# Nomogram to Predict Graft Thickness in Descemet Stripping Automated Endothelial Keratoplasty: An Eye Bank Study

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**Purpose:** The purpose of this study was to develop a nomogram to predict postcut thickness of corneal grafts prepared at an eye bank for Descemet stripping automated endothelial keratoplasty (DSAEK).

**Methods:** Retrospective chart review was performed of DSAEK graft preparations by 3 experienced technicians from April 2012 to May 2017 at the Eye Bank of Canada—Ontario Division. Variables collected included the following: donor demographics, death-to-preservation time, death-to-processing time, precut tissue thickness, postcut tissue thickness, microkeratome head size, endothelial cell count, cut technician, and rate of perforation. Linear regression models were generated for each microkeratome head size (300 and 350 μm).

**Results:** A total of 780 grafts were processed during the study period. Twelve preparation attempts resulted in perforation (1.5%) and were excluded. Mean precut tissue thickness was 510 ± 49 μm (range: 363–670 μm). Mean postcut tissue thickness was 114 ± 22 μm (range: 57–193 μm). Seventy-nine percent (608/768) of grafts were ≤130 μm. The linear regression models included precut thickness and donor age, which were able to predict the thickness to within 25 μm 80% of the time.

**Conclusions:** We report a nomogram to predict thickness of DSAEK corneal grafts prepared in an eye bank setting, which was accurate to within 25 μm 80% of the time. Other eye banks could consider performing similar analyses.

**Key Words:** DSAEK, graft thickness, nomogram, eye bank, ultrathin, DSEK, endothelial keratoplasty

(*Cornea* 2018;0:1–4)

Received for publication October 1, 2017; revision received December 12, 2017; accepted December 14, 2017.

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Supported by the Odd Fellows and Rebekahs of Ontario of the Grand Lodge of Ontario, IOOF, and the Rebekah Assembly of Ontario IOOF.

The authors have no conflicts of interest to disclose.

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Descemet stripping automated endothelial keratoplasty (DSAEK) is a partial thickness corneal transplant procedure, first described by Melles in 1999,[1] that is used to treat numerous conditions causing endothelial dysfunction including Fuchs endothelial dystrophy and bullous keratopathy.

The thickness of the donor graft is believed to affect outcomes,[2] with increasing preference for thinner donor grafts. A recent multicenter randomized controlled trial showed that ultrathin DSAEK, using donor grafts thinner than 100 μm, resulted in better visual outcomes and quicker recovery, with similar rates of complications and endothelial cell loss compared to those of conventional DSAEK.[3] However, preparing these ultrathin tissues can be challenging, with one study reporting that donor tissue loss was over 20% when preparing ultrathin DSAEK grafts using a double-pass technique.[4]

Predicting postcut graft thickness using a nomogram based on preparation variables can help achieve ultrathin grafts. One study created a nomogram incorporating corneal thickness, microkeratome head size, and advancement speed, and reported a 97% success rate of achieving a donor graft thickness between 70 and 120 μm.[5] However, the sample size was limited, ranging from 3 to 18 eyes in each subgroup of the nomogram. Another study created a nomogram based on graft thickness, whether or not epithelial debridement was performed, and the microkeratome head size.[6] They reported a 97% success rate achieving a graft thickness of <130 μm, and a 90% success rate achieving <100 μm. The sample size of that study was limited as well, ranging from 5 to 19 eyes in each subgroup. Two previous studies have reported nomograms based on larger sample sizes at eye banks.[7,8] However, the statistical analysis performed to reach these nomograms is unclear.

No previous study has used linear regression modeling to produce a predictive nomogram, to our knowledge. Our eye bank database has the advantage of larger sample sizes compared with a single surgeon's practice. Preparing donor grafts in an eye bank has several advantages, including decreasing stress for the surgeon during the operation, and decreasing cost associated with using donor grafts.[9]

The purpose of this study was to develop a nomogram to predict donor tissue thickness based on tissue preparation variables and donor characteristics.

## METHODS

A retrospective chart review of consecutive corneal graft preparations for DSAEK from April 2012 to May 2017 at the Eye Bank of Canada—Ontario Division was performed. Variables collected included the following: donor demographics, death-to-preservation time, death-to-processing time, precut tissue thickness, postcut tissue thickness, microkeratome head size, endothelial cell count, cut technician, history of laser in situ keratomileusis, and rate of perforation. This study adhered to the tenets of the Declaration of Helsinki.

To predict postcut tissue thickness, a linear regression model was generated for each microkeratome head size (300 and 350 μm). To do this, the data set was separated according to the size of the microkeratome head. For each group, 80% of the data was randomly selected to create the linear regression model based on 2 predictor variables: donor age and precut tissue thickness. The remaining 20% was used to test the accuracy of the model. The formula of the regression model is of the form *postcut thickness* = $A + B \times$ *(precut thickness)* + $C \times$ *(donor age)* for some constant numbers $A$, $B$, and $C$.

The regression model was then converted into a nomogram, a 2-dimensional image consisting of 3 parallel scales, one for each of our 3 variables. Knowing the values of our 2 predictor variables enables calculation of the postcut thickness by drawing a straight line, or *isopleth*, between the known values on these 2 scales and seeing where the line intersects the third scale (Fig. 1).

### Statistical Analysis

Statistical tests were performed using the programming language R version 3.4.1 (https://www.r-project.org/). Several $t$ tests were run before creating the linear model to test the validity of our decision to exclude or include certain variables in the final model. Pairwise $t$ tests revealed no statistically significant difference in postcut thickness among the 3 technicians ($P > 0.05$; Bonferroni correction). Which technician performed the operation was therefore assumed to be independent from postcut graft thickness. Additionally, we found no significant difference in the postcut thickness among donors with laser in situ keratomileusis corrective surgery and those without ($P > 0.05$). These variables were excluded from the linear models we created.

### Tissue Preparation

Corneal tissue for DSAEK was prepared by 3 experienced technicians using the Moria Evo 3 microkeratome (Moria SA, Antony, France) with 300- or 350-μm head sizes. Given that our eye bank services 30 corneal surgeons and grafts are not prepared with a specific surgeon in mind, the target thickness range was 70 to 160 μm. The tubing height was kept constant at 55 inches. The distance of tubing clamped was individualized to each technician based on personal experience to achieve consistent thicknesses. Endothelial cell density (ECD) was measured with specular microscopy. Sterile surgical tubing assembly was used to

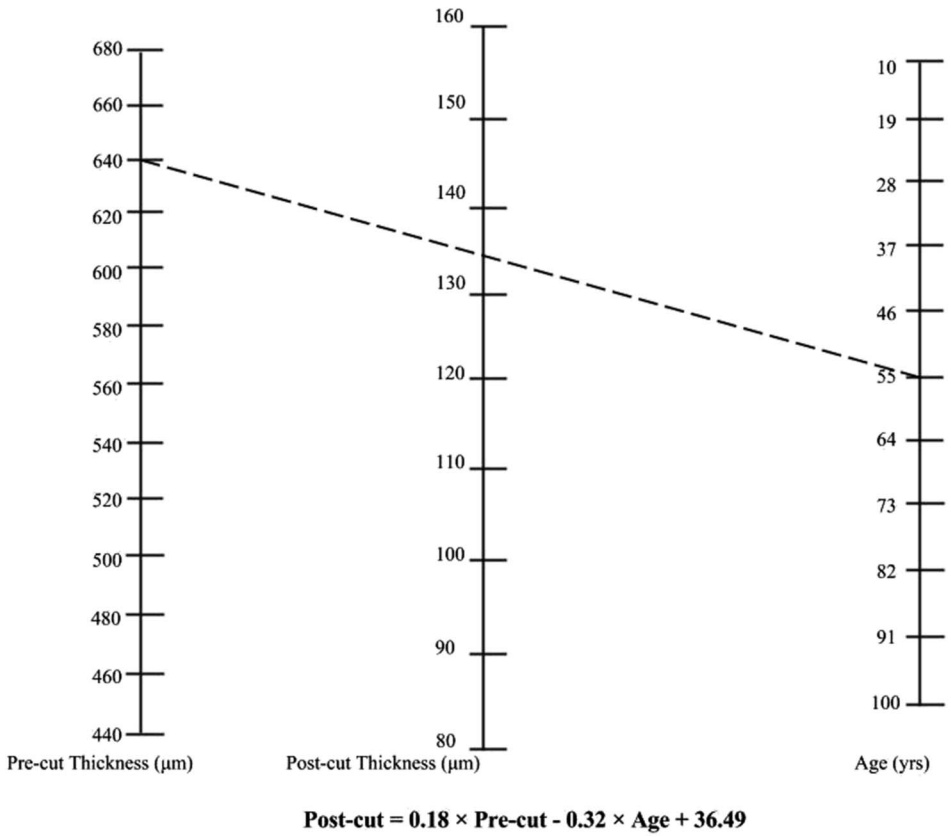


**FIGURE 1.** Nomogram to predict postcut thickness of DSAEK grafts using the 350-μm microkeratome head. For example, a 640-μm cornea from a 55-year-old donor cut with a 350-μm head is estimated to produce a 135-μm graft (dotted line).

connect the artificial anterior chamber supplied with balanced salt solution. The donor corneoscleral rim was carefully centered and locked on the chamber. The corneal epithelium was removed with a sponge, and balanced salt solution was used to lubricate the corneal surface. Central corneal thickness was measured with a handheld pachymeter (PalmScan AP2000; Micro Med Inc, Calabasas, CA), and the average of 3 readings was taken. A 300- or 350-μm microkeratome head was selected based on the precut tissue thickness. Typically, the 300 μm head was used if precut tissue thickness measured <450 μm. The head was attached to the handpiece and mounted on the base of the chamber. Using a foot pedal to activate the microkeratome, the blade was carefully advanced by the technician in a smooth constant motion to cut tissue in approximately 4 seconds. Handheld pachymetry was repeated to measure the postcut graft thickness. The anterior cornea cap was replaced onto the cornea and centered, and spear sponges were used to ensure adherence and absorb excess liquid. The cornea was removed and stored in a viewing chamber with fresh preservation medium. Specular microscopy and slit-lamp examination were performed.

## RESULTS

A total of 780 donor grafts were prepared during the study period. Of these, 474 (60.7%) were from male donors and 306 (39.3%) were from female donors. The mean age of the donors was 59.9 ± 11.5 (range: 15–78). The mean death-to-corneal preservation time was 18.8 ± 5.5 hours (range: 3.3–28.3 hours). The mean death-to-corneal processing time was 5.1 ± 1.7 days (range: 2–12 days).

Twenty-eight of 780 grafts (3.6%) were discarded. Twelve graft preparation attempts resulted in tissue perforation (1.5%). These 12 perforations for which postcut tissue thickness and ECD could not be determined were excluded from the nomogram and further analysis. The other 16 grafts were discarded for the following reasons: endothelial cells could not be visualized after cut (7); excess storage artifacts (3); graft too thick after cut (1); cap or stromal tear (3); stromal gap (1); and cornea slipped off the piston after cut (1). Postcut ECD was not obtained in these instances and were excluded from ECD calculations.

Mean precut tissue thickness was 510 ± 49 μm (range: 363–670 μm). Mean postcut tissue thickness was 114 ± 22 μm (range: 57–193 μm). Of note, 79% (608/768) of grafts were ≤130 μm.

A linear regression model was generated for each microkeratome head size (300 and 350 μm) based on precut tissue thickness and donor age. In both models, over 80% of the predictions made on the test data set were within 25 μm of the actual postcut thickness (25/31 on the 300-μm microkeratome and 99/123 on the 350-μm microkeratome). Based on the models, nomograms were created to predict postcut tissue thickness for each microkeratome head size. Figure 1 shows the nomogram for the 350 μm case.

Mean precut ECD was 2761 ± 245. Mean postcut ECD was 2673 ± 229, indicating mild endothelial cell loss ($P < 0.0001$). Seventy-five percent of tissues had a decreased ECD after processing, with an average loss of 5.1%.

## DISCUSSION

The earliest report of precut DSAEK tissue was published in 2008 by Chen et al,[10] who reported a mean postcut thickness of 169 μm with significant variability (range: 88–257). Since the era of ultrathin DSAEK, new techniques have emerged to achieve thinner grafts, including double-pass techniques,[11] and drying of the graft with an alcohol sponge before cutting.[12] Recent studies of ultrathin DSAEK graft preparation (130 μm or less) have reported success rates of 88% immediately after cut,[13] and 97% postoperatively.[5,6]

Mean postcut graft thickness in our study was 114 μm, which is in line with our technicians' goal to cut between 70 and 160 μm. Another recent eye bank study using a single-pass technique reported an average postcut thickness of 114 ± 30 μm, which is similar to our study.[14] Our nomogram estimated postcut thickness 80% of the time to within 25 μm. This suggests that the regression model can provide effective estimates, but there remains considerable variability that is unaccounted for by the nomogram. Because of the retrospective nature of our study, the distance of tubing clamped, which affects the artificial anterior chamber pressure, was not captured as a variable. A prospective study including this variable may improve the accuracy of the nomogram.

It is well recognized that after cutting, the graft deturgesces and becomes thinner. Tang et al[15] reported an average graft thickness of 189 μm immediately after cut, which decreased to 148 μm 4 hours later in preservation medium. Di Pascuale et al[16] reported that mean graft thickness decreased from 243 μm postoperatively on day 1 to 148 μm at the last visit, stabilizing approximately 6 months after surgery. Even with thinner grafts as in the study by Romano et al[12] with mean postcut thickness at 83 μm, mean thickness decreased to 70 μm at 3 months postoperatively. Many studies reporting on ultrathin DSAEK outcomes have measured graft thickness postoperatively, and thus, our mean 114 μm postcut thickness measured immediately after cutting would decrease with time.

Our study is the first to use linear regression modeling to produce a predictive nomogram that provides a precise estimate of postcut thickness. Using the nomograms for each head size to produce estimates, the technician can then make an informed decision to select a head size that will achieve closer to his/her desired thickness. Previous nomograms have been reported that provide decision guidance (eg, cut or leave epithelium, microkeratome head selection) but do not attempt to estimate postcut thickness.[5–7] To our knowledge, only one study has reported such a nomogram, using variables including technician, cut depth goal, microkeratome head size, precut thickness, and duration of tissue storage.[8] However, this study did not clarify the statistical method used to reach this nomogram, nor did it clarify whether all included factors were statistically significant. The average reported standard deviation of its predictions was 12 μm.

We found mild, statistically significant endothelial cell loss after tissue processing. This is consistent with some reports[10,13] but not others that have reported a slight increase

in ECD.[14,17] One study found no change.[18] The mild endothelial loss seen in our study is likely a result of tissue handling or bias in measurements. The explanations proposed by Kelliher et al[17] and Choulakian et al[14] for their findings of increased ECD postcut include regression to the mean and other biases. Overall, the change in ECD is mild, and in the majority of cases, grafts remain viable with an acceptable ECD after tissue processing.

In conclusion, we report a nomogram based on eye bank data to predict postcut graft thickness using linear regression modeling. The constants for the variables in our nomogram are difficult to generalize to other institutions because of differences in equipment and technicians. However, other eye banks can use linear regression modeling based on their own data as a way to produce precise estimates of graft thickness. Furthermore, donor age may warrant further study as a contributing variable to graft thickness, because we are unaware of previous studies reporting a significant impact. Prospective studies to measure additional variables such as distance of tubing clamped or direct "intraocular" pressure of the artificial anterior chamber system may improve accuracy of the nomogram.

## ACKNOWLEDGMENTS

*The authors acknowledge the technicians at the Eye Bank of Canada—Ontario Division, Jenny Chai, Dan Abramovic, and Linda Nham—for their skill and expertise in corneal tissue preparation.*

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