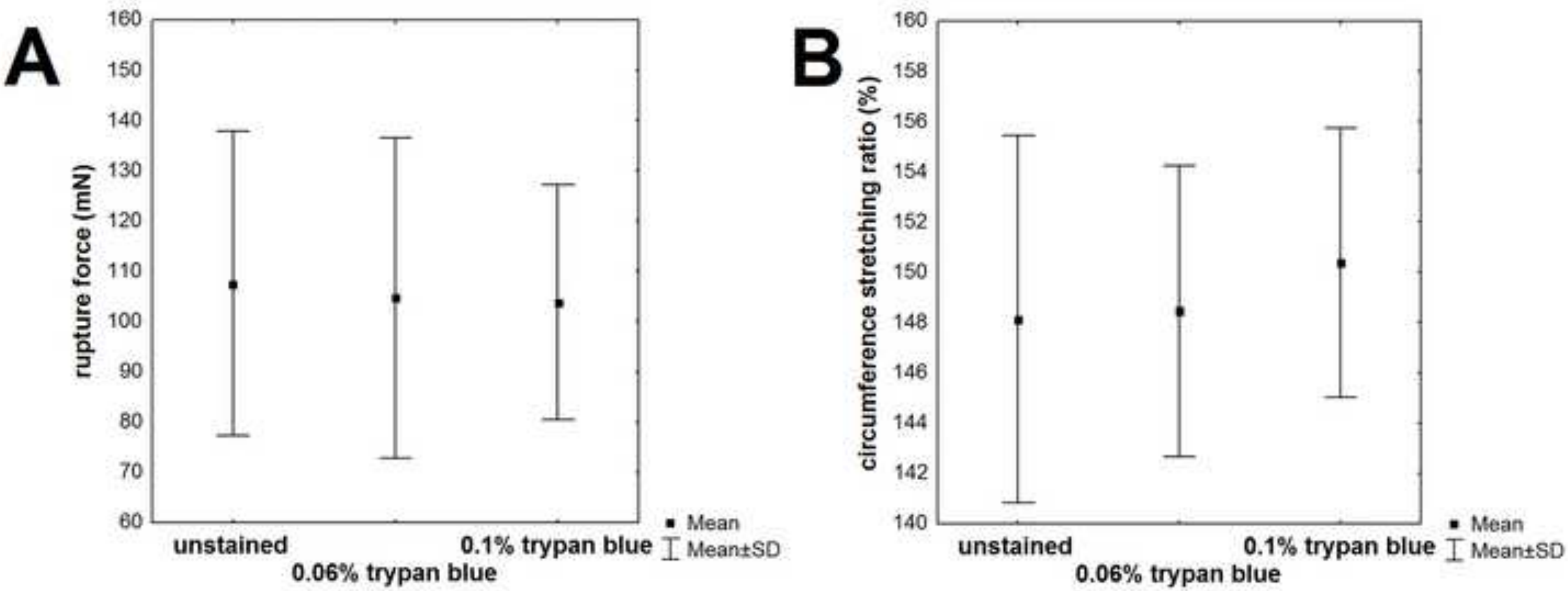

Figure 3
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