

Technique for Preparing Ultrathin and Nanothin Descemet Stripping Automated Endothelial Keratoplasty Tissue

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Purpose: To describe and report outcomes of our single-pass microkeratome technique for preparation of ultrathin (UT, $\leq 100 \mu\text{m}$) and nanothin (NT, $\leq 50 \mu\text{m}$) Descemet stripping automated endothelial keratoplasty (DSAEK) grafts.

Methods: To prepare NT-DSAEK grafts, a pachymetry nomogram specific to each technician and individual microkeratome head was developed based on accumulated precut and postcut pachymetry data from previous DSAEK grafts. Mean graft thickness as well as precut and postcut endothelial cell counts (ECCs) of NT-DSAEK, UT-DSAEK, and Descemet membrane endothelial keratoplasty (DMEK) grafts between July 2015 and July 2017 were calculated and compared statistically. Endothelial cell loss was evaluated using calcein AM stains and ImageJ analysis. Postcut graft thickness and rates of perforation/tissue loss for NT-DSAEK grafts between May and July 2017 were calculated to determine overall graft preparation success rates.

Results: Mean postcut graft thickness for all grafts within the NT range was $41.0 \pm 6.4 \mu\text{m}$ (range 26–50 μm). Mean ECC did not differ between NT-DSAEK, UT-DSAEK, and DMEK grafts ($P = 0.759$ and 0.633 , respectively). The overall tissue loss rate from attempted NT-DSAEK was 4.8%. Excluding cases of perforation, the chance of achieving NT thickness was 60% and within the traditional UT range was 100%.

Conclusions: We propose the term “NT-DSAEK” for grafts $\leq 50 \mu\text{m}$. The described nomogram allows for standardized creation of NT grafts with a low tissue loss rate. This technique is safe and does not result in significant ECC loss compared with UT-DSAEK and DMEK grafts. Further studies are necessary to corroborate the postsurgical results of NT grafts.

Key Words: Descemet stripping automated endothelial keratoplasty (DSAEK), microkeratome, nanothin DSAEK (NT-DSAEK), ultrathin DSAEK (UT-DSAEK)

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Endothelial keratoplasty procedures, such as Descemet membrane endothelial keratoplasty (DMEK) and Descemet stripping automated endothelial keratoplasty (DSAEK), have become the preferred treatment for endothelial dysfunction and disease as they allow selective replacement of the diseased endothelium.¹ Although DMEK has grown in popularity because it provides true anatomic replacement of recipient diseased Descemet membrane and endothelium,² surgical difficulty and unpredictability associated with the procedure have limited its widespread adoption. As a result, DSAEK remains the most popular endothelial keratoplasty technique in the United States.²

However, overall visual acuity and rejection rates for DSAEK have been shown to be inferior when compared with DMEK. This is often attributed to the fact that DSAEK transplants retain a variable degree of stroma in addition to Descemet membrane and endothelium compared with DMEK. Evidence suggests that minimizing the amount of residual stroma on a DSAEK graft and using thinner DSAEK grafts can significantly improve visual outcomes, making the procedure more comparable to DMEK. We previously demonstrated that DSAEK grafts $\leq 131 \mu\text{m}$ demonstrated better postoperative best-corrected visual acuity compared with the thicker $>131\text{-}\mu\text{m}$ DSAEK group.³

Because of this, many DSAEK surgeons have become increasingly interested in using thinner tissue. Over time, as surgeons have grown more comfortable with handling thinner DSAEK grafts and insertion techniques have become more advanced, increasing demand has been placed on eye banks to provide these thinner grafts. Target graft thicknesses have decreased from 70 to 100 μm range to even thinner target thicknesses ($<70 \mu\text{m}$) based on surgeon preference. This growing demand for ultrathin DSAEK (UT-DSAEK) requires that eye banks have a reliable processing technique to cut these extremely thin grafts.

Multiple studies have demonstrated that reliably achieving thinner DSAEK grafts is possible with the right technique. However, to date, no specific techniques have been published that describe how to cut DSAEK tissue that is $\leq 50 \mu\text{m}$. Busin et al published their double-pass microkeratome technique to

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achieve UT-DSAEK grafts ($\leq 131 \mu\text{m}$) routinely, even by relatively inexperienced eye bank technicians.⁴ Other techniques (both single and double pass) have since been published with target graft thickness ~ 70 to $130 \mu\text{m}$.^{5–14}

Most DSAEK grafts processed in US eye banks are cut to a targeted depth using a microkeratome. The Moria microkeratome applanates and cuts as it pivots over a cornea mounted on an artificial anterior chamber (AAC). The depth of the cut is largely determined by the various microkeratome heads available, which vary the height of the blade (in millimeters) from the applanated corneal surface. Because of operator variability and variations of tissue biomechanics in deeper corneal stroma, the specific height of the blade in each microkeratome head does not accurately predict the depth of cut. As a result, cutting extremely thin DSAEK grafts can be challenging because of the risk of perforation from cutting too deep. The Minnesota Lions Eye Bank has refined a single-pass technique for cutting extremely thin tissue by creating an operator-specific nomogram to more accurately predict the cut depth of each of the various Moria microkeratome heads. With this technique and associated nomogram, even thicknesses $\leq 50 \mu\text{m}$ have been attained. We are proposing the phrase “nanothin DSAEK” (NT-DSAEK) as a new description of DSAEK grafts that achieve $\leq 50 \mu\text{m}$. In this study, we describe the Minnesota Lions Eye Bank technique for NT-DSAEK graft preparation and associated graft preparation outcomes.

METHODS

Pachymetry Nomogram

A nomogram based on the single-pass rotational style microkeratome (Moria Inc, Antony, France) used at the Minnesota Lions Eye Bank was developed to help achieve NT-DSAEK grafts with targeted thickness $\leq 50 \mu\text{m}$. Using pachymetric data from DSAEK cuts performed from July 2015 to July 2017, an individualized pachymetry nomogram specific to each technician and each microkeratome head was developed over the study period. Each operator began with cut depth data from 10 previous DSAEK procedures. Additional data points were then added continuously over time with refinements to the cut depth predictions made after each additional new cut.

NT-DSAEK Tissue Selection

In all cases in which an NT-DSAEK was attempted with target thickness ≤ 50 , appropriate tissue selection was first performed. When performing NT-DSAEK, it is preferable to start with a cornea that is $\leq 550 \mu\text{m}$ thick centrally. Deep microkeratome cuts produce more variability in cut depth than shallow cuts because of the elasticity of the corneal stroma. Consequently, thinner corneas, which require less removal of tissue, are preferable when trying to hit a narrow target ($\leq 50 \mu\text{m}$), with little margin for error. Variations in stromal thickness, either because of laser in situ keratomileusis or anterior scarring within the cutting area, can also create unpredictable and irregular cuts when preparing NT-DSAEK tissue. Therefore, a suitable NT donor cornea should have an even stromal layer thickness and no history of laser in situ keratomileusis.

Tissue Preparation

For tissue preparation, precut and postcut pachymetry data were obtained and were used to determine the depth of cut. All precut pachymetry data were obtained intraoperatively, immediately before the cut using ultrasound pachymetry. In all cases, donor tissue was first centered on a Moria AAC and mounted with storage medium backed by a constant 100 mm Hg of hydrostatic pressure from a balanced salt solution bottle hung at a fixed height above the work area. The loose epithelium was removed with eye spears wetted with balanced salt solution in cases in which the epithelium was irregular. In cases of slightly thicker corneas in which target graft thickness was unlikely to be achievable based on the predicted cut depth of even the deepest blade, the epithelium was completely removed to thin the cornea. Precut ultrasound pachymetry was then obtained immediately before the cut. Based on the pachymetry nomogram, the authors aimed to be as close as possible to $50 \mu\text{m}$.

During the cut, the tubing leading into the AAC was pinched and clamped with a hemostat to hold the pressure constant during the blade pass. A single pass of the microkeratome blade was performed. Four sterile ink marks were added at cardinal points of the graft bed edge to help the surgeon center their trephine in the operating room. The anterior stromal cap was then replaced on the graft bed, and the cornea was placed in fresh storage media in a viewing chamber.

Final (after microkeratome) graft thickness was then obtained 30 minutes after processing by optical coherence tomography (OCT) (Fig. 1E) or specular microscopy. Both these methods for measuring postgraft thickness correlate strongly with each other.¹⁵ The depth of the cut was then calculated for each tissue processed by calculating the difference between the precut and postcut pachymetry measurements. Mean cut depths were then calculated by averaging the results of all accumulated DSAEK cuts. This was then used as a predictive guide for the next expected depth of cut for a given microkeratome head in the hands of a given operator.

Table 1 illustrates a sample pachymetry nomogram for a single operator using a single set of reusable microkeratome heads. The central pachymetry measurement of a cornea mounted on an AAC determines the expected final graft thickness. Small shortfalls between the predicted cut depth and the desired target were addressed by taking advantage of techniques to thin the precut corneal thickness with epithelial removal or increased drying time or by taking advantage of additional cut variables such as cut speed and hand pressure. Longer pass times and firmer hand pressure result in deeper than average cuts, creating thinner than average grafts for the given operator. A lighter hand pressure and faster pass will have the opposite effect. Greater time on the AAC allows for tissue to thin by dehydration, which helps if the cornea is too thick for the nearest microkeratome head to achieve the target. Conversely, leaving the epithelium intact increases intraoperative thickness 15 to $30 \mu\text{m}$ relative to when it is removed. It is important to observe the epithelium just before the microkeratome cut because exposure can give focal irregular areas. In addition, younger donors have more pliable stroma and therefore tend to become thinner with the same head than older donors. Using

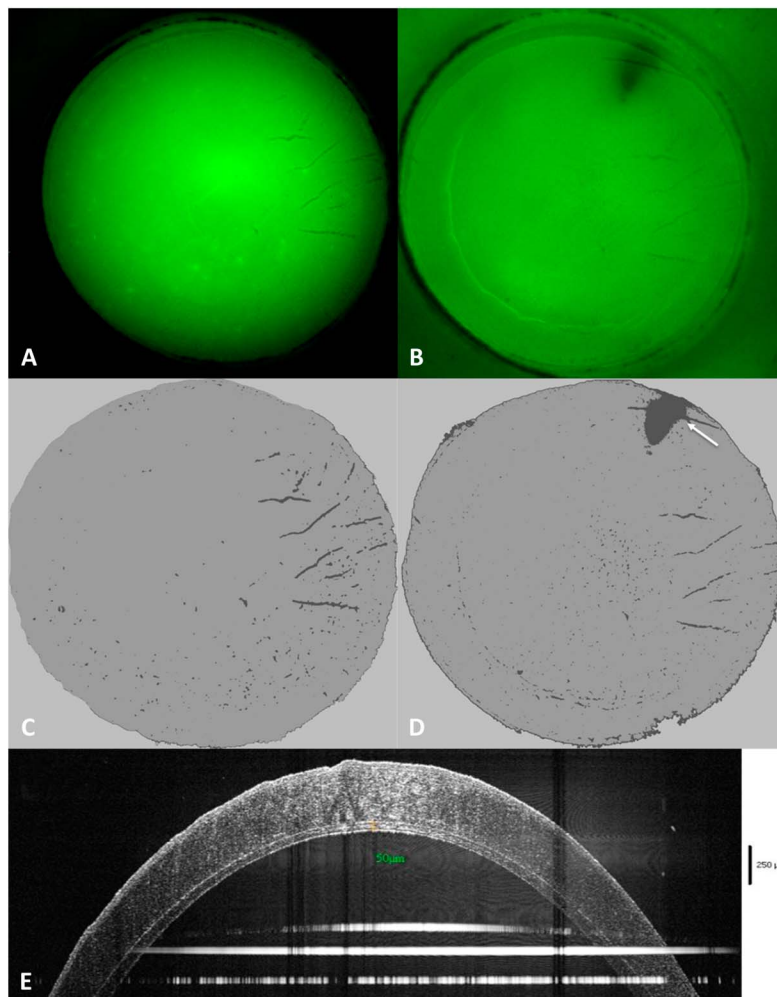


FIGURE 1. Corneal endothelium is stained with calcein AM to highlight representative cell loss before and after NT-DSAEK processing (A, B). Images are analyzed using ImageJ Trainable Weka Segmentation plugin to quantify total ECL, represented by dark areas after image segmentation (C, D). A, Demonstrates the cornea before processing with some peripheral stress lines, whereas B shows the same cornea after an NT-DSAEK cut with additional central cell loss and peripheral cell loss associated with gentian violet ink placement (ie, S-stamp). The preprocessing baseline of 2% ECL (C) increases to 5% (D) after the microkeratome cut with a focal area of ECL over the area of gentian violet ink placement (white arrow). E, Anterior segment OCT image of the NT graft.

these variables alone or in combination greatly assists in closing any gaps between target cut depths and mean head cut depths.

Because of individual operator variables (ie, operator setup time allowing time for graft dehydration, hand size, and physical strength), it is difficult to make a standardized, generic nomogram that applies to all operators. An individualized nomogram based on many previous cuts accounts for individual subtleties and allows for accurate selection of microkeratome heads for each operator. The challenging corneas to cut in the NT range are typically thicker corneas (as thinner corneas typically fall into the nomogram). To compensate in these cases, we adjust additional variables after using the individualized nomogram. The graft is allowed to dehydrate longer on the AAC. This is checked every 1 minute (up to 5 minutes) to decrease the graft thickness by an additional total of 20 to 30 μm (for the full 5 minutes) or to the target thickness goal if achieved earlier than 5 minutes.

With experienced operators, each operator develops a consistent, standard cut (with their own standard speed and weight/firmness) that they achieve 95% to 99% of the time. Each operator also develops a consistent deeper cut (slower speed and increased weight/firmness) to obtain a ~ 20 to 30 μm deeper cut. With 2 consistent cuts, each operator is

able to produce a meaningful nomogram even when deeper cuts are necessary. Combining these additional variables has allowed us to target the NT range more consistently.

Evaluating the Safety of NT-DSAEK

To assess what impact cutting NT-DSAEK has on corneal endothelium, tissue data for all DSAEK and DMEK grafts distributed by the eye bank from July 2015 to July 2017 were reviewed. DSAEK grafts were separated into 3 groups based on graft thickness. Grafts with thicknesses ≤ 50 μm were included in the NT-DSAEK group. Grafts with thicknesses ranging from 51 to 70 μm were included in the Other UT-DSAEK group, and grafts with thickness 71 to 100 μm were included in the Other UT-DSAEK group. Mean precut and postcut endothelial cell counts (ECCs) were then calculated for each of the DSAEK groups and for all DMEK grafts distributed by the eye bank over the course of the study period. For statistical analysis, the normality of data was tested with D'Agostino–Pearson normality tests. Based on the normality of data, analysis of variance or Kruskal–Wallis was used to determine whether there was any statistically significant difference among groups. If there was significance, the *t* test was

TABLE 1. Sample Nomogram for Ultrasound Pachymetry-Single Set, Single Operator

Microkeratome head (Mean cut depth, μm)	250 (310)	300 (375)	350 (464)
Intraoperative Pachymetry Values, μm		Predicted Residual Bed Thickness, μm	
400	90	25	-64
425	115	50	-39
450	140	75	-14
475	165	100	11
500	190	125	36
525	215	150	61
550	240	175	86

used to compare the groups pairwise. $P < 0.05$ was considered statistically significant. Endothelial cell loss (ECL) was also evaluated using calcein AM stains, and images were analyzed using ImageJ (National Institutes of Health; <http://rsbweb.nih.gov/ij/>) Trainable Weka Segmentation plugin.¹⁶

Tissue Loss Rate

To calculate the efficacy of the Minnesota Lions Eye Bank's nomogram for cutting NT-DSAEK, all outcomes from attempted NT-DSAEK ($\leq 50 \mu\text{m}$) preparations for planned surgical distribution from May 2017 to July 2017 were identified and reviewed. In all cases, careful selection of appropriate donor corneas was performed as described above. Final graft thickness from all cuts was evaluated. Outcomes, including endothelial perforation or unacceptable endothelial damage after processing, were also documented. Tissue loss rates were then calculated and compared with tissue loss rates for DMEK and DSAEK.

RESULTS

Graft Preparation Outcomes

Over the past 2 years, the Minnesota Lions Eye Bank has distributed a total of 39 DSAEK grafts for surgical transplantation that, intentionally or unintentionally, had a final thickness of $\leq 50 \mu\text{m}$ after being prepared with a single-pass microkeratome technique. Mean final graft thickness for these NT grafts was $41.0 \pm 6.4 \mu\text{m}$ (range

26–50 μm). Table 2 demonstrates the postprocessing ECC outcomes for these NT grafts compared with other DSAEK and DMEK grafts that were distributed by the eye bank over that same period. For NT-DSAEK tissue, mean ECC was 2726 before cutting and 2814 after cutting. This was similar to preprocessing and postprocessing ECCs for UT-DSAEK (both at the 51–70 μm thickness and at the 71–100 μm thickness) and DMEK grafts. Mean ECC (before and after) did not differ in NT-DSAEK, UT-DSAEK, and DMEK grafts ($P = 0.759$ and 0.633, respectively). Pairwise comparisons between each DSAEK group and the DMEK group are shown in Table 2.

From May 2017 to July 2017, there were 21 intentional attempts to cut NT-DSAEK grafts for transplantation (based on surgeon request). The graft processing outcomes are displayed in Table 3. The overall tissue loss rate was 4.8% (1/21). In comparison, the overall DSAEK (all types, $n = 610$) and DMEK ($n = 236$) tissue loss rates were 3.6% and 2.9%, respectively, over the past 12 months. All nonperforated grafts had acceptable endothelium for transplantation. Of the nonperforated corneas, there was a 60% (12/20) success rate for obtaining NT tissue (graft thickness $\leq 50 \mu\text{m}$). In terms of final graft thickness for the processed tissue cuts, 85% (17/20) achieved ≤ 51 to 70 μm thickness and 100% (20/20) achieved ≤ 71 to 100 μm thickness. All nonperforated grafts were released for transplantation, and no tissues were wasted.

NT tissue was also stained with calcein AM to highlight representative cell loss before and after DSAEK processing (Figs. 1A, B). Calcein AM is not visible in dead cells but fluoresces green when cleaved by active cellular metabolism. The images were also analyzed using ImageJ Trainable Weka Segmentation plugin to quantify total ECL (Figs. 1C, D).

DISCUSSION

Because our group first presented evidence correlating thinner DSAEK grafts with better visual acuity,³ subsequent studies from other groups have found conflicting results regarding the relationship between DSAEK graft thickness and visual acuity.^{17–21} These studies were mainly retrospective, and the subgroup cutoff values of compared graft thicknesses vary between studies. Dickman et al²² presented the first multicenter, prospective, double-masked, randomized, controlled clinical trial that demonstrated that UT-DSAEK (mean 101 μm) results in faster and better recovery of best-corrected visual acuity with similar refractive

TABLE 2. Minnesota Lions Eye Bank Graft Endothelial Cell Count Comparison by Graft Thickness: July 2015 to June 2017

	NT-DSAEK ($\leq 50 \mu\text{m}$)	UT-DSAEK (51–70 μm)	Other UT-DSAEK (71–100 μm)	DMEK
Grafts (n)	39	119	257	453
Mean thickness, μm	41.0 ± 6.4	61.0 ± 5.7	88.8 ± 8.7	NA
Mean ECC before microkeratome	2726 ± 296.5	2772 ± 326.9	2742 ± 279.4	2728 ± 268.3
Mean ECC after microkeratome	2814 ± 333.7	2772 ± 298.3	2789 ± 291.7	2800 ± 272.5
P^*	0.991	0.777	0.961	NA

ECC, endothelial cell counts; DMEK, Descemet membrane endothelial keratoplasty; DSAEK, Descemet stripping automated endothelial keratoplasty; NA, not applicable; NT-DSAEK, nanothin DSAEK; UT-DSAEK, ultrathin DSAEK.

* P value of pairwise comparison of mean ECC after microkeratome to DMEK.

TABLE 3. Minnesota Lions Eye Bank Nanothin DSAEK Graft Processing Outcomes: May 2017 to July 2017

	NT-DSAEK ($\leq 50 \mu\text{m}$)
NT requests	16
No. tissue loss	1
Loss rate/request	6.3% (1/16)
No. tissue cuts to fulfill requests	21
Total no. tissue loss	1
Loss rate/cut	4.8% (1/21)
No. tissue in NT range	12
NT ($\leq 50 \mu\text{m}$) success rate/processed cuts	60.0% (12/20)
UT (51–70 μm) success rate	85.0% (17/20)
UT (70–100 μm) success rate	100.0% (20/20)

DSAEK, Descemet stripping automated endothelial keratoplasty; NT, nanothin; NT-DSAEK, nanothin Descemet stripping automated endothelial keratoplasty; UT, ultrathin.

outcomes, ECL, and incidence of complications compared with DSAEK (mean 209 μm).

Quality of vision may also be improved with thinner DSAEK grafts. Graft thickness correlates with graft asymmetry, which in turn may be associated with higher-order aberrations.²³ Besides graft thickness, factors such as stromal scarring, interface opacity, graft shape, posterior curvature, and total corneal thickness may play a role in poor visual outcomes in DSAEK. Although it may improve with time, irregularity or stromal scarring at the anterior graft surface may also limit visual function.²⁴ Thus, thinner grafts with minimal stromal substance could enhance graft symmetry, regularity of shape, and quality of vision. Microkeratome-assisted DSAEK graft preparation has led to increased reproducibility and improved quality/smoothness of the stromal surface.²⁵

With these potential benefits of thinner DSAEK grafts, we have requested donor tissue cut at least $<70 \mu\text{m}$ for the last couple of years and ideally $\leq 50 \mu\text{m}$ (NT-DSAEK range) over the past 3 months. For the past few years, the Minnesota Lions Eye Bank has refined their single-pass technique for cutting extremely thin tissue as described here. This is the first study looking at preparation of tissue with target graft thickness $\leq 50 \mu\text{m}$; our final graft thickness measurements were taken 30 minutes postcut.

Since Busin et al published their double-pass microkeratome technique, other techniques (both single- and double-pass) have since been described to target graft thickness <70 to 130 μm .^{5–14} Of these, only the single-pass technique described by Nahum et al demonstrated extremely thin graft thickness (average 63 μm , range 23–177 μm) 3 months postoperatively; however, they did not report immediate postcut graft thicknesses.⁹ Romano and colleagues achieved graft thickness in the

NT range for 1 eye postcut. In this study, 3 eyes thinned to the NT range 3 months after surgery.¹⁴

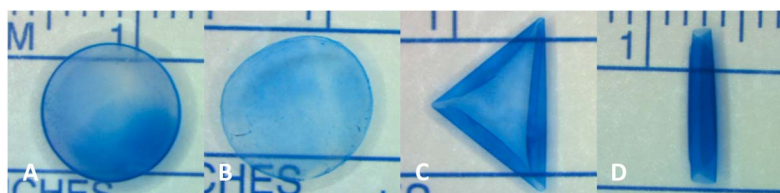
The advantages of UT- and NT-DSAEK include the similar surgical technique for graft insertion and positioning as traditional DSAEK. This translates to more predictable operating room (OR) time. Figure 2 demonstrates the scrolling of the different endothelial keratoplasty grafts. DSAEK detachment rates are potentially lower than those of DMEK.^{20,22,26} Although the postoperative ECL and visual acuity results need to be further studied with longer follow-up, NT-DSAEK may be a viable option that is comparable to DMEK.

Other UT techniques have used air drying, continuous drying with polyvinyl alcohol sponges, controlling AAC pressure, and using a THIN-C medium to reduce donor corneal thickness before a single microkeratome pass.^{8,14,27,28} Alternatively, the double-pass microkeratome technique has been used to achieve UT tissue with a second microkeratome pass after a standard single-pass microkeratome cut.^{4,5} Each of these techniques uses a tailored nomogram.

A single-pass may be safer and less detrimental compared with a double-pass technique. Busin et al²⁰ reported that all microkeratome-related complications (7.2%), such as buttonholing and perforation, occurred during the second pass. Waite et al⁵ demonstrated an increase in endothelial cell damage with the double-pass technique. Furthermore, Suh et al²⁹ noted that the double-pass technique involved a longer duration of raised intraocular pressure and the risk of obtaining a smaller diameter cut after the second pass.

A limitation of microkeratome dissection can be its poor accuracy in consistently determining the final thickness of the DSAEK graft. However, using our nomogram-guided single-pass technique has allowed us to target thinner graft thicknesses in a consistent manner. This often requires manipulating multiple variables based on the final graft thickness predicted by the nomogram. The presented nomogram can be even further refined by including more variables (ie, hand pressure, speed, etc). With larger numbers, NT-DSAEK graft loss rates can be more accurately compared with UT-DSAEK and DMEK loss rates. For reference, the SCUBA technique used for DMEK graft preparation yielded successful rates of graft peeling approaching 95% or greater.^{30,31} Our own annual DSAEK (all types) and DMEK loss rates were 3.6% and 2.9%, respectively, for the past year. Microkeratome dissection may also give variability to the peripheral thickness, with the disparity most noticeable at the entrance and exit of the microkeratome blade. However, this peripheral part of the graft is not typically included for the final graft after the surgeon trephines the lenticule. If the central 6-mm disparity is minimal, we will still accept the

FIGURE 2. Comparison of tissue scrolling for grafts in balanced salt solution: DSAEK (A, 147 μm), NT-DSAEK (B, 29 μm), pre-Descemet endothelial keratoplasty graft from air dissection (C, 37 μm), and DMEK (D, ~ 10 –15 μm). Note that with NT-DSAEK (B), the tissue does not scroll compared to the similarly thick pre-Descemet endothelial keratoplasty (C) because of the thicker periphery in NT-DSAEK.



grafts. A clinical correlation with further studies will determine whether this is visually significant.

A disadvantage of our described technique is the learning curve. Formulating a personalized nomogram for each technician requires multiple previous cuts and careful analysis of the data. Each nomogram is continually changing as additional cuts are added for analysis. In reality, one may encounter scenarios when tissue supply is short, leading to the necessary use of tissue that is less than ideal. Our failure rate for cutting grafts in the NT-thin range may be an underestimation of what is actually possible in eye banks, where tissue selection may be limited and suboptimal tissue may need to be used. But with ideal tissue and training, this failure rate may be even lower. Of note, this nomogram was originally optimized for tissue in the UT-thickness range. As we continue to cut additional tissue in the NT-thin range, the nomogram is likely to refine itself further for these extremely thin cuts.

In conclusion, the described customized, single-pass microkeratome nomogram allows for standardized creation of NT grafts. Although these cuts are deep (final graft thickness as thin as 26 μm), this process seems to be safe for endothelial cells. The tissue loss rate was very low, measuring 4.8%, whereas the chance of achieving target thickness was 60%. All the tissue cuts that were not within the NT range were still within the traditional UT range, and in this study, no tissue was wasted. Given this, NT-DSAEK does seem to be viable for most eye banks with an adequate number of surgeons willing to accept UT-DSAEK grafts in cases in which graft thickness falls outside the NT range. Further studies are necessary to corroborate the postsurgical results of grafts in the NT range.

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