In Vitro Evaluation of Endothelial Cell Loss Using the Neusidl Corneal Inserter

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Purpose: To determine the immediate endothelial cell loss (ECL) resulting from insertion of a precut donor button using the Neusidl Corneal Inserter (NCI) and compare it with the previously published ECL resulting from insertion of a folded donor button using non-coapting forceps.

Methods: Ten corneas were precut for Descemet stripping automated endothelial keratoplasty and trephinated to a diameter of 8.0 mm (n = 5) or 8.5 mm (n = 5). Each tissue was placed onto the platform of a new NCI spatula and inserted into a cadaveric whole globe through a 5.2 mm incision. The tissue was carefully removed and stained with trypan blue and alizarin red to detect damaged endothelium. ECL was estimated using Adobe Photoshop planimetry. Mean ECL was compared with previously reported studies of forceps insertion with a one-sample t test, using SPSS v. 19. Geographic patterns of ECL were also documented.

Results: Mean ECL was 15.6% (95% confidence interval, 13.8–17.4). We were unable to detect a difference in ECL compared with previous insertion methods studied (P < 0.001). The pattern of damage from the NCI was different than that previously seen with forceps insertion.

Conclusion: Immediate endothelial damage resulting from use of the NCI for insertion of Descemet stripping automated endothelial keratoplasty tissue is comparable with that seen with a standard forceps technique, but with a different damage pattern.

Key Words: DSAEK, Neusidl Corneal Inserter, Adobe Photoshop, endothelial cell loss, corneal transplantation

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Descemet stripping automated endothelial keratoplasty (DSAEK) has enjoyed a rapid gain in popularity over the past 10 years. As DSAEK became the standard of care for treating endothelial dysfunction, it spurred the development of new surgical instruments to refine the procedure and improve graft survival. The Neusidl Corneal Inserter (NCI; Fischer Surgical, Arnold, MO) is one such instrument, a delivery spatula designed to protect the corneal endothelium from wound compression during tissue insertion for DSAEK. Many surgeons enjoy the simplicity of tissue insertion and orientation provided by the NCI and other inserter devices. The potential to protect the donor endothelium from damage during insertion is a valuable development for DSAEK.

In this study, we performed an in vitro simulation of the NCI-assisted DSAEK procedure to validate the NCI’s protection to the endothelium during insertion. Ten human corneas were inserted into cadaver eyes using the NCI, removed through an open sky technique, and stained with vital dyes for analysis with Adobe Photoshop planimetry. This standardized method1 for evaluating various corneal surgery techniques provides an objective measure of the total endothelial cell loss (ECL), enabling a clear and quantifiable comparison of the NCI with other endothelial keratoplasty devices and techniques in terms of the pattern and amplitude of endothelial damage.

The NCI was compared with a previously published study of various forceps and pull-through insertion techniques.2 In that study, the use of non-coapting forceps to insert a folded graft through a 5.0 mm incision resulted in a mean ECL of 18.4%. This study was only powered to determine “noninferiority,” meaning that we can only conclude whether ECL resulting from DSAEK with the NCI is comparable with and not worse than DSAEK with Charlie forceps. To establish noninferiority of the NCI to the forceps technique, we set our benchmark at an ECL of 18.4% and included a noninferiority margin of 5 percentage points. A confirmative result required the 95% confidence interval (CI) for the mean percent ECL to fall below 23%.

MATERIALS AND METHODS

Tissue Selection, Preparation, and Evaluation

Fifteen corneoscleral rims were selected from the research tissue pool at Lions VisionGift. All tissues came from donors less than 76 years old and had endothelial cell densities of at least 1500 cells per square millimeter before lamellar dissection. Tissues were evaluated under slit lamp to rule out significant endothelial defects or visible guttae. Tissues were then precut with a microkeratome (Moria, Inc, Doylestown, PA) to a target thickness of 125 µm and reexamined at the slit lamp to verify postcut endothelial integrity.
To mitigate variability of the vital dye process and subjectivity during Photoshop analysis, 5 tissues were randomly selected to serve as masked controls. After microkeratome preparation and postcut evaluation, these tissues were trephinated to a diameter of 8.0 mm and stained without any further manipulation. Each tissue was given a masked ID number and entered randomly into the Photoshop analysis queue.

Four whole globes were also obtained from Lions VisionGift. All globes contained the original crystalline lens, reducing the potential for mechanical damage from a dislodged intraocular lens. The globes were secured with gauze in a styrofoam training model (Hairless Corporation, Crown Point, IN) and prepared for DSAEK according to the NCI package insert. A 5.2 mm scleral tunnel was formed 1 mm from the temporal limbus. Directly across from the insertion site, a 180-degree clear corneal incision was formed and then closed with 10-0 nylon sutures to create a watertight seal. This flap was opened after each tissue insertion to allow for atraumatic removal, per the earlier study by Terry et al.2

### Tissue Insertion
Each cornea was trephinated with a Barron trephine (Katena Products, Denville, NJ). Five tissues were cut to a diameter of 8.0 mm and 5 were cut to 8.5 mm. After trephination, each tissue was coated with a thin layer of Healon (Abbott Medical Optics, Abbott Park, IL) and loaded onto the NCI platform as specified in the NCI’s standard operating procedures. A new NCI spatula was used for each tissue. The NCI was irrigated through plastic tubing attached to a bottle of balanced saline solution (BSS), suspended on an intravenous bottle stand raised to a height of 100 cm above the globe. Each tissue was then inserted into a globe through the 5.2 mm incision and released from the NCI platform according to the standard operating procedures. After the spatula was withdrawn from the anterior chamber, the tissue was removed through the large incision opposite the main wound and placed in a petri dish with the endothelial side up for staining.

### Staining
Each tissue was stained according to the protocol described by Park et al.3 The tissue was first carefully rinsed with BSS in a syringe through a 30-gauge cannula to remove the coating of Healon. The tissue was then gently dried with a Weck-Cel optical spear (Beaver-Visitec International, Waltham, MA) and covered with a 0.4% solution of trypan blue (MP Biomedicals, Solon, OH) for 90 seconds. The tissue was rinsed with BSS and then rinsed again with deionized water to prevent precipitation of the subsequent stain. A 0.5% solution of alizarin red was prepared by diluting a 1% stock solution (GFS Chemicals, Columbus, OH) in deionized water. Water was preferred because the high salt concentration of BSS causes precipitation of alizarin red, interfering with the vital dye process. The 0.5% alizarin red solution was applied to each tissue for 120 seconds and then rinsed with deionized water. The tissues were then suspended in BSS within a glass vial and mounted on the slit lamp for photography.

### Photoshop and Statistical Analysis Methods
JPEG images of all 15 stained tissues were imported into Photoshop Elements v. 7 (Adobe Systems, Inc, San Jose, CA) for quantification of ECL as described by Saad et al.1 Donor characteristics were compared between control and study tissues using one-way analysis of variance. The mean ECL from the 10 study tissues was compared with the 23.4% noninferiority value using a one-sample t test. Assuming a true mean ECL of 18%, a standard deviation of 5%, and a significance level (alpha) of 0.025, the sample achieved a power of 0.80. A secondary analysis compared the ECL between the grafts trephinated to 8.0 or 8.5 mm in diameter using independent samples t test. Statistical analyses were performed with SPSS v. 19 (IBM, Chicago, IL). Power calculations were performed with G*Power v. 3.1.3 (Franz Faul, Universität Kiel, Germany).

### RESULTS

#### Donor Characteristics
Donor characteristics are summarized in Table 1. Endothelial cell density was statistically significantly higher among the control tissues, but study and control tissues did not differ significantly with respect to donor age, tissue thickness, or death-to-surgery interval.

#### Endothelial Cell Loss
For the 10 NCI tissues, mean ECL was 15.58% (95% CI, 13.78–17.38). This represents a mean difference of 7.8 percentage points less than the 23.4% noninferiority benchmark we set for our comparison with the ECL reported from tissue insertion using Charlie forceps.2 This difference is statistically significant (t = −9.83, P < 0.001). The 95% CI for the true difference lies between -9.62 and –6.02. For the control group of 5 grafts, mean ECL was 7.26% (95% CI, 5.81–8.72).

### TABLE 1. Results of One-Way Analysis of Variance to Compare Preoperative Demographics Among Groups

<table>
<thead>
<tr>
<th>Tissue Group</th>
<th>Control</th>
<th>NCI: 8.0 mm</th>
<th>NCI: 8.5 mm</th>
<th>ANOVA Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor age, yr</td>
<td>61.0 (17.2)</td>
<td>66.2 (8.6)</td>
<td>64.6 (7.3)</td>
<td>F = 0.252, P = 0.782</td>
</tr>
<tr>
<td>Donor ECD, cells/mm²</td>
<td>2573.0 (363.2)</td>
<td>2130.0 (508.7)</td>
<td>2256.8 (513.1)</td>
<td>F = 1.194, P = 0.237</td>
</tr>
<tr>
<td>Tissue thickness, μm</td>
<td>141.2 (18.8)</td>
<td>176.2 (21.7)</td>
<td>160.0 (44.8)</td>
<td>F = 1.626, P = 0.0337</td>
</tr>
<tr>
<td>Death-to-surgery time, d</td>
<td>4.2 (1.5)</td>
<td>4.6 (2.1)</td>
<td>4.6 (2.1)</td>
<td>F = 0.074, P = 0.929</td>
</tr>
</tbody>
</table>

Values are mean (SD). ANOVA, analysis of variance.
The tissues with 8.00 mm diameter had a mean ECL of 14.57 (95% CI, 11.27–17.87). The tissues with 8.50 mm diameter had a mean ECL of 16.60 (95% CI, 13.94–19.26). This difference was not statistically significant with independent samples t test (t = −1.33, P = 0.222).

**Staining Pattern**

Figure 1 provides raw images for the highest and lowest ECL in the study, presented beside their corresponding Photoshop-processed images to demonstrate the analysis process. In the interest of space, the remaining figures present only the processed images. Several of the study tissues displayed a pronounced pattern of lines along one central axis of the tissue (Figures 1A, 2), which probably corresponded to the center of the folding platform and the point of forceps manipulation when moving the tissue from the trephine block to the platform. The remaining tissues displayed a more diffuse damage pattern that was not readily attributed to the NCI mechanisms (Figure 3). None of these patterns were seen in the control tissues.

**DISCUSSION**

This in vitro study provides a standardized analysis of ECL resulting from the NCI. The study was designed to allow for a direct comparison with a previous in vitro study examining ECL from Charlie forceps, the Busin glide (Moria, Inc), and suture-assisted pull-through insertion. That study found no real difference in ECL between techniques, as long as the incision was sufficiently large (5.0 mm). The mean ECL resulting from insertion of folded tissue through a 5.0 mm scleral tunnel using non-coapting forceps was found to be 18.4%. The NCI yielded a mean ECL of 15.6%, successfully passing our noninferiority benchmark of 23.4% (18.4 ± 5)

ECL. We have shown that ECL from insertion with the NCI is comparable with insertion of a folded donor graft using the Charlie forceps. No significant difference was observed between grafts cut to 8.0 mm or 8.5 mm; however, the study was not adequately powered to answer this question. A larger sample size would be needed to determine the relationship between graft diameter and ECL.

**FIGURE 1.** Examples of raw images and corresponding Photoshop analysis. A, The tissue that had the lowest ECL. B, The tissue that had the highest ECL.

**FIGURE 2.** Four tissues with ECL patterns suggestive of the NCI’s rolling mechanism.

**FIGURE 3.** The 4 remaining tissues in the sample, with no discernible pattern of ECL.
The NCI was designed to protect the donor tissue from endothelial damage during insertion by eliminating the need for folding the tissue and protecting the tissue from compression by the wound. Tissues that were folded and inserted with forceps revealed an obvious line of damage along the point of folding and a sharp mark of cell death at the point of forceps application.\(^2\) The decrease in mean ECL seen with the NCI suggests that its protective mechanisms do provide a significant advantage over standard forceps techniques. However, it is interesting to note the unique pattern of diffuse stress lines observed in several of the study tissues (Figures 1A, 2). These stress lines were not seen in any of the control tissues studied, indicating that the damage was caused by the NCI itself. Although the need for folding and forceps manipulation is eliminated, the NCI’s rolling mechanism was still predictably damaging to the endothelium.

The NCI is one of the several devices designed for endothelial keratoplasty graft insertion, including the Endo-Glide (Angiotech, Vancouver, Canada) and the EndoSerter (Ocular Systems, Inc, Winston-Salem, NC). There are no publications reporting in vitro laboratory studies using vital dyes for either instrument. The Tan EndoGlide has several reports of endothelial cell density as measured postoperatively via specular microscope, and these reports provide a favorable comparison with other standard techniques.\(^4\)\(^-\)\(^6\) Other insertion techniques use more traditional surgical instruments that lack the moving parts seen in the new generation inserters. These techniques include the Busin glide and the Rosenwasser shovel (Katena Products, Inc, Denville, NJ). The Busin glide produced a favorable staining profile in our previous article\(^2\) and other publications,\(^6\) and favorable clinical results from the Rosenwasser shovel were recently reported at the lamellar keratoplasty scientific paper symposium at the Annual Conference for the American Society of Cataract & Refractive Surgery (April 2012, Chicago, IL). The available data seem to indicate that all the popular techniques and devices are adequate methods for DSAEK insertion, provided the proper technique and incision size are used.

This in vitro study was designed to evaluate the protective mechanisms built into the NCI, specifically the avoidance of wound compression damage. However, it is not a study of the overall ECL resulting from clinical use of the NCI. The tissues were removed from the eye immediately after insertion, and no attempt was made to properly orient the graft or to attach it to the recipient. Each insertion technique presents its own set of challenges after insertion, and surgical maneuvers such as unfolding the graft, injecting the air bubble, and centering the tissue might cause additional ECL. The total effect of DSAEK surgery with the NCI is best measured in a clinical study. We are currently conducting a large, prospective, randomized controlled clinical trial comparing the postoperative endothelial cell densities in DSAEK eyes with tissue inserted using either the NCI or the Charlie forceps. For now, our laboratory testing of the NCI indicates that the device protects the endothelium at least as well as forceps. Finally, from a clinical perspective, the NCI may offer other clinical and “ease of use” advantages to the individual surgeon that are not addressed by this study.

REFERENCES