Increase in lens capsule stiffness caused by vital dyes

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PURPOSE: To assess potential changes in lens capsule mechanical properties after staining with brilliant blue, indocyanine green (ICG), and trypan blue.

SETTING: Department of Ophthalmology and Applied Physics and Center for NanoScience, Ludwig-Maximilians-University, Munich, Germany.

DESIGN: Experimental study.

METHODS: Fifteen unstained lens capsules were dissected into 7 wedge-shaped parts. Three fragments were stained with brilliant blue 0.025%, ICG 0.05%, and trypan blue 0.06%, respectively, for 1 minute. Another 3 specimens were additionally illuminated using a standard light source. The seventh part served as an untreated control. All specimens were analyzed using atomic force microscopy (AFM) in contact mode with a scan rate of 0.6 Hz. Two scan regions of 10 μm × 10 μm were chosen, and stiffness was determined using AFM in a force spectroscopy mode. The force curves were performed with a data rate of 5000 Hz.

RESULTS: Staining of the samples resulted in an increase in tissue stiffness (brilliant blue: $P<.001$; ICG: $P<.01$; trypan blue: $P<.05$). Additional illumination after staining further increased tissue stiffness, but not significantly. Mean increase in the relative elasticity values were 1.61 ± 0.15 (SD) for brilliant blue, 2.04 ± 0.21 for brilliant blue with illumination, 1.63 ± 0.22 for ICG, 2.01 ± 0.22 for ICG with illumination, 1.23 ± 0.11 for trypan blue, and 1.39 ± 0.11 for trypan blue with illumination. In relation to unstained tissue, the relative elasticity of the stained tissue increased 1.2-fold after illumination.

CONCLUSION: Staining significantly increased the mechanical properties of the human lens capsule.

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Cataract surgery was among the first ophthalmic surgical procedures in which dyes were introduced to assist the surgeon in more challenging cases, such as eyes with mature cataract. In mature white cataracts, a controlled capsulorhexis of the anterior capsule is often difficult to perform due to absence of the red fundus reflex. Therefore, vital dyes were introduced to better visualize the lens capsule in these cases; the dyes include fluorescein, indocyanine green (ICG), and trypan blue.[1–3] Trypan blue is the most frequently used blue dye at present, and the concentrations used to stain the lens capsule vary between 0.0125% and 0.1%.[1,4,5] Brilliant blue is a relatively new dye that is mainly used to assist removal of the internal limiting membrane of the retina during vitreomacular procedures[6]; however, it also has potential as a capsule stain.[7]

Dye-enhanced cataract surgery has become the preferred method to allow the surgeon to perform a safe capsulorhexis in complex cases. However, the interaction of the dye and the tissue is relatively unknown. In macular surgery, it is a common intraoperative observation that the stained internal limiting membrane can be removed easier and in large fragments, indicating increased stiffness and elasticity of the tissue. The present study used atomic force microscopy (AFM) to assess potential changes in lens capsule stiffness after staining using brilliant blue, ICG, and trypan blue in commercially available concentrations (brilliant blue and trypan blue) or in concentrations described in the literature (ICG). The advantage of AFM over other microscopy techniques is that biological samples can stay in physiological conditions. To

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mimic the intraoperative situation, in which the surface of the lens is illuminated by the surgical microscope and the known photosensitizing effects of 1 dye is evaluated (ICG), we measured the increase in stiffness after staining alone and after subsequent illumination.

MATERIALS AND METHODS

The AFM measurements were performed using human lens capsules obtained during conventional cataract surgery. The study was performed at the Department of Ophthalmology Department of Applied Physics and Center for NanoScience and was approved by the local institutional review board.

Tissue Preparation

Human lens capsules were obtained during routine cataract surgery after a curvilinear capsulorhexis was created using a bent needle. Lens capsules were immediately placed in a balanced salt solution and handed over to a technician.

Immediately after removal from the eye, the lens capsule specimen was placed on a glass slide with the convex surface pointing down to allow a reliable orientation and tissue measurement. Then, the tissue was divided into 7 wedge-shaped parts. Each piece was placed inside a balanced salt solution droplet on a microscope slide (Superfrost Plus, Gerhard Menzel GmbH) that had been coated with poly-L-lysine. The balanced salt solution was removed slowly while the tissue was carefully smoothed on the glass using a glass needle and avoiding dehydration of the specimen during preparation. A circle was drawn around each fragment with a PAP pen (Sigma-Aldrich Chemie GmbH). Then, a drop of brilliant blue 0.025% (Brilliant Peel, Fluoron GmbH), ICG 0.05% (Pulsion, Pulsion Medical Systems AG), and trypan blue 0.06% (Vision Blue, DORC International BV) was placed on 2 fragments each. One of these specimens was then illuminated with a standard light source used for vitreoretinal surgery (Penta Lux × 50, Fritz Ruck Ophthalmologische Systeme GmbH) for 1 additional minute.The remaining unstained fragment served as a control.

Fifteen lens capsules were evaluated, meaning that for each dye, AFM measurements could be performed on 15 different specimens.

Atomic Force Microscopy Measurements

All AFM imaging and force indentation experiments were performed using a Nanowizard II atomic force microscope (JPK Instruments AG) mounted on an inverted microscope. For imaging and stiffness measurements with the atomic force microscope, standard silicon–nitride triangular cantilevers (MLCT, Veeco Instruments, Inc.) with integrated sharp silicon–nitride pyramidal tips (nominal spring constant k = 0.1 N/m, apex angle $\alpha \sim 35$ degrees) were used. Before every measurement, the spring constant value was determined with the thermal fluctuation method. The samples were imaged in contact mode with a scan rate of 0.6 Hz while kept in phosphate-buffered saline at room temperature. Two scan regions of 10 µm × 10 µm were chosen on the sample. Stiffness was determined using AFM in force spectroscopy mode. Briefly, indentations were made over an 8 point × 8 point grid at a rate of 1 load–unload cycle per 2.4 seconds with a maximum load of 5 nN. The force curves were performed with an approaching speed of 3 µm/s, a retraction speed of 7 µm/s, and a data rate of 5000 Hz. Assessment of the dynamic indentation modulus E of each force-indentation curve was performed offline using JPK data processing software (JPK Instruments AG). The AFM raw data obtained from the scan regions were corrected for the additional height by the deflection of the cantilever. These corrected distance data were plotted against the force for each indentation.[8] After the Sneddon model[9] was fit to the data, the resulting elasticity moduli were averaged for each scan region.

RESULTS

Staining of the samples resulted in a statistically significant increase in tissue rigidity compared with controls (Figure 1). Compared with unstained controls of human lens capsules, the stiffness of the tissue significantly increased with brilliant blue ($P < .001$), ICG ($P < .01$), and trypan blue ($P < .05$) staining. There was a trend toward a further increase in stiffness after illumination for each dye; however, this effect did not reach statistically significant levels for brilliant blue with illumination and ICG with illumination (Table 1). Although trypan blue provided a significant increase in stiffness compared with the control, the effect after illumination was significantly less

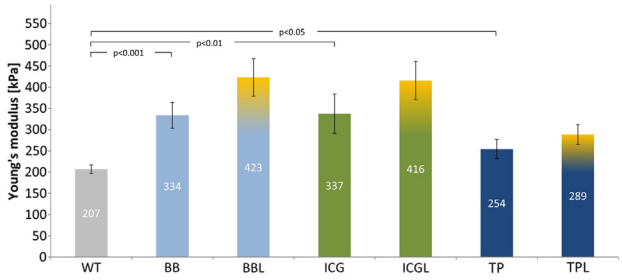

Figure 1. Mean values of all map lens capsule results are plotted, with error bars showing the standard error of the mean. Indicated *P* values have a significance level of 5% and are calculated from a *t* test (BB = brilliant blue; BBL = brilliant blue and illumination; ICG = indocyanine green; ICGL = indocyanine green and illumination; TP = trypan blue; TPL = trypan blue and illumination; WT = control).

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Table 1. Significance levels comparing dyes and controls with regard to the increase in stiffness measured at a defined surface of the lens capsule.

	P Value					
Parameter	WT	BB	BBL	ICG	ICGL	TP
BB	<.001	—	—	—	—	—
BBL	<.001	NS	—	—	—	—
ICG	<.01	NS	NS	—	—	—
ICGL	<.001	NS	NS	NS	—	—
TP	<.05	NS	<.01	NS	<.01	—
TPL	<.01	NS	<.01	NS	<.05	NS

BB = brilliant blue; BBL = brilliant blue and illumination; ICG = indocyanine green; ICGL = indocyanine green and illumination; TP = trypan blue; TPL = trypan blue and illumination; WT = control

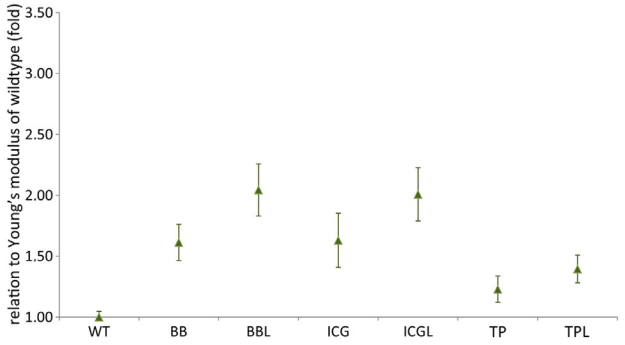

Figure 2. Young moduli of human lens capsule (*green triangles*) normalized to corresponding control (WT) Young modulus (BB = brilliant blue; BBL = brilliant blue and illumination; ICG = indocyanine green; ICGL = indocyanine green and illumination; TP = trypan blue; TPL = trypan blue and illumination).

pronounced than for brilliant blue with illumination and ICG with illumination (Table 1).

An assessment of the relative changes in elasticity using Young moduli in relation to the corresponding control sample found that the relative elasticity of the lens capsule increased compared with that of an unstained control. Normalized to the Young modulus of the control (1.00 ± 0.05 [SD]), the mean increase in the relative elasticity values were 1.61 ± 0.15 for brilliant blue, 2.04 ± 0.21 for brilliant blue with illumination, 1.63 ± 0.22 for ICG, 2.01 ± 0.22 for ICG with illumination, 1.23 ± 0.11 for trypan blue, and 1.39 ± 0.11 for trypan blue with illumination (Figure 2). This indicates that for trypan blue, the increase in stiffness was less pronounced than the increase for brilliant blue and ICG. The gradient comparing illuminated versus nonilluminated tissue was approximately 1.2 for all 3 dyes tested (Figure 2).

DISCUSSION

Atomic force microscopy is a well-established examination technique that has been used to measure the thickness and the rigidity of human ocular basement membranes, such as the unstained internal limiting membrane.[10,11] The aim of the present study was to assess and quantify the changes in the rigidity of another human basement membrane, such as the lens capsule, after the application of vital dyes currently used for ophthalmic surgical procedures.

We found that there was an increase in tissue stiffness after staining using brilliant blue, ICG and—although to a lesser degree—trypan blue. Illumination using a standard surgical light source did not increase the mechanical properties of the lens capsule in a statistically significant manner. However, the observed increase in stiffness was quite consistent for all 3 dyes tested, with an increase of approximately 1.2-fold.

It remains rather unclear what mechanisms contribute to the observed increase in the mechanical properties of the lens capsule in our setting. Indocyanine green is a known photosensitizing agent that has been reported to increase the stiffness of the ICG-stained internal limiting membrane (another human basement membrane) in an animal model in a hypothesized light-induced crosslinking process as the result of a type I reaction of photooxidation.[12,13] We observed very similar increases in stiffness for brilliant blue, a triarylmethane dye without known photosensitizing properties, as those described for ICG. This observation underlines that there are other tissue–dye interactions that we have to take into account and that are still unknown.

However, the increased mechanical stability of the human lens capsule may be an interesting aspect for ophthalmic surgeons because one may hypothesize that the rim of a capsulorhexis after a curvilinear capsulorhexis created on a stained anterior capsule may be more stable than on an unstained capsule. Therefore, in addition to improved contrast, the increased mechanical properties of the stained anterior capsule may contribute to successful cataract surgery in complex cases, such as in eyes with white cataract with liquefied cortex.

A recent study by Jaber et al.[14] did not find a significant difference in the tear resistance of the capsulorhexis after staining with trypan blue 0.06% solution using a model for biomechanical measurements of the entire capsular bag. In general, the methodology of our AFM study differs from this setting in several aspects. It is important to consider that AFM provides measurements of the surface of the tissue with a penetration of less than 1 µm. It is known that trypan blue does not penetrate the tissue but rather stains the surface. Therefore, a stiffening effect measured by AFM might be more pronounced at the surface of the stained tissue and may not necessarily correlate to tear resistance of the whole capsular bag. In addition, in the

present study trypan blue 0.06% provided the weakest stiffening effect as measured by AFM, and one may therefore hypothesize that this effect might not be detectable in the experimental setting used by Jaber et al.[14] Nevertheless, it may be of interest to determine whether a significant effect on tear resistance would be seen using dyes other than those tested in the present study or using different dye concentrations.

The increase in lens capsule stiffness differed between the dyes evaluated in our study; thus, it might be interesting to use dyes that provide a more pronounced stiffening effect in situations in which increased tissue stiffness may be helpful. Nevertheless, one may hypothesize that increased stiffness of stained tissue might be disadvantageous in other surgical interventions, such as posterior lamellar keratoplasty, where trypan blue is used during the preparation of the donor tissue. However, our study focused on commercially available substances (brilliant blue and trypan blue) in given concentrations as provided by the manufacturer. Future investigations may further evaluate the effect on the mechanical properties of the lens capsule and other tissues using different concentrations of the dyes tested here and using alternative experimental models as described previously.[13]

WHAT WAS KNOWN

- In cataract surgery, vital dyes are used in situations in which lens opacification interferes with the red fundus reflex.
- The contrast enables the surgeon to perform a controlled capsulorhexis.

WHAT THIS PAPER ADDS

- To date, there has been little information on tissue–dye interactions beyond the staining effect of the dye used for surgery.
- In addition to staining of the lens capsule, there was significant stiffening of the tissue as a result of dye application. This may be a beneficial or disadvantageous effect depending on the situation.

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