Ingress of India Ink Into the Anterior Chamber Through Sutureless Clear Corneal Cataract Wounds

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Background: Sutureless clear corneal cataract incisions may be associated with an increased risk of endophthalmitis.

Objective: To assess the degree of ocular surface fluid ingress into the anterior chamber of cadaveric human globes with clear corneal wounds.

Methods: Self-sealing clear corneal incisions were created in 4 eyes, and intraocular pressure was controlled with an infusion cannula. To evaluate possible flow of surface fluid through the corneal wound, india ink was applied to the corneal surface while the intraocular pressure was varied, so as to simulate the intraocular pressure fluctuations secondary to blinking or eye squeezing. The optical density from aqueous samples of globes were measured both before and after india ink application using a spectrophotometer.

Results: Aqueous aspirates from the 3 globes with sutureless clear corneal wounds revealed a significant increase in spectrophotometric readings ($P < .01$), in contrast to the sutured wound, which did not show an increase in absorbance level relative to the baseline. Ink particles were both grossly and microscopically visible inside the sutureless corneal wounds.

Conclusions: Fluctuations of intraocular pressure following sutureless clear corneal cataract surgery may allow entry of surface fluid into the anterior chamber during the initial postoperative period when the wound is not healed.


Since the introduction of sutureless clear corneal cataract incisions in 1992, the procedure has gained increasing popularity worldwide because it offers several advantages over the traditional sutured scleral tunnels, such as faster visual recovery and minimizing induction of astigmatism. In recent reports, clear corneal incisions were preferred by 56% of cataract surgeons in the United States and 64% of cataract surgeons in New Zealand. However, numerous studies within the past several years have suggested that there is an increased risk of endophthalmitis with the use of sutureless clear corneal incisions. In a study by John and Noblit, patient records from 1992 and 1996 revealed a rate of 0.29% and 0.02%, respectively, while Colleaux and Hamilton reported a 0.13% and 0.05% incidence of endophthalmitis following cataract extraction with sutureless clear corneal and scleral tunnel incisions, respectively. More recently, Nagaki et al also reported a statistically increased risk with clear corneal incisions (0.29%) compared with sclerocorneal incisions (0.05%). These studies indicate a several-fold increase in endophthalmitis risk associated with clear corneal incisions compared with scleral and sclerocorneal wounds.

Although rare, acute endophthalmitis is still a devastating complication with high potential for ocular morbidity including permanent severe vision loss. With more than 2.5 million cases of cataract surgery performed each year in the United States alone, an increase in the incidence of this complication can result in large absolute numbers of surgical failures.

Integrity of the surgical wound is an important factor in the prevention of postoperative infection. Maxwell et al reported that 80% of postsurgical cases of endophthalmitis were associated with wound defects including wound gape and malapposition. However, problems with clear corneal wounds may not be readily apparent intraoperatively. Wound integrity may vary as a function of intraocular pressure (IOP). Intraocular pressure is known to vary in the postoperative period following clear corneal phacoemulsification with 21% of eyes having an IOP of 5 mm Hg or less. Variations of IOP in both human and animal subjects following squeezing of the lids...
or even normal unconscious blinking further supports the notion that large fluctuations in IOP can occur during the postoperative period.\textsuperscript{10-12}

Optical coherence tomographs of clear corneal incisions created in cadaveric human and rabbit eyes demonstrate that transient fluctuations in IOP of a magnitude not uncommon in the postoperative period resulted in gaping of the wound margins.\textsuperscript{13} This effect was more noticeable at lower IOP and less pronounced when the eye was well pressurized for standard self-sealing incisions. Furthermore, histologic examination of the incisions showed that India ink particles could migrate from the ocular surface into the stroma even though there were no particles seen adhering to the Descemet membrane to prove a full-thickness penetration.\textsuperscript{13}

The present study was designed to further investigate the potential of extraocular microorganisms gaining access to the anterior chamber and hence creating a risk for endophthalmitis following clear corneal incisions. Using India ink and measuring optical density of the aqueous humor, we attempted to qualitatively and quantitatively assess the possibility and degree of surface fluid ingress into the anterior chamber. India ink was chosen because of its broadly similar size to bacteria and simple spectrophotometric analysis.\textsuperscript{14-16}

**METHODS**

**TISSUE PREPARATION AND SURGICAL PROCEDURES**

Five human cadaveric eyes ranging from 1 to 4 days post mortem were obtained from the Central Florida Lions Eye & Tissue Bank (Tampa). All globes were kept at 4°C in a moist chamber prior to use. Globes were placed in a globe holder and oriented so that the temporal cornea was placed at the 6-o’clock position under the operating microscope. A 23-gauge butterfly needle was inserted through the limbus at approximately the 12-o’clock position, 180° away from the incision site (arrow), connected by intravenous tubing to a 250-mL bottle of normal saline (Figure 1). Intracocular pressure was varied by adjusting the height of the bottle.

Standard self-sealing, 2-planed clear corneal cataract incisions were created under microscopic visualization using a 2.5-mm disposable keratome (Alcon, Forth Worth, Tex) with extension of the wound to 3.0 mm. Incisions were made approximately 1 to 2 mm anterior to the limbus and tunnel lengths varied from 2.0 to 2.5 mm. In 1 globe, the corneal wound was closed using a single 10-0 nylon, 13.24-cm monofilament suture (Alcon). The corneal surfaces peripheral to the incisions were depressed with a cellulose acetate sponge to test for leakage. All surgical incisions were performed by an experienced ophthalmic surgeon.

**SPECTROPHOTOMETRY**

To examine for evidence of fluid ingress into the corneal wound, black India ink (Sanford Corp, Bellwood, Ill) was applied to the corneal surface over the region of the incision. The ink solution was a commercially available waterproof drawing ink with a specific gravity of 1.03 to 1.04. We used a UV-visible recording spectrophotometer (UV160U; Shimadzu Corp, Kyoto, Japan) to quantitatively assess the India ink concentration in a given sample by measuring its absorbance level.

To analyze the level of India ink in a sample, a standard curve was created using the spectrophotometer. Serial dilutions with normal saline were performed starting from the original India ink solution (1:1 or 100%) down to 1:100000 dilution.

The India ink was applied over the incision using a super-saturated cellulose sponge placed above the wound site. Using a dropper, the sponge was kept moist continuously with the India ink. The IOP was then varied by raising and lowering the infusion bottle. The infusion bottle height was modified abruptly (so as to simulate blinking) once every 15 seconds for a period of 2 minutes, followed by once every minute for another 10 to 13 minutes. The pressure was varied over a period of a few seconds. The IOP was varied from approximately 0 to 75 mm Hg. The corneal surface was then gently irrigated with normal saline to wash away the excess India ink present on the corneal surface.

One globe without any incision was used as a negative control. Clear corneal wounds were created in 4 globes. Three remained sutureless, while 1 was closed with a suture. Following cannulation, anterior chamber aspirations of 0.2 mL were obtained from all eyes using a 27-gauge needle. Samples were obtained prior to creation of the corneal incision, after creation of the incision but prior to India ink application, and following India ink application and IOP variation. Absorbance levels of the samples were then measured after addition of 0.8 mL of normal saline to the cuvette.

**LIGHT MICROSCOPY**

To find morphologic evidence of ingress of surface fluid into the sutureless corneal wound, histologic testing of the corneal tissue was performed to determine if India ink particles were present along the incision edges. After all procedures described earlier, the cornea was excised from the limbus and fixed in 10% buffered formaldehyde for 72 hours. Histologic corneal sections were then prepared, with sections oriented radially from 3 to 9 o’clock so as to demonstrate the course of the clear corneal incisions from the corneal surface to the Descemet membrane.

**STATISTICAL ANALYSIS**

Unpaired t tests were performed to compare differences between the measured absorbance levels and dilution factors.
RESULTS

SPECTROPHOTOMETRY

The spectrophotometric absorbance levels of different india ink concentrations are represented in both tabular (Table 1) and graphical (Figure 2) forms. We were able to accurately measure india ink concentrations down to 1:100,000 dilution. As an example, the 1:1000 sample corresponded to 1 µL of the india ink in 999 µL of normal saline.

CONTROL AQUEOUS SAMPLES

Table 2 represents the spectrophotometric analysis of anterior chamber taps. The absorbance levels of the aqueous samples prior to india ink application was very close to those of normal saline. The absorbance levels of the aqueous samples after creation of the corneal incision but before applying ink were also the same as prior to creation of the incision. Thus, simply making clear corneal incisions with the keratome did not alter absorbance levels. In addition, application of india ink to the surface of unincised control corneas did not result in changed absorbance levels of the aqueous samples, indicating that the india ink particles were unable to traverse intact stroma.

SUTURELESS CLEAR CORNEAL INCISIONS

Aqueous samples from the 3 gloves with sutureless corneal incisions revealed a significant increase in spectrophotometric readings (Table 2) relative to control (P<.01). Thus, absorbance level measurements were consistent with a small volume of india ink traversing the corneal incision and being diluted in aqueous samples.

SUTURED CORNEAL INCISION

The aqueous aspirate from a corneal incision that was sutured prior to application of india ink did not show an increase in absorbance level above the baseline (Table 2).

INDIA INK PENETRATION

When india ink was applied to the corneal surface, the dye quickly became visible through the operating microscope within the sutureless clear corneal incisions. However, following the application of the dye and rinse, black particles were visible even with the naked eye. Histologic examination of the wound confirmed full penetration of india ink particles along the edges of the corneal incision (Figure 3). Ink particles were present within the incision in a confluent distribution involving the deeper one third of the wound length.

COMMENT

The findings of the present study suggest that there is a potential for microorganisms such as bacteria to gain access to the intraocular compartment through apparenently self-sealing clear corneal wounds as demonstrated by the entry of india ink into the anterior chamber. In the sutureless clear corneal wounds, a spectrophotometer detected a significant level of absorbance in aqueous samples following india ink application and IOP variations within a physiologic range (<5-75 mm Hg) performed to mimic fluctuations of IOP that have been demonstrated to occur after cataract surgery or during normal activities (eye blinking, eye rubbing, eye squeezing). When the clear corneal incision was sutured, however, the absorbance level of the aqueous samples did not increase relative to the baseline.

The light micrographs of the sutureless clear corneal incision show penetration of india ink along the entire length of the incision, which indicates the potential for self-sealing incisions to draw surface tear fluid into and along the incisions before the occurrence of any wound healing or closure of the surface epithelial defect. The technique used in this study may have possibly washed away some india ink particles that entered the corneal incision. Thus, we believe that the results underestimate the degree to which surface fluid penetrates into the sutureless clear corneal incisions when IOP is low (≤5 mm Hg).

The level of pressure applied to the globe by squeezing of the lids or normal unconscious blinking is substantial. The presence of this force has been recognized for decades. In the 1940s, Burton demonstrated that the upper eyelid or orbicularis oculi muscle exerts a squeeze force of up to 50 to 70 g on the globe. Coleman and Trokel measured IOP variations from blinking and squeezing in a human subject by inserting a 23-gauge needle directly into the anterior chamber. They showed that blinking resulted in pressure increases of up to 10 mm Hg, while squeezing of the lids produced prompt elevation to levels up to 110 mm Hg (or a 90 mm Hg increase), followed by 8 mm Hg undershoot after lid opening relative to baseline IOP. Using a different technique, Miller also measured lid tension on 10 human subjects and determined that the average pressure developed during a blink was 10.3 mm Hg, while a mean of 51 mm Hg was produced during a hard lid squeeze.

### Table 1. Spectrophotometric Measurements of India Ink

<table>
<thead>
<tr>
<th>India Ink Concentration or Dilution Factor</th>
<th>Log of Dilution Factor</th>
<th>Absorbance (λ = 800 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1 = 100%</td>
<td>0.000</td>
<td>2.287</td>
</tr>
<tr>
<td>1:50</td>
<td>1.699</td>
<td>2.280</td>
</tr>
<tr>
<td>1:100</td>
<td>2.000</td>
<td>2.288</td>
</tr>
<tr>
<td>1:500</td>
<td>2.699</td>
<td>2.207</td>
</tr>
<tr>
<td>1:1000</td>
<td>3.000</td>
<td>1.923</td>
</tr>
<tr>
<td>1:2000</td>
<td>3.301</td>
<td>0.846</td>
</tr>
<tr>
<td>1:4000</td>
<td>3.602</td>
<td>0.397</td>
</tr>
<tr>
<td>1:8000</td>
<td>3.903</td>
<td>0.185</td>
</tr>
<tr>
<td>1:10000</td>
<td>4.000</td>
<td>0.162</td>
</tr>
<tr>
<td>1:20000</td>
<td>4.301</td>
<td>0.067</td>
</tr>
<tr>
<td>1:40000</td>
<td>4.602</td>
<td>0.031</td>
</tr>
<tr>
<td>1:80000</td>
<td>4.903</td>
<td>0.008</td>
</tr>
<tr>
<td>1:100000</td>
<td>5.000</td>
<td>0.006</td>
</tr>
</tbody>
</table>

*The spectrophotometer was calibrated relative to normal saline. Normal saline was used for the serial dilutions from the original india ink solution.
In his article, Miller also cited an experiment performed by Comberg during the 1920s where the reported effect of lid pressure on a human globe caused the IOP to go from 18 mm Hg to 70 mm Hg during a hard lid squeeze. These pressure recordings confirm the clinically apparent fact that blinking or squeezing of the lids can exert tremendous forces on the globe that may be important following intraocular surgery.

We examined the ex vivo dynamic changes in unhealed clear corneal cataract incisions that might adversely affect the risk of intraocular infection. Optical coherence tomography demonstrated variation of corneal wound morphology in response to IOP fluctuations. High IOP was associated with close apposition of the wound edges in standard self-sealing incisions. At low IOP (≤5 mm Hg), however, wound edges were seen to gape, starting at the internal wound aspect. The variation in wound apposition and ability of surface fluid to traverse the wounds suggest a mechanism by which microorganisms from the ocular surface can gain access to the anterior chamber during the early postoperative period, at a time when little, if any, wound healing has taken place.

We propose that a physiological mechanism analogous to the lacrimal sac and tear drainage system may allow inflow of organisms present in the tear film. Maurice observed that a negative pressure is present within the lacrimal sac, rising to positive values during a blink. The negative pressure in the sac during the time between blinks is due to elastic expansion of the sac after compression during the blink. This elastic force causes

Table 2. Spectrophotometric Readings and Corresponding India Ink Concentrations From Anterior Chamber Aspirations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance (λ = 800 nm)*</th>
<th>Log of Dilution Factor</th>
<th>Corresponding Log of Dilution Factor†</th>
<th>Final Concentration or Dilution Factor†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no incision)</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Incision with suture</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sutureless incision 1</td>
<td>0.033</td>
<td>4.50</td>
<td>1:32 000 (1:6400)</td>
<td></td>
</tr>
<tr>
<td>Sutureless incision 2</td>
<td>0.026</td>
<td>4.64</td>
<td>1:43 450 (1:8690)</td>
<td></td>
</tr>
<tr>
<td>Sutureless incision 3</td>
<td>0.015</td>
<td>4.78</td>
<td>1:60 260 (1:12 050)</td>
<td></td>
</tr>
</tbody>
</table>

*For each globe, the absorbance level from the initial anterior chamber tap prior to India ink application was subtracted from that after India ink application to obtain this value.
†Multiplied by 5 since each anterior chamber sample was diluted 5 times prior to spectrophotometric measurement.

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![Figure 3. India ink penetration into clear corneal incision. A, Low-power micrograph demonstrates a heavy, confluent accumulation of India ink particles (arrowhead) toward the Descemet side of the corneal incision (hematoxylin-eosin, original magnification ×20). B, Higher-power micrograph demonstrates accumulation of India ink particles (arrowhead) along the margins of the wound extending the full length of the incision (hematoxylin-eosin, original magnification ×40).](https://archopht.jamanetwork.com/)
a partial vacuum or negative pressure to form within the canalici and sac, leading to the suction of fluid. Using high-speed close-up cinematography, the findings of Doane\textsuperscript{19} further supported these observations and those of Rosengren\textsuperscript{20} in the early 1970s.

McGowan\textsuperscript{21} noted that a suction mechanism might be responsible for the development of endophthalmitis following clear corneal cataract surgery, making an analogy to a squash ball with a puncture, that when squeezed and placed in a fluid medium, results in aspiration of fluid. Doane\textsuperscript{22,23} demonstrated the dramatic posterior movement of the globe associated with lid motion. He attributed this to the pressure of the lid on the globe; indeed, the tendency for the globe to come forward in conjunction with the relaxation of the lids supports this view. Doane described that during each blink cycle, the upper lid sweeps down over the eye with its intimate and forceful contact with the cornea acting much like a windscreen wiper blade. This pressure is often great enough to push the anterior surface of the globe in a posterior direction. The amount of posterior movement of the globe varies considerably, ranging between 0.7 to 1.6 mm, perhaps reflecting differences in eyelid tightness and orbital resistance to retropulsion.\textsuperscript{21,22,23} The elastic expansion of the globe during the opening phase of the blink potentially creates a scenario during which suction might draw tear fluid and microorganisms into the anterior chamber through a sutureless clear corneal wound.

The use of spectrophotometric measurement of India ink in the present study proved to be a valuable tool in assessing the degree of ocular surface fluid inflow. India ink has proved to be a valuable research tool as evident in numerous studies, including some in ophthalmology. It is often used in the dental industry as an indicator of root canal sealing ability and for the detection of root canal orifices.\textsuperscript{15,16,24-30} In ophthalmology, India ink has been used for the visualization of uveoscleral drainage routes,\textsuperscript{31} the investigation of neovascularization,\textsuperscript{22,23} the morphologic examination of conjunctival mucus,\textsuperscript{34} the examination of the structure of corneal stroma and its fibroblasts,\textsuperscript{35} and for the creation of an experimental glaucoma model.\textsuperscript{36} India ink is broadly similar in size to most bacteria.\textsuperscript{14,15} Although the biomass of bacteria varies more than 10 orders of magnitude, from 0.2 to 750 µm in diameter, most are less than 10 µm.\textsuperscript{37,38} Using a particle size apparatus, Ahlberg et al\textsuperscript{30} reported that the average particle size of India ink is 10 µm. In another report, Youngson et al\textsuperscript{27} determined that the India ink particle sizes ranged from less than 1 to 600 µm in diameter with a mean particle size of 236 µm, which is considerably greater than the average value of 10 µm noted by Ahlberg et al.\textsuperscript{30} However, by excluding larger particles, Youngson derived a mean diameter of 9.6 µm, agreeing closely with the findings of Ahlberg. In yet another study of corneal fibroblasts, Fujita et al\textsuperscript{37} determined that ink particles were 0.5 to 10 µm in diameter. Yoshikawa et al\textsuperscript{32} measured India ink particles using a microtrack particle size distribution indicator and reported a mean size of 3.8 to 5.9 µm. Therefore, it should be recognized that a range of particle sizes exists within an ink suspension. In a recent in vitro study investigating different tracers and assessment methods in the sealing ability of retrograde root fillings, it was noted that bacterial ingress and India ink penetration provided a similar rank order for the sealing ability of the material tested.\textsuperscript{15} Hence, we believe that the body of literature supports our use of measured increases in aqueous sample absorbance levels due to India ink penetration as a reasonable surrogate for bacterial penetration.

The concentration of India ink detected by spectrophotometry in our samples was quite small, indicating that the volume of surface fluid that traverses the clear corneal incisions is small. While some ingress of fluid may be common, the low volume may explain the relative rarity of clinical infection. According to the mechanism we propose, endophthalmitis may only develop when the quantity of bacteria traversing the wound overwhelms the natural clearing mechanism of the aqueous humor, or when a particularly virulent organism gains access.

In a previous study, we were able to demonstrate macroscopically the inflow of extraocular India ink through clear corneal incisions using a Miyake microscopy approach.\textsuperscript{29} Although this method was useful to qualitatively detect ingress of surface fluid in some of the eyes where the IOP was experimentally lowered at certain levels, not all eyes showed the same response. Additionally, external mechanical forces were required to demonstrate the penetration of India ink into the eye. The more sensitive method used in this study allows us to detect ink inflow at a level not observable with the Miyake approach. Using spectroscopy measurements, we were able to quantitatively show the intraocular penetration of minimal amounts of ink into the eye in all globes after inducing IOP changes, without need for manual globe compression. In addition, we were able to demonstrate in this study that suturing the wound prevents the passage of India ink through these incisions, similar to the behavior of an intact globe. We believe this model of intraocular pressure variation more closely mimics the clinical situation.

If confirmed, our findings have several implications in the postsurgical management of patients. First, the physician should carefully evaluate the wound for signs of leakage and gaping and should consider having a low threshold for placing a suture if there is any question about wound integrity. Second, the demonstrated possibility of imbibition of surface fluid into the aqueous humor within the setting of constantly fluctuating IOP underscores the need for prophylactic antibiotic therapy in the early postoperative period. A broad-spectrum antibiotic frequently applied to the ocular surface may be useful in reducing the risk of introducing pathogenic organisms into the eye by eliminating them from the tear film. Even if surface tear fluid did gain access to the interior of the eye, the fluid would contain concentrations of antibiotic sufficiently high to suppress bacterial replication in the aqueous humor.

The present study represents an effort to evaluate in vitro the stability of clear corneal wounds and the potential of ocular surface fluid ingress in the first several hours after surgery before wound healing. Further in vivo studies are warranted to confirm our concerns about these wounds because of possible limitations of ex vivo ex-
experiments such as lack of a functional endothelial pump and eyelid dynamics, and to address possible solutions.

Considering the relevance of the current hypothesis on the etiology of such a potentially devastating condition, we believe it is most appropriate to create different experimental approaches to mimic the in vivo situation. Ethical limitations in the study of these events in real patients may justify further research using ex vivo models of wound construction and incision biomechanics.

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