Endothelial Keratoplasty: The Influence of Insertion Techniques and Incision Size on Donor Endothelial Survival

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Purpose: To determine the acute endothelial cell damage from trephination and tissue insertion in endothelial keratoplasty (EK) surgery. The influence of insertion technique (forceps insertion vs "pull-through" insertion) of donor tissue and incision size (3 vs 5 mm length) was assessed.

Methods: Forty precut 8.-mm-diameter donor posterior buttons were used in this study. Thirty-five buttons were inserted through a limbal incision of either 3 or 5 mm length into the anterior chamber of cadaver eyes and then removed through an open sky technique without further trauma. Five buttons that were trephined but not inserted served as a control group. Vital dye staining and computer digitized planimetry were used to analyze the tissue and quantify the total damaged area over the entire endothelial surface. Five buttons for each of 7 insertion techniques were used. The 8 tissue groups evaluated were as follows: group 1: control group of trephination only, with no insertion; group 2: forceps with folded tissue through 5-mm incision; group 3: suture pull through of nonfolded tissue through a 5-mm incision; group 4: forceps pull through of Busin glide folded tissue through a 5-mm incision; group 5: forceps with folded tissue through a 3-mm incision; group 6: suture pull through with folded tissue through a 3-mm incision; group 7: suture pull through with nonfolded tissue through a 3-mm incision; and group 8: forceps pull through of Busin glide folded tissue through a 3-mm incision.

Results: The control group demonstrated 9% ± 2% peripheral cell damage from simple trephination of the tissue but without insertion. In the 5-mm incision surgeries, forceps insertion (group 2) caused 18% ± 3% loss, suture pull-through insertion (group 3) caused 18% ± 2% loss, and Busin glide pull through (group 4) caused 20% ± 5% loss. There were no significant differences in damage between any of the 5-mm incision group techniques ($P > 0.99$). In the 3-mm incision surgeries, forceps insertion (group 5) caused a 30% ± 3% loss, pull through with folded tissue (group 6) caused 30% ± 5% loss, pull through with nonfolded tissue (group 7) caused 56% ± 4% loss, and Busin glide pull through (group 8) caused a 28%± 5% loss. There was no difference in damage among the 3-mm groups ($P > 0.96$), with the exception of group 7 where pulling the unfolded tissue through a 3-mm incision was significantly worse than all other techniques ($P < 0.001$). There was significantly greater cell area damage in the 3-mm groups (36%) than in the 5-mm groups (19%) ($P < 0.001$). Large patterns of striae with cell loss were seen in the 3-mm groups emanating from the peripheral traction site, regardless of whether the traction to pull the tissue through the incision and into the chamber was generated by a suture or cross-chamber forceps. Direct forceps insertion caused circular patterns of injury at the tip compression site regardless of incision size, but this damage was multiplied and exacerbated by insertion through a smaller incision.

Conclusions: Smaller size (3 mm) incisions for EK surgery result in greater acute endothelial area damage than larger size (5 mm) incisions. Pull-through insertion techniques through a 5-mm incision seem equivalent in the amount of induced area damage to that of forceps insertion. Compressive injury from the incision appeared less when the tissue was folded than when not folded. Insertion with any technique through a 3-mm incision resulted in larger areas of endothelial damage. All these iatrogenic death zones outside the central endothelial area would be missed clinically by standard early specular microscopy after EK surgery.

Key Words: cornea, endothelial keratoplasty, deep lamellar endothelial keratoplasty, Descemet stripping endothelial keratoplasty, DSAEK, vital dye staining, Adobe Photoshop, trypan blue, alizarin red S, endothelial damage, quantitative assessment

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Endothelial keratoplasty (EK) is quickly becoming the preferred method of corneal transplantation for endothelial dysfunction.[1] We performed the first EK surgery in the United States in March of 2000.[2,3] Although the EK technique at our institution has evolved over the past 9 years from deep lamellar endothelial keratoplasty (DLEK)[4,5] to Descemet stripping endothelial keratoplasty[6] to Descemet stripping automated endothelial keratoplasty (DSAEK),[7,8] since 2001, we have consistently used a donor insertion access incision of 5 mm length, placed at the surgical scleral limbus, in more than 700 cases of EK. In our purely DSAEK series,[7] we have recently documented a low dislocation rate of 1.5% and an iatrogenic primary graft failure

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rate of 0% in our initial 200 consecutive cases, and these complication rates are lower than those in other published reports.[9–16] We believe that our complication rate is directly related to our surgical technique, as this low complication rate was achieved by 4 different surgeons of varying experience.[7] Although surgeon's experience certainly can be a factor in rates of dislocation and graft failure,[12–14] differences in surgical technique may also be inherently responsible for more endothelial cell damage, resulting in the complications of dislocation and iatrogenic primary graft failure rate.[15]

There have been extensive changes by many surgeons to the method in which the donor tissue is delivered into the anterior chamber, including the use of smaller access incisions,[16,17] folding the tissue with glides and cartridges,[17–19] and placement of the tissue by pulling the tissue into the eye with either peripheral traction sutures or cross-chamber forceps.[19–21] Although there are 2 reports on acute endothelial cell death resulting from forceps insertion,[17,22] to date, there have been no reports directly comparing the acute donor endothelial damage from these various techniques using various incision sizes.

In this study, we evaluated 7 methods used in EK for donor tissue delivery into the anterior chamber. We isolated the specific role of trephination and tissue insertion to assess what role these 2 steps play in acute donor endothelial damage. Other manipulations that may contribute to tissue damage, such as unfolding and tissue positioning, were deliberately not assessed to limit the confounding variables. Finally, the influence that incision size has on endothelial trauma with various insertion techniques was quantitatively evaluated in the laboratory setting.

MATERIALS AND METHODS

Cadaver Eyes

Forty precut corneas were obtained from the Lions Eye Bank of Oregon, which were unacceptable for clinical transplantation but had normal and abundant endothelium as determined by standard eye bank procedures. Average donor age was 57 ± 13 years. Death to preservation time averaged 8.9 ± 3.1 hours. Precut central endothelial cell density (ECD) averaged 2674 ± 449 cells/mm^2, and the postcut central ECD averaged 2613 ± 428cells/mm^2. The ECD both precut and postcut was determined using an EB-3000 XYZ Eye Bank specular microscope (HAI Laboratories Inc, Lexington, MA). Microkeratome resection of the anterior lamella was performed with a Moria artificial anterior chamber using a 300-micron CB head (Moria Systems, Doylestown, PA), the anterior cap of tissue was placed back on the stromal bed, and the corneal–scleral tissue was placed back into Optisol and stored at 4°C until use, usually within 24 hours.

The "recipient" eye was a whole globe that had been treated with dextran solution to provide a clear cornea for experimentation. We have described our method of providing corneal detergesence and clarity for ophthalmic experimentation in our previous publications.[23,24]

The insertion incisions for the recipient tissue were created with a diamond knife set to a depth of 350 μm. The length was either 3 or 5 mm and verified with calipers. The location for all incisions was 0.5 mm peripheral to the junction of the clear corneal limbus and the scleral limbus. A beveled crescent metal blade was used to create a 2-mm beveled entry into the anterior chamber for all incisions.

To avoid further damage to the donor button after its insertion into the anterior chamber of the whole globe, a 180-degree limbal perforating incision was made beforehand directly opposite from the area of the surgical incision. This 180-degree incision was kept closed with a running suture during the time of donor insertion to create a normally pressurized anterior chamber and to allow the use of an anterior chamber maintainer when needed. After donor insertion, the 180-degree incision could be opened, the entire cornea retracted, and the donor tissue removed from the anterior chamber through an open sky technique, completely avoiding further trauma to the donor tissue.

Surgical Techniques

For all 40 donor tissues, the corneal–scleral tissue was trephined centrally with an 8-mm Barron Punch trephine (Katena Products, Denville, NJ). In cases where the tissue was to be folded, a thin strip of highly cohesive viscoelastic (Healon; AMO, Santa Monica, CA) was placed centrally, and the tissue was folded into a 40%/60% "taco," as we were the first to describe in our clinical techniques of EK.[1,4] The tissue was then inserted with the various techniques described below, always with the 60% side of the taco positioned superiorly. In cases where the Busin glide (Moria Systems) was used, a similar strip of Healon was placed on the endothelium before pulling the tissue into the glide. In all cases where the tissue was pulled through with either a suture or a cross-chamber forceps, the entire sclera adjacent to the insertion wound was covered with Healon to protect any exposed endothelial surface of the donor tissue as it was pulled into the wound. This protective coating covered the entire sclera adjacent to the length of the wound in cases where the tissue was pulled through without prior folding of the tissue.

There were 8 groups (1 control group and 7 groups of insertions), with 5 donors in the control group and 5 donors in each of the 7 insertion technique groups.

Group 1: This control group of donor tissues was simply trephined, stained, and then analyzed for percentage area of cell damage.

Group 2: A 5-mm incision was used. The tissue was folded and then inserted with Charlie non-coapting single-point forceps (Bausch and Lomb Surgical, St. Louis, MO).

Group 3: A 5-mm incision was used. A 10-0 Prolene suture on a CIF straight needle (Ethicon) was placed through the far peripheral edge of the donor button and a loop knot tied to secure the tissue without suture compression. The needle was then passed through the access incision and exiting through the opposite limbal wound, and the unfolded tissue was pulled through the 5-mm wound.

Group 4: A 5-mm incision was used. The tissue was rolled up with a Busin Glide (Moria Systems) and then pulled through a 5-mm wound and placed into the anterior chamber with a cross-chamber fine forceps (retinal membrane forceps; Grieshaber, Germany). The anterior chamber was maintained at all times with balanced

saline solution (BSS) through an anterior chamber maintainer (Moria Systems).

Group 5: A 3-mm incision was used. The tissue was folded and inserted with a Charlie non-coapting single-point fixation insertion forceps.

Group 6: A 3-mm incision was used. The tissue was folded, the edge sutured with a CIF 10-0 Prolene traction suture, and then the needle passed through the incision, and the tissue pulled through the wound and into the chamber by the traction suture.

Group 7: A 3-mm incision was used. The tissue was not folded but secured at the edge with the Prolene suture and then pulled through the wound and into the chamber.

Group 8: A 3-mm incision was used. The tissue was rolled up with a Busin Glide (Moria Systems) and then pulled through a 3-mm wound and placed into the anterior chamber with a cross-chamber fine forceps (retinal membrane forceps; Grieshaber). The anterior chamber was maintained at all times with BSS through an anterior chamber maintainer (Moria Systems).

Staining and Quantification of Endothelial Damage

After removal from the anterior chamber through the open sky technique, the tissue was gently irrigated with BSS to unfold and normalize the configuration and then stained with trypan blue 0.25% (MP Biomedicals, LLC, Solon, OH) and alizarin red S 0.2% (GFS Chemicals Inc, Columbus, OH). The technique of vital dye staining is described in detail in our previous publication.[25]

Quantitative analysis of the endothelial damage was performed. The graft was placed in a clear glass vial containing BSS and mounted on the slit lamp and photographed using the highest available magnification (×16) that allowed us to capture a panoramic digital picture (MicroFire; Optronics, Goleta, CA) for the whole graft after adjusting the illumination to avoid shadows or excessive light reflections. This digital image then underwent planimetry quantitative processing using standard Adobe Photoshop 7.0 software. We have described this technique in detail previously.[25] By determining the number of pixels that make up the stained damaged areas and dividing this number of pixels by the number of pixels that make up the entire endothelial area, a percentage of "endothelial damage" from any given manipulation can be determined. The consistency and reproducibility of this quantitative analysis of endothelial area damage have already been established.[25]

Statistical Analysis

All data were analyzed using SPSS version 12.0. An analysis of variance was performed using Tukey Honest Statistical Determination (HSD) post-hoc analysis to compare differences between the various surgical techniques. Alpha was held at $P = 0.05$.

RESULTS

The typical vital dye staining and processed image depicting patterns of cell damage that occurred with each of the 8 groups are shown in Figure 1. The control group trephination damage and the total quantified area damage from the various insertion techniques of all 8 groups are detailed in Table 1 and graphically demonstrated in Figure 2. The statistical analysis between each of the 8 groups is detailed in Table 2. Comparison of mean cell damaged areas between the 3-mm incision groups with the 5-mm incision groups are detailed in Table 3.

Patterns of Damage

The patterns of geographic cell damage from the various techniques were quite distinctive (Fig. 1). Simple trephination of the tissue caused a ring of damage at the edge of the donor button, which was fairly consistent in width. In some controls, very faint and random pattern striae damage across the paracentral cornea could be seen on occasion. Although these faint striae could have been caused by the stretching "trampoline effect" of trephination on the posterior tissue, this damage could also have been caused by the microkeratome and other precut maneuvers in the eye bank before the experiments. When forceps were used for insertion through a 5-mm incision, the tips of the forceps caused a distinctive circular area of damage on either side of the folded tissue. This pattern has been documented by others as well.[17,22] The forceps tip damage was accentuated when the tissue was inserted through a 3-mm incision with secondary tip injury patterns seen in the adjacent peripheral cornea, multiplying the damage from the tips. In addition, although some striae emanating from the forceps tips across the central cornea were seen with 5-mm insertions, these striae were more prominent and in greater numbers when the tissue was passed with forceps through a 3-mm incision, which again, accentuating the damage to the tissue. Both the Busin glide and the suture pull-through techniques produced a similar pattern of damage. The peripheral cornea had a distinct circular area of damage where either the traction suture was placed or the cross-chamber forceps were used to grasp the tissue. Most distinctive with both of these "pull-through" techniques was the pattern of striae damage that appeared to emanate from the point of traction, often extending through the central region of the endothelial layer. Once again, the striae were more prominent and in greater numbers when the tissue was pulled through a 3-mm incision than through a 5-mm incision. Again, the 3-mm incision appeared to accentuate the pattern of damage to the tissue.

Percentages of Areas of Endothelial Damage

In the control group (group 1), simple trephination of the donor tissue caused a ring of peripheral endothelial damage, which was measured at 8.64%.

Tissue that was inserted with a non-coapting forceps (group 2) created an area of damage of 18.39% with a 5-mm incision, but this same technique of insertion through a 3-mm incision (group 5) caused an area of damage of 30.47%. The advantage of a 5-mm incision was statistically significant ($P = 0.001$).

Tissue that was not folded and then inserted with a Prolene suture "pull-through" technique caused an area of damage of 17.7% when pulled through the 5-mm incision

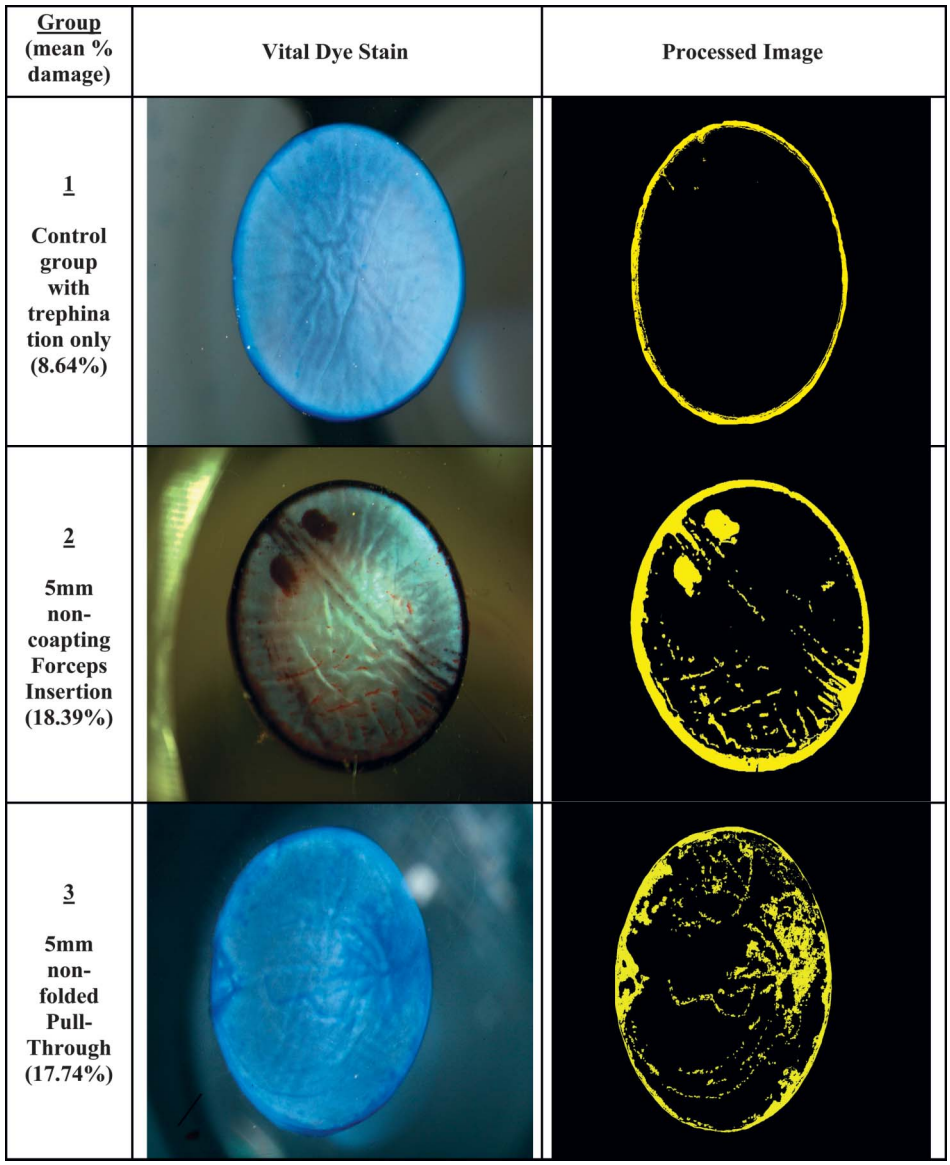

FIGURE 1. Vital dye–stained images next to Adobe Photoshop images for each of the 8 experimental groups. These images are 1 of the 5 donor disks processed for each group and most closely represent the mean value of donor endothelial damaged areas and the pattern of damage seen with each experimental group.

(group 3), but the damage was 55.8% when pulled through a 3-mm incision (group 7). The advantage of a 5-mm incision was statistically significant ($P < 0.001$).

Tissue that was rolled up with a Busin Glide and then pulled through the 5-mm incision with a forceps created an area of damage of 20.26% (group 4), but the damage was 27.94% when pulled through a 3-mm incision (group 8). The advantage of a 5-mm incision did not reach statistical significance ($P = 0.075$).

The average damage of all the 5-mm incision groups was 18.80%, and this was significantly better than the average damage of 36.06% incurred by the 3-mm incision groups ($P < 0.001$).

DISCUSSION

EK has provided patients with faster visual rehabilitation and less irregular astigmatism than penetrating keratoplasty (PK). However, there is growing concern with the increased degree of central endothelial cell loss that is measured with EK in the first year compared with that after PK surgery.[4,5,8,9,26–30] It was evident from our studies of DLEK surgery that placing an unfolded tissue through a 9-mm incision resulted in endothelial damage that was significantly less at 2 years after surgery than the damage incurred by the same DLEK surgery placing folded tissue through a 5-mm incision.[4,31] What was interesting was that the 6-month specular microscopy results were not significantly different between the 5- and 9-mm access incisions, yet the 2-year results were significantly worse for a smaller incision approach than a larger incision approach.[31] As McCarey and colleagues[32] have pointed out, central ECD by specular microscopy may continue to be affected by peripheral trauma for at least 5–8 years after the initial surgery. Similarly, the acute damage that occurs in EK at the time of surgery to peripheral areas of the donor endothelial layer may not be recognized or may not accurately appreciated by central specular microscopy until years after the original

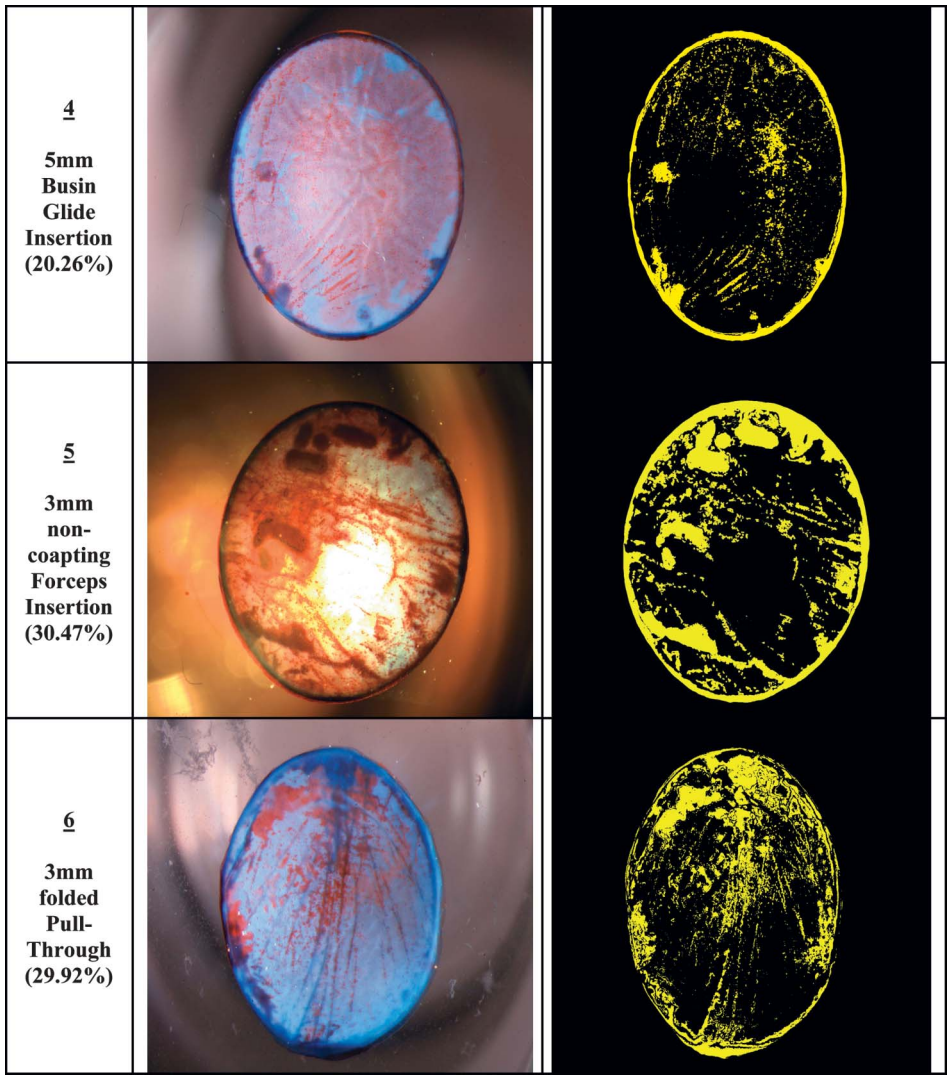

FIGURE 1. continued.

surgery. Therefore, specular microscopy performed in the first few months after any EK procedure will likely underestimate the full degree of endothelial damage.[33] Furthermore, any surgical technique that can minimize the acute trauma to the central and the peripheral donor endothelium and minimize complications that contribute to endothelial cell loss should be prized.[7]

We have felt that the benefits of improved astigmatism and a stronger wound that resulted from a 5-mm incision DLEK outweighed the disadvantage of an additional 16% cell loss when compared with a 9-mm incision DLEK. Therefore, we have continued with this 5-mm scleral access incision approach in all our EK surgery.[5,7,27,31,34] With an insignificant average astigmatic refractive effect of 0.06 diopters from the scleral 5-mm incision in our EK surgery,[4] we have not been motivated to further reduce the size of our access incision and to risk further endothelial damage.

Over the last couple of years, some EK surgeons have moved the access incision from the scleral limbus to a clear cornea location and have reduced the size of the incision to 3 mm. A 3-mm clear corneal access incision allows a surgeon to arguably save time, use topical anesthesia, and avoid a limbal conjunctival peritomy and wound sutures. However, unlike change from a 9-mm incision to a 5-mm incision, there are no data to support any further refractive advantage for the EK patient by making the incision shorter than 5 mm. Although a 3-mm clear corneal incision has refractive advantage over a 5.0-mm clear corneal incision, the literature indicates that there is actually more astigmatism (0.50 diopters)[35,36] induced by a 3.0-mm *corneal* incision than that (0.06 diopters) induced by the 5.0-mm *scleral* incisions documented in our EK series.[4] This higher astigmatism induction by the clear corneal incision than by the scleral incision is likely due to the closer proximity of the corneal incision to the visual axis. Direct comparison of the wound strength of a clear cornea unsutured wound with that of a sutured, beveled, and vascularized scleral wound has not been done, but it is likely to favor the scleral wound. The 5-mm scleral wound of EK has already demonstrated stability against significant postoperative trauma.[37,38] Therefore, despite our desire to make our access incisions ever smaller, in EK surgery using noninjector techniques, we may have reached the point of diminishing returns for the patient at the

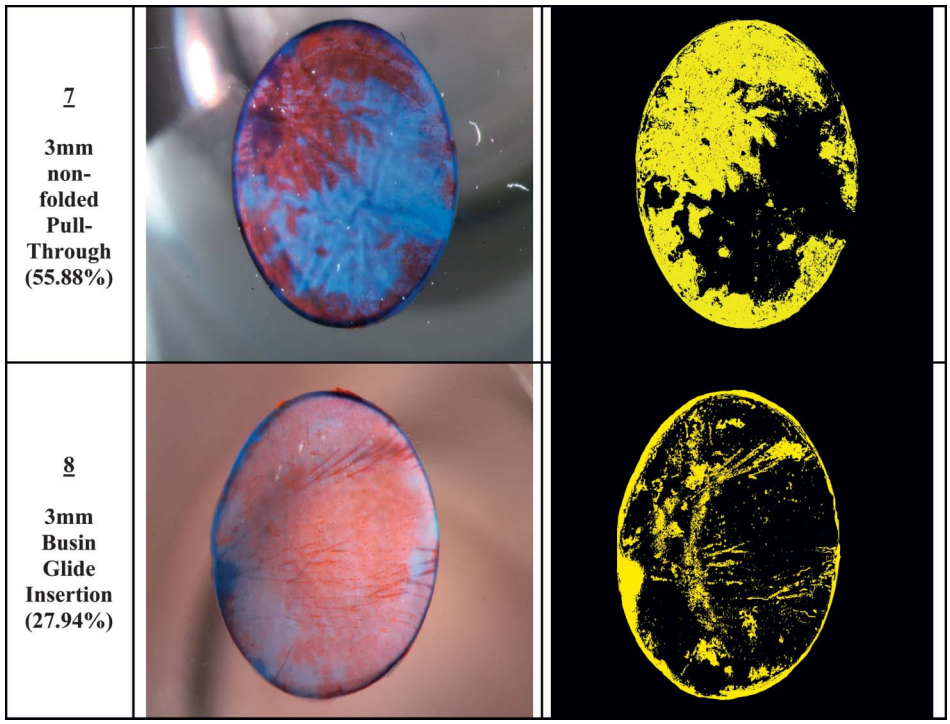

FIGURE 1. continued.

5-mm incision size. For, unlike cataract surgery where only inert plastic is inserted through a 3-mm incision, EK involves the insertion of delicate endothelial cells whose cell death must be justified by gains in either lower astigmatism or a safer wound. In the absence of these patient-centered advantages, minimizing wound compression trauma to donor cells should be a higher priority for the surgeon than surgical speed or convenience.

Primary graft failure in DSAEK surgery is clearly iatrogenic and nearly entirely technique dependent.[7] The central ECD after EK surgery continues to diminish over time,[4,5,8,28,31] and therefore, similar to PK surgery,[29] the goal should be to have as many cells at the completion of surgery as possible to extend the life of the graft. Success in DSAEK surgery should not be measured in the short term by early graft clearance and attachment but by long-term donor endothelial

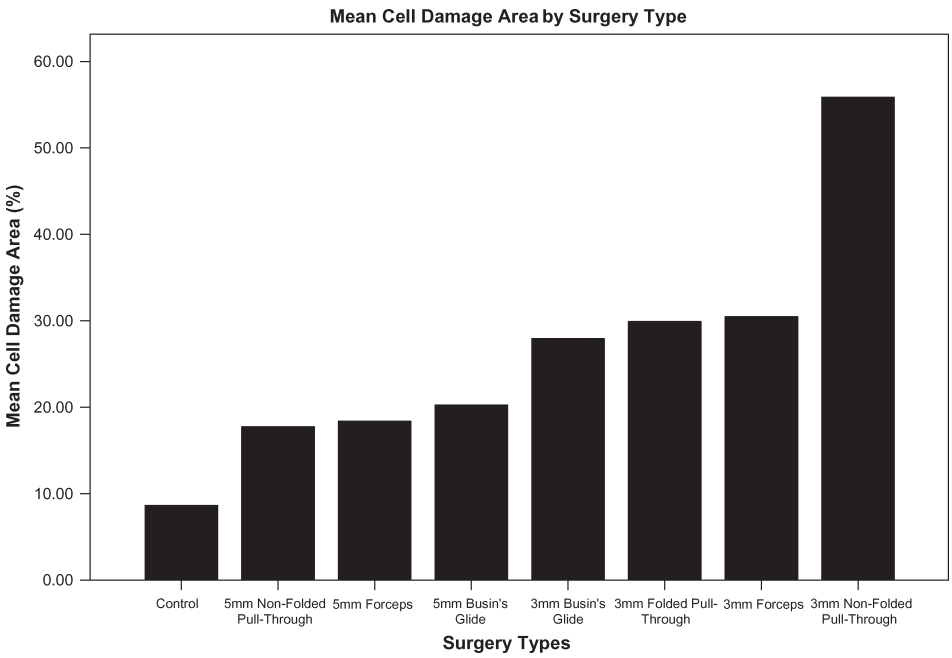

FIGURE 2. Bar graph demonstrating the means of the total area of cell damage in each experimental group from least amount of damage (group 1–control) to most amount of damage (group 7–3-mm incision, non-folded pull through).

TABLE 1. Mean Cell Area Damage by Surgery Type

Surgery Type	Mean % Cell Damage	Range
Control group with trephination only (group 1)	8.64% ± 2.43%	5.93%–12.33%
5-mm Non-coapting forceps insertion (group 2)	18.39% ± 2.55%	14.05%–20.53%
5-mm Nonfolded pull through (group 3)	17.74% ± 2.22%	15.7%–20.93%
5-mm Busin glide insertion (group 4)	20.26% ± 5.17%	16.51%–28.90%
3-mm Non-coapting forceps insertion (group 5)	30.47% ± 2.74%	28.06%–34.42%
3-mm Folded pull through (group 6)	29.92% ± 5.33%	21.94%–34.63%
3-mm Pull through (group 7)	55.88% ±4.05%	51.47%–61.85%
3-mm Busin glide insertion (group 8)	27.94% ± 5.45%	22.42%–35.97%

survival and avoidance of late graft failure. Therefore, any surgical steps regarding wound construction, which the surgeon can take to enhance donor endothelial survival, should be embraced.

As in all laboratory experiments, there are limitations to the clinical interpretation of the information that is obtained. The experiments of this report were designed to isolate the specific surgical maneuver of donor tissue insertion and then evaluate the endothelial damage that resulted from that single maneuver. Obviously, other maneuvers in EK surgery such as tissue unfolding, touch of the endothelium to the iris or lens after unfolding, centering of the tissue, air bubble exposure time, and other potential "real-life" sources of damage were not evaluated. The various clinical techniques for donor insertion may or may not have damage from these other factors, and this would add to the damage they all incur from the wound size–related damage. We also tested a bracket of wound size (3 vs 5 mm) but did not assess sizes intermediate to that bracket (4 mm). We kept the tunnel length of the insertion incision uniform at 2.0 mm for all experiments to further minimize confounding variables, as there has been some clinical evidence from 6-month specular microscopy results that a 5-mm clear corneal incision with a shorter tunnel length may yield less damage than a 5-mm scleral incision with a longer tunnel length.[28] Laboratory vital dye staining work is needed to clarify the acute endothelial damage that results from incisions with varying tunnel length. We restricted our testing to the most common described techniques of EK insertion in the literature, and it was beyond the scope of this experiment to test every form of modification of insertion technique or instrumentation that may be currently in use by individual surgeons. For example, the Busin glide is most commonly used as a means of rolling the tissue for insertion (to avoid folding), and the glide is then placed *adjacent* to the incision and the tissue is pulled through the incision with a cross-chamber forceps, as described in these experiments. If the distal edge of the Busin glide is placed completely into the anterior chamber, it requires an incision much larger than 3 mm, the device changes in function from a glide to a tissue injector, and therefore, this modification was not within the parameters of these experiments that determined the effects of incision size. Such a modification of use of the Busin glide, however, could potentially avoid wound compression of the tissue and enhance endothelial survival. Once again, further vital dye staining on this and other applications and devices needs to be done.

In the experiments of this report, what was most evident was that, regardless of the technique used to insert the tissue, the overriding factor in endothelial damage was the size of the incision. Regardless of the technique of donor insertion, whether it was by forceps, suture pull through, or forceps pull through, there was more endothelial damage to the tissue if a 3-mm incision rather than a 5-mm incision was used. The obvious explanation of these results is that a smaller incision causes more compression of the tissue than a larger incision. Therefore, further innovations of donor tissue insertion should be directed at reducing the direct compression forces of the incision either by increasing the length of the access incision (and therefore decreasing the compressive forces) or by using a method of insertion that eliminates the compression of the tissue entirely, allowing the tissue to be "delivered" into the anterior chamber without any damaging compression from the wound. The calculated dimensions and requirements of such

TABLE 2. *P* Value for Post-Hoc Analysis of Mean Cell Area Damage by Surgery Type

	5-mm Non-Coapting Forceps	5-mm Nonfolded Pull Through	5-mm Busin Glide	3-mm Non-Coapting Forceps	3-mm Folded Pull Through	3-mm Nonfolded Pull Through	3-mm Busin Glide	Control
5-mm Non-coapting forceps	NA	1.0	0.995	0.001	0.001	<0.001	0.012	0.01
5-mm Nonfolded pull through	1.0	NA	0.971	<0.001	0.001	<0.001	0.006	0.02
5-mm Busin glide	0.995	0.971	NA	0.006	0.011	<0.001	0.075	0.001
3-mm Non-coapting forceps	0.001	<0.001	0.006	NA	1.0	<0.001	0.97	<0.001
3-mm Folded pull through	0.001	0.001	0.011	1.0	NA	<0.001	0.993	<0.001
3-mm Nonfolded pull through	<0.001	<0.001	<0.001	0.97	<0.001	NA	<0.001	<0.001
3-mm Busin glide	0.012	0.006	0.075	0.97	0.993	<0.001	NA	0.001
Control	0.01	0.02	0.001	<0.001	<0.001	<0.001	0.001	NA

NA, not available
All results are compared using Tukey HSD post-hoc analysis.

TABLE 3. *P* Value for Post-Hoc Analysis of Mean Cell Area Damage by Incision Size

Surgery Type vs	Surgery Type	*P*
5-mm Incision size vs (mean area cell damage: 18.80%)	3-mm Incision size	<0.001
	Control	0.098
3-mm Incision size vs (mean area cell damage: 36.06%)	Control	<0.001

All results are compared using Tukey HSD post-hoc analysis.

a tissue delivery system have already been worked out in previous publications,[39,40] and initial laboratory work with cartridge tissue injectors looks promising.[17] However, until such devices are thoroughly tested and commercially available, the EK surgeon using forceps or pull-through techniques is advised to avoid using incisions smaller than 5 mm for donor tissue insertion.

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