Comparison of Donor Insertion Techniques for Descemet Stripping Automated Endothelial Keratoplasty

Jodhbir S. Mehta, MD, FRCS(Edin); Yong Ming Por, MD, FRCS(Edin); Rebekah Poh, BSc; Roger W. Beuerman, PhD; Donald Tan, MD, FRCS(Edin)

Objective: To evaluate the difference in endothelial cell damage between 2 donor insertion techniques for Descemet stripping automated endothelial keratoplasty (DSAEK).

Design: Experimental study and prospective case series. Thirty donor corneas and 10 patients undergoing DSAEK with glide insertion were included. Donor cornea lenticules were prepared and a wet lab DSAEK model established. Donor lenticules were inserted either by a "taco" fold (n=15) or glide insertion (n=15). Endothelial cell damage was assessed by scanning electron microscopy (n=20) and trypan blue exclusion (n=10). Endothelial cell count was assessed by specular microscopy in the clinical patients.

Results: Endothelial cell viability and scanning electron microscopy demonstrated 2 different patterns of cell damage in either group. Cell viability and scanning electron microscopy showed there was mean cell damage of 9% and 9.2%, respectively, following glide insertion and 32% and 38%, respectively, following the taco-folded insertion (P=.004). The mean (SD) cell loss in the clinical patients following glide insertion was 25.3% (4.3%) at 6 months.

Conclusion: Endothelial cell damage was higher in a wet lab model following taco-folded insertion compared with glide insertion. Initial clinical results with glide insertion showed satisfactory endothelial cell loss at 6 months.

Clinical Relevance: Folding of the corneal tissue during DSAEK causes more endothelial damage than glide insertion.

Arch Ophthalmol. 2008;126(10):1383-1388

NEW FORMS OF ANTERIOR and posterior lamellar keratoplasty are now enabling targeted replacement of only diseased layers of the cornea.1 Descemet stripping automated endothelial keratoplasty (DSAEK) is a form of small-incision and essentially sutureless surgery. The DSAEK procedure involves stripping of the diseased Descemet membrane and endothelial cells through a small corneal incision and replacement with a posterior lamellar donor corneal lenticule prepared with an automated lamellar therapeutic keratoplasty unit.2

With the adoption of any new surgical technique there is an inevitable learning curve for the surgeon and an accompanying evolution in techniques.3-7 One of the most challenging aspects of this procedure is insertion of the donor posterior lenticule into the anterior chamber (AC) through a small incision. The current widely performed technique requires insertion of the donor lenticule through a small (5 mm or less) corneal or scleral incision by folding the lenticule and gripping the folded tissue with noncompressing forceps, ie, "taco-folded insertion."

Preliminary in vitro work has alluded to endothelial cell damage occurring with the conventional folding technique despite the use of commercially available noncompressing forceps (Goosey forceps, model No. 19090; Moria, Antony, France). Damage primarily occurred as a consequence of direct contact of folded endothelial surfaces where the folding forceps are applied as well as along the folding crease.8,9 The aim of this study was to assess the damage to donor cornea endothelium by comparing a glide insertion technique with conventional taco insertion in a laboratory DSAEK model with human eye bank corneas. In a second arm of this project, clinical outcomes are reported in a prospective case series of patients who underwent the glide insertion technique in a manner identical to that of the laboratory arm of the study.
After approval by the institutional review board of the Singapore National Eye Centre, 30 human corneal-scleral buttons with healthy endothelium, but otherwise unsuitable for clinical transplantation, were procured from the Singapore Eye Bank and stored in Optisol GS solution (Bausch & Lomb, Irvine, California) at 4°C. The mean (SD) death to experimentation time of the corneas was 5 (1.7) days. The corneas were sequentially assigned to 1 of 2 groups (Fig 1).

**PREPARATION OF DONOR LENTICULES**

Microkeratome dissection was performed using an automated lamellar therapeutic keratoplasty unit (Carrazio-Barraquer microkeratome; Moria) equipped with a 250-µm head (2 corneas), 300-µm head (13 corneas), or 350-µm head (15 corneas). Ultrasound pachymetry (mean of 3 values) (CorneoGage; Sonogage, Cleveland, Ohio) was performed before and after dissection. We selected either a 250-, 300-, or 350-µm Carrazio-Barraquer head, aiming for a donor lamella of 150 to 200 µm. Following dissection, the cornea was trephined with an 8-mm Hanna trephine (Moria).

**EXPERIMENTAL DSAEK MODEL**

A human corneal-scleral button was mounted onto an artificial anterior chamber (AAC) (Coronet Network Medical Products, North Yorkshire, England). A 5 x 2-mm scleral tunnel incision was made 1 mm from the limbus. The AAC was infused with balanced salt solution (BSS) to ensure physiological intraocular pressure (determined digitally). The donor cornea was then removed per preassigned insertion group. Following donor insertion, the AAC was reinfused to maintain the chamber. The tightening ring was loosened, the chamber inverted upside down, and the base assembly removed (Fig 2). The donor lenticule was then removed and inserted into fixative or Optisol GS.

**DONOR INSERTION**

The insertion technique for 15 buttons (group 1) was the standard taco-folded method. The donor corneal lenticule, endothelial surface up, was coated with a small amount of cohesive ophthalmic viscosurgical device. Using Kelman Macpherson forceps (Rumex International Co, St Petersburg, Florida), the right edge of the posterior endothelial lamella was grasped and folded over the donor tissue, leaving more donor tissue on the upper half, ie, a 60:40 overfold ratio. Noncompressing DSAEK donor folding forceps (Goosey forceps) were then used to grasp the folded tissue. The infusion into the AAC maintainer was turned off because this is the standard procedure in folded insertion. During folded insertion, if the infusion remains on, the donor disc often exits the anterior chamber once released, owing to the efflux of fluid following lifting of the incision. The scleral incision was then gently lifted and the taco-folded donor tissue inserted fully into the AC. The forceps were gently removed without pulling out or dislodging the donor tissue. The BSS was reinfused into the AAC maintainer once the donor tissue had been disengaged from the forceps. A 5-mL syringe of BSS with a 30-gauge cannula was used to carefully unfold the donor tissue in the AC, ensuring that the cannula was advanced below the upper edge of the folded donor tissue. A small air bubble was injected under the donor cornea with a 30-gauge needle to prevent descent of the donor cornea.

The insertion technique for the other 15 buttons (group 2) was with our previously described sheets glide-insertion technique. A paracentesis was first made in the peripheral cornea opposite the scleral tunnel wound for insertion of Kawai intracapsular capsulorhexis forceps (ASICO, Westmont, Illinois). A standard AC intraocular lens sheets glide was trimmed and inserted into the AAC through the scleral tunnel while the BSS infusion was maintained. A dispersive ophthalmic viscosurgical device was liberally applied over the endothelial surface of the donor cornea and on the anterior surface of the glide. The donor cornea was gently everted, corneal endothelial surface–down, onto the ophthalmic viscosurgical device–covered portion of the glide. The Kawai forceps were passed through the paracentesis, over the sheets glide, and out through the scleral incision, grasping the donor cornea edge and pulling the donor cornea through the scleral incision. With this technique, a deep chamber was maintained throughout the maneuver. A small air bubble was injected under the donor cornea with a 30-gauge needle to prevent descent of the donor cornea, and the donor cornea was released from the Kawai forceps.

**ENDOTHELIAL STAINING FOR CELL VIABILITY**

The donor buttons were initially stored in Optisol GS at 4°C (for less than an hour) and then rinsed in BSS and placed in 0.06% trypan blue solution (Dutch Ophthalmic Research Cen...
Donor tissue insertion was by the glide-insertion technique. The diameter of those used in the laboratory arm of this study. The diameter of the glide-insertion technique for the patients were the same as microkeratome and the protocol used to prepare the tissue and masked observer. Both the number of cells with disrupted cell membranes and percentage of disrupted cells was determined by counting center and in 1 in each clock hour around the button. The measurement of endothelial cell density were performed using a fixed frame protocol, counting the apices of at least 100 cells from endothelial images of each cornea. Manufacturer’s recommendations for calibration of magnification were followed. Postoperative specular microscopy measurements of endothelial cell density were performed using a noncontact specular microscope (Konan Medical Corp, Hyogo, Japan). Tests were performed at 3 and 6 months postoperatively by the same technician using the same specular microscope at each visit. The postoperative cell counts were obtained using the manufacturer’s calibrations for magnification and manual adjustment of focal length of scan. A fixed-frame method was used, marking at least 50 to 100 cells per image. Statistical analysis was performed with SPSS version 11 (SPSS Inc, Chicago, Illinois). Comparison of mean cell loss was performed by Mann-Whitney U test; α < .05.

SCANNING ELECTRON MICROSCOPY

The donor buttons were fixed in 2% glutaraldehyde, 2% paraformaldehyde, and 0.1M sodium cacodylate overnight at 4°C. The tissues were secondarily fixed in 1% osmium tetroxide and then dehydrated, dried, and mounted on scanning electron microscopy (SEM) stubs. They were then sputter coated and examined by SEM (JSM-5600; JEOL, Tokyo, Japan) at 15 W. Low-power (×18) and high-power (×500) SEM images were taken using a previously described technique. Thirteen micrographs were taken per button, 1 in the center and in 1 in each clock hour around the button. The percentage of disrupted cells was determined by counting both the number of cells with disrupted cell membranes and the total number of cells on scanning electron micrographs (×500). Assessment of cell damage was performed by a masked observer (Figure 3).

DESCEMET STRIPPING AUTOMATED ENDOTHELIAL KERATOPLASTY

Descemnet stripping automated endothelial keratoplasty was performed in 10 consecutive patients by a single surgeon using a similar technique (D.T.), as described by Price and Price. However, donor tissue insertion was by the glide-insertion technique. The microkeratome and the protocol used to prepare the tissue and the glide-insertion technique for the patients were the same as those used in the laboratory arm of this study. The diameter of the donor buttons ranged from 8.00 to 8.75 mm (mode, 8.50 mm).

ENDOTHELIAL CELL DENSITY MEASUREMENTS

Preoperative specular microscopy of the donor tissue was performed either by certified technicians in an Eye Bank Association of America–certified eye bank (Lions Eye Institute for Transplant and Research, Tampa, Florida) or by a certified eye bank technician at the Singapore Eye Bank. Preoperative cell counts were obtained using a fixed frame protocol, counting the apices of at least 100 cells from endothelial images of each cornea. Manufacturer’s recommendations for calibration of magnification were followed. Postoperative specular microscopy measurements of endothelial cell density were performed using a noncontact specular microscope (Konan Medical Corp, Hyogo, Japan). Tests were performed at 3 and 6 months postoperatively by the same technician using the same specular microscope at each visit. The postoperative cell counts were obtained using the manufacturer’s calibrations for magnification and manual adjustment of focal length of scan. A fixed-frame method was used, marking at least 50 to 100 cells per image.

STATISTICAL ANALYSIS

Statistical analysis was performed with SPSS version 11 (SPSS Inc, Chicago, Illinois). Comparison of mean cell loss was performed by Mann-Whitney U test; α < .05.

RESULTS

LABORATORY STUDY OF BASELINE CHARACTERISTICS

All of the corneas in the study were 7 days or fewer post mortem with a mean storage time of 5 days. The mean (SD) age of the donors was 60 (7) years. The mean (SD) pachymetry of the donor corneas was 568 (59.6) µm. There was no significant difference between the preoperative donor thickness of the 2 groups (P = .29). The mean (SD) depth of microkeratome dissection was 317 (62) µm. The mean deviation from intended microkeratome cut was 33 µm. Following microkeratome dissection, the mean (SD) pachymetry of the posterior donor button was 251 (62) µm. There was no significant difference between the thickness of the donor buttons between the 2 groups (P = .49). The mean (SD) endothelial cell count of the human donor corneas was 2613 (381) cells/mm² (range, 1976-3649 cells/mm²). There was no significant difference between the 2 groups (P = .7) with respect to endothelial cell counts.

ENDOTHELIAL DAMAGE ON SEM

Two patterns of cell damage could be seen on low-power SEM. Following conventional taco-folded insertion (group 1), there were 2 discrete longitudinal bands of cellular damage. This corresponded to compression of tissue along the shaft of the forceps (Figure 4A.) In these areas there was often complete endothelial cell loss and baring of the Descemet membrane visible on high-power SEM. Where the cornea was folded, fine linear streaks of endothelial cell damage at the area of the maximal folding could be seen. In the corneas from group 2, there were fine linear streaks of endothelial cell damage.
radiating out from where the donor tissue had been pulled with intraocular forceps. A second area of damage was also seen in the periphery of the donor tissue, presumably where there may have been some contact with the sheets glide-insertion surface. This rim of damage varied from between 1 and 3 cells in width (Figure 4B).

Quantitative analysis of the endothelial cell damage showed there was mean (SD) cell damage of 38% (12.79%) (range, 22.2%–52.4%) in group 1 buttons. The mean (SD) cell damage from corneas in group 2 buttons was 9.24% (5.16%) (range, 3.9%–16.8%). There was significantly less endothelial cell damage following glide insertion (group 2 corneas) compared with taco-folded insertion (group 1) (P = .004).

### CELL VIABILITY

Following donor insertion, the mean (SD) percentage of graft endothelial injury from forceps-folded insertion was 32% (10%) (range, 20% to 50%). The mean (SD) percentage of graft endothelial injury from glide insertion was 9% (4%) (range, 5% to 15%) (Figure 5). The same pattern of damage seen by SEM was seen on cell viability staining.

### INTERVENTIONAL CLINICAL CASE SERIES

All patients had improvement in their visual acuity following DSAEK. Details of patients are listed in the Table. Six patients had DSAEK alone and 4 had phaco-DSAEK. All of the patients had a routine postoperative course with resolution of the corneal edema. There were no cases of primary graft failure or graft dislocation in the immediate postoperative period. Mean (SD) follow-up was 7.5 (1) months. Mean preoperative logarithm of the minimum angle of resolution of best spectacle-corrected visual acuity was 1.4; postoperative it was 0.38. The mean (SD) endothelial cell count at 6 months was 2109 (120) cells/mm² (range, 1960-2304 cells/mm²). This represented a 25.3% (4.3%) (range, 16.7% to 29.4%) cell loss at 6 months.

### COMMENT

Endothelial keratoplasty is becoming an increasingly popular alternative to penetrating keratoplasty. The excellent refractive results and faster visual rehabilitation achieved after this procedure is an inherent advantage over traditional penetrating keratoplasty. However, the operation does require a completely different skill set relating to handling of the thin donor tissue, donor tissue insertion, and manipulation of the donor tissue within the AC. There is a steep learning curve, and higher rates of primary (iatrogenic) graft failure are reported. Our study showed less endothelial cell damage following a nonfolded (glide) insertion compared with a typical taco-folded insertion. The results were translated clinically with respectable endothelial cell loss at 6 months postoperatively.

We chose to use corneas that were less than 7 days old because both pachymetry and endothelial cell morphology may be affected by prolonged storage in Optisol GS. This may have biased the laboratory experiments; if the donor tissue was swollen, greater damage would then be expected following folding with forceps. There was no significant difference in the preexperiment donor thickness or endothelial cell counts used for both groups. Some of the tissue we used had less endothelial cell counts than we would have used for clinical DSAEK, eg, 1976 cells/mm². However, because we were assessing the percentage of cell loss, we felt it was acceptable to use this quality of tissue for laboratory experiments.

The pattern of endothelial cell damage documented on SEM and vital dye staining was consistent with each of the insertion techniques. Essentially, following forceps insertion (group 1), there were 2 bands of cellular damage analogous to where the forceps compressed the tissue. Where the cornea was folded, linear (crease-like) streaks of endothelial cell damage could be seen. The results are consistent with our preliminary in vitro work and similar to that reported by other authors with vital dye staining. It is important when comparing these results to examine which forceps are being used. In 2 of the previous reports, noncompressing forceps (Goosey) were used, while in the third, Kelman-McPherson forceps were used. Noncompressing forceps (eg, the former) have been shown to cause less endothelial cell damage than compressing forceps. It is interesting to note in all of the reported cases that the mechanical damage induced by the forceps is along the forceps arms and that the resultant damage causes complete loss of endothelial cells with baring of the Descemet membrane.

The pattern of damage following glide insertion was completely different. There were no bands of bare Descemet membrane, but streaks of dead endothelial cells radiating from where the graft was pulled into the eye was probably related to donor creasing from the traction of the forceps. Also, in the periphery of the graft there was a rim of bare Descemet membrane that we postulate to be related to some degree of rim contact with the plastic glide surface. It was encouraging to note that minimal central endothelial damage occurs, confirming the important role of copious ophthalmic viscosurgical device use beneath the donor. Kuo et al described the effects of passing an 8-mm graft via a cartridge system through a 3-mm incision. There was approximately 9% endothelial cell damage with this...
technique. However, mathematical modeling has shown that a 3-mm incision will only allow an 8-mm graft of 225-µm thickness to pass through a cartridge without the edges overlapping and damaging endothelium. One of the advantages of DSAEK over conventional penetrating keratoplasty is the ability to transplant a larger reserve of healthy donor endothelial cells. Grafts of 9 mm are frequently used for DSAEK that allow 26% more surface area of cells than an 8-mm graft. A 9-mm graft would have conversely resulted in greater endothelial cell damage when passing through such a small (less than 5-mm) incision. As such, we feel that the aim to perform refractively neutral smaller incisions in DSAEK should not be a priority at the expense of the potential for increased endothelial cell damage.

We assessed endothelial cell damage using 2 techniques. The cell damage following glide insertion after assessment by both vital dye staining and on SEM was 9%. This was significantly less than the 32% and 38% cell damage seen with the forceps delivery on vital dye staining and SEM, respectively. The protocol for SEM assessment has been previously described. Because the magnification of the photomicrographs on SEM is ×500, to visualize the endothelial cells we felt that vital dye staining would provide additional confirmatory data of the overall damage we were seeing on SEM.

Our early clinical series provides some encouraging results. The mean endothelial cell loss at 6 months was 25.3%. Recently, Terry et al and Price and Price have studied endothelial cell loss in more than 340 eyes. Interestingly, in their individual series they both reported a mean endothelial cell loss at 6 months of 34% (Terry et al, n=80 eyes; Price and Price, n=263 eyes). This was followed by further cell loss of only 2% and 1% at 12 months postoperatively. Although our results are encouraging, our sample size is small compared with the large series reported by Terry et al and Price and Price. Both of these other studies offer an important insight into longer-term endothelial cell attrition following DSAEK. Our 25.3% mean cell loss is higher than our mean in vitro

### Table. Demographics of Patients Who Underwent DSAEK and Glide Insertion

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Indication for DSAEK</th>
<th>Preoperative Donor</th>
<th>Donor at 6 mo</th>
<th>Preoperative LogMar BSCVA</th>
<th>Follow-up, mo</th>
<th>LogMar BSCVA at Last Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PK failed</td>
<td>2590</td>
<td>1960</td>
<td>2</td>
<td>6</td>
<td>1a</td>
</tr>
<tr>
<td>2</td>
<td>FECD</td>
<td>2874</td>
<td>2053</td>
<td>1.3</td>
<td>8</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>PBK</td>
<td>2770</td>
<td>1960</td>
<td>0.5</td>
<td>7</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>PBK</td>
<td>2743</td>
<td>2136</td>
<td>2</td>
<td>9</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>FECD</td>
<td>3135</td>
<td>2212</td>
<td>2</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>6</td>
<td>PBK</td>
<td>3003</td>
<td>2304</td>
<td>2</td>
<td>8</td>
<td>0.4</td>
</tr>
<tr>
<td>7</td>
<td>PBK</td>
<td>2941</td>
<td>2083</td>
<td>0.9</td>
<td>7</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>FECD</td>
<td>2680</td>
<td>2232</td>
<td>0.6</td>
<td>7</td>
<td>0.4</td>
</tr>
<tr>
<td>9</td>
<td>FECD</td>
<td>2820</td>
<td>2105</td>
<td>2</td>
<td>9</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>PBK</td>
<td>2483</td>
<td>2049</td>
<td>1</td>
<td>6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Abbreviations: DSAEK, Descemet stripping automated endothelial keratoplasty; FECD, Fuchs endothelial dystrophy; LogMar BSCVA, logarithm of the minimum angle of resolution of best spectacle-corrected visual acuity; PBK, pseudophakic bullous keratopathy; PK, penetrating keratoplasty.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogMar</td>
<td>Logarithm of the minimum angle of resolution of best spectacle-corrected visual acuity</td>
</tr>
<tr>
<td>BSCVA</td>
<td>Best spectacle-corrected visual acuity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 5. Representative photomicrographs of quantitative analysis (shaded color) showing area of cell damage following “taco-folded” insertion (A) (mean damage, 32%) and glide insertion (B) (mean damage, 9%) techniques.</td>
<td></td>
</tr>
</tbody>
</table>
cellular damage of 9%. This may be owing to additional cell loss from air bubble tamponade. Increasing numbers of cases and longer follow-up are needed with our technique to assess long-term endothelial cell damage.

Although our model was a useful pressurized model to assess donor insertion, it does not fully mimic the complete DSAEK procedure. However, considering that we were assessing donor insertion, we may assume that the endothelial cell loss with air tamponade will be similar following both donor insertion techniques. The surgeon performing the donor insertion with both techniques was in the learning curve for this procedure, i.e., less than 20 cases. A higher rate of complication occurs during this period. This may explain the higher rate of endothelial cell loss from forceps folding compared with the clinical series of Terry and colleagues and Price and Price. Terry et al also use different forceps than those described in this study, and more recently, Price has also stopped using the forceps described in this study (information obtained through oral communication). However, the surgeon was also in the learning curve for the glide insertion technique and was able to maintain moderate cell loss compared with forceps delivery.

In conclusion, we describe an alternative surgical technique for donor insertion compared with conventional forceps folding. Our in vitro studies appear to implicate forceps-related damage as a significant cause of endothelial cell loss despite specifically designed noncompressive DSAEK forceps, as compared with a glide insertion. Our early clinical series showed a relatively respectable level of 25.3% endothelial cell loss at 6 months postoperatively.

Submitted for Publication: April 4, 2008; final revision received June 1, 2008; accepted June 4, 2008.

Correspondence: Donald Tan, MD, FRCS(Edin), Singapore National Eye Centre, 11 Third Hospital Ave, Singapore 168751, Singapore (jodmehta@gmail.com).

Financial Disclosure: None reported.

REFERENCES


Archives of Ophthalmology and Archives of Pathology and Laboratory Medicine will publish a joint theme issue on pathology in August 2009. Submissions of articles on clinical and translational research in retinoblastoma, melanoma, lymphoma, and orbital and adnexal tumors in ophthalmology will have the best chance for consideration for this theme issue. Please submit papers online (www.archophthalmol.com) no later than February 1, 2009. All submissions will undergo our usual peer-review process.