Factors Associated with Thickness of Eye Bank-Prepared DSAEK Graft Tissue

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ABSTRACT

Purpose: To identify factors associated with corneal thickness of eye bank-prepared lamellar tissue for endothelial keratoplasty.

Methods: Data from all corneal tissues processed for DSAEK (Descemet stripping automated endothelial keratoplasty) at a single eye bank from 2008-2012 were included. Multivariable linear regression models were used to identify factors contributing to DSAEK graft thickness.

Results: Eye bank-prepared DSAEK grafts have become thinner over time, on average 48 microns thinner in 2012 than in 2008. Decreased pre-processing corneal thickness and higher pre-processing endothelial cell density were associated with thinner lamellar grafts ($p<0.0001$). Older donor age was associated with thinner grafts ($p<0.0001$). There was no significant interaction between donor age and endothelial cell density in relation to graft thickness ($p=0.16$). Grafts processed during the "workday" (8a-5p, n=5,397) were on average 3.8 microns thinner than those processed "after-hours" (5p-8a, n=1,897; $p<0.0001$). Death-to-procurement time and death-to-processing time were not associated with graft thickness ($p=0.94$ and $p=0.93$). In comparing technician experience, no significant difference was found between number of tissues processed and DSAEK graft thickness.

Conclusions: Eye bank-prepared DSAEK graft thickness has decreased during the five year study period, likely reflecting surgeon preference. Thinner grafts were associated with thinner initial corneal tissue thickness, higher endothelial cell density, increased donor age, and daytime tissue preparation. There was no association between graft thickness and death-to-procurement time, death-to-processing time, or technician experience. While the identified associations show statistical significance, no individual association conveys substantial clinical significance.

Keywords: eye bank; DSAEK graft thickness; DSAEK; DSAEK graft; DSAEK processing; endothelial keratoplast

Descemet stripping automated endothelial keratoplasty (DSAEK) is a safe and effective surgery that has become the main surgical technique for treatment of corneal endothelial diseases 1. The ability of DSAEK graft tissues to be prepared by eye banks has further popularized this procedure 2, 3. Eye bank-prepared graft tissue is of high quality and is well-accepted by surgeons 4. As eye banks perform more complex processing of corneal tissue, it is important for surgeons and eye bank professionals to identify factors associated with high quality transplant tissue. In recent years, there has been a growing trend of surgeon requests for thinner graft tissues and several techniques have been developed to produce ultrathin DSAEK grafts 5-7. In this study we analyzed factors associated with thickness of eye bank-prepared lamellar graft tissues to be used for DSAEK from a single eye bank in an effort to identify ways to achieve consistent graft preparations of the desired thickness.

MATERIALS AND METHODS

We obtained IRB exemption for this study through University of Michigan IRB-MED. We conducted a retrospective analysis of data from 7,668 consecutive corneal tissues processed for DSAEK at a single eye bank (Michigan Eye Bank, Ann Arbor, Michigan) from 2008 to 2012. Due to missing data, 374 tissues were excluded from the analysis, and 7,294 tissues were included in the model. All data were acquired from the Midwire database, a software program designed to track eye bank tissue information (Midwire Systems, Ann Arbor, MI).

A uniform protocol was followed for all DSAEK donor tissue preparation in this study 8. Briefly, donor tissues

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were mounted on an artificial anterior chamber filled with balanced salt solution. The Moria microkeratome system was used for the lamellar dissections. A nomogram taking into account pre-processing corneal thickness was used to determine whether a 300 or 350 micron microkeratome head was used. Corneal tissues were cut with a smooth manual pass. Technicians are rigorously trained prior to preparing tissue for surgical use. All tissues were processed with a single pass technique, with technician control over the speed of the microkeratome pass. The main outcome, post-processing graft thickness, was measured by ultrasound pachymetry in the central cornea.

Statistical analysis was performed with SAS 9.3 (SAS Institute, Cary, NC). Values more than an order of magnitude outside the plausible range of the variable were determined to be erroneous and removed from the data. Continuous covariates were checked for multicollinearity. We performed multivariable linear regression modeling in order to identify factors associated with DSAEK graft thickness. Covariates in the analysis included: donor age, pre-processing corneal thickness, pre-processing endothelial cell count, death-to-procurement time (in hours), death-to-processing time (in hours), year of processing, day of week of processing, and time of day of processing (divided into two periods, 8am to 5pm to represent the normal workday, and 5pm to 8am to represent after-hours). We used a stepwise selection procedure as o consideredwere nd death to processing as covariates. We also considered using both forward and backward selection to determine which covariates to include in the final regression model. Variables were entered into the model with a p<0.05 criterion, and removed with a p>0.10 criterion. In the final model, a p-value of <0.05 was considered statistically significant.

RESULTS

The primary outcome measure of post-processing DSAEK graft thickness had a continuous and normal distribution (mean + SD = 161.1 + 40.8 microns, Figure 1). Our data set included tissues processed by 46 technicians with varying experience (median, min – max number tissues per technician = 12.5, 1 – 1506). We performed regression analyses at the technician level, but determined that there was no significant relationship between the number of tissues processed and the average post-processing graft thickness. These results held when considering the number of tissues processed as a continuous covariate (natural logarithm of number of tissues processed) and as a categorical covariate (quartiles of number of tissues processed). We concluded that there was no systematic effect of technician experience on DSAEK graft thickness, and the data were pooled across all technicians for further analyses.

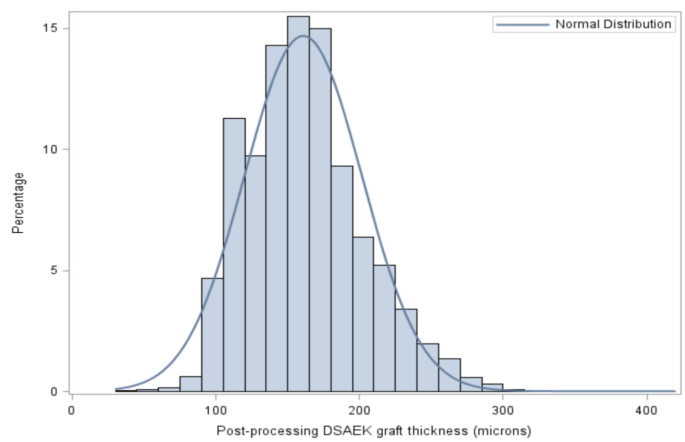

Figure 1. *Distribution of DSAEK graft thickness. A total of 7,294 corneal graft tissues from a single eye bank over four years had a nearly normal distribution.*

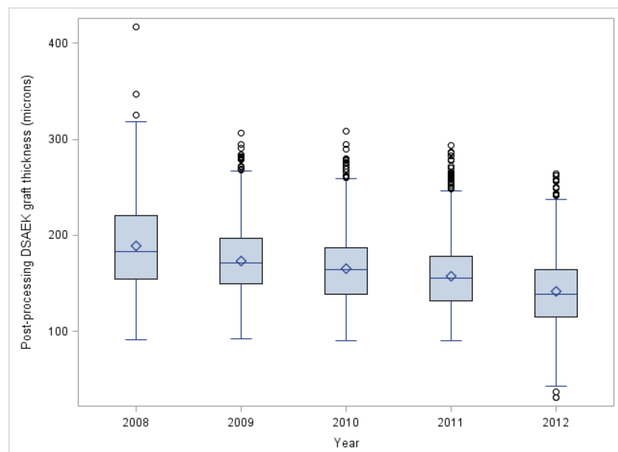

Figure 2. *DSAEK graft thickness over time. From 2008 to 2012, DSAEK grafts have become increasingly thinner (p<0.0001). Line=median, diamond=mean, open circle=outlier values*

Average DSAEK graft tissue thickness decreased over time, with the average graft tissue in 2012 being 48.1 microns thinner than the average graft tissue from 2008 (Figure 2). Stepwise linear regression was utilized to analyze associations between the covariates and the outcome of post-processing DSAEK graft thickness (Table 1). The mean, median, and range of the continuous variables, and frequency of categorical variables are shown in Table 2. Decreased pre-processing corneal thickness was associated with thinner lamellar grafts (p<0.0001); for each additional 1μm thickness of the pre-processing corneal tissue, we observe on average a 0.30 μm increase in the thickness of the processed DSAEK graft. Higher endothelial cell density was also associated with thinner lamellar grafts ($p<0.0001$), with on average a 1 µm decrease in the processed graft for each additional 100 endothelial cells/mm2. Older donor age was associated with thinner grafts ($p<0.0001$), for each year of increase in age, a 0.24 µm decrease in processed graft thickness was observed. As older age and higher pre-processing cell density were both associated with thinner final DSAEK grafts, we performed a secondary analysis to further elucidate these associations. We hypothesized that there might be an interaction between donor age and pre-processing cell density, but when we included an interaction term in the model it was not significant ($p=0.23$). Furthermore, the Spearman correlation coefficient between donor age and pre-processing endothelial cell count in our data set is -0.12, showing only a weak inverse relationship. Death-to-procurement time and death-to-processing time were not associated with graft thickness changes and were not included in the multivariable linear regression model ($p=0.94$ and $p=0.93$).

Unequal numbers of grafts were processed on each day of the week. The numbers of tissues processed on Sundays, Mondays, Tuesdays, and Wednesdays were comparable to each other, and much higher than the numbers processed on Thursdays, Fridays, and Saturdays (Table 2). Similarly, the number of tissues processed during the work-day was much higher than those processed during after-hours (5,397 tissues, or 74% during the work-day; 1,897 tissues, or 26% during after-hours). Most days of the week when grafts were processed had no significant association with graft thickness differences (with grafts processed on Sunday as the reference group), except Monday, which was associated with thinner tissues ($p=0.0002$). DSAEK grafts processed during the "work-day" (defined as 8am to 5pm) were on average 3.9 microns thinner than those processed "after-hours" (5pm to 8am; $p<0.0001$).

DISCUSSION

In this analysis, we confirm the trend towards thinner DSAEK graft preparation over the past several years. We found that a number of factors were associated with thickness of eye bank-prepared DSAEK grafts, including donor age, pre-processing thickness, endothelial cell density, year, day of the week, and time of day. We also found that some variables, including technician experience, death-to-procurement time, and death-to-processing time, had no association with final DSAEK graft thickness.

The year of processing showed a strong association to graft thickness, likely reflecting increased surgeon requests for thinner graft tissues over the years. While a number of studies have demonstrated no association between donor tissue thickness and post-surgical visual acuity 9-11, some studies have shown just the opposite—that donor tissue thickness does play a role in the visual outcome, whether by inducing greater and uneven refractive shifts 12, 13 or due to the donor-host stromal interactions. Although the effect of graft thickness on final visual outcome is unclear, this eye bank began creating thinner tissue likely in an attempt to accommodate explicit or implicit surgeon requests.

Pre-processing donor corneal thickness was directly related to the post-processing graft thickness, as would make sense based on the technique of microkeratome-assisted donor cornea dissection 8. In this eye bank, the technicians use nomograms to guide the decision on which microkeratome head depth to use to provide the final graft thickness that they would like to achieve. Additionally, eye bank technicians are aware that they can control the final lamellar thickness by varying the speed of the microkeratome pass. Even with the availability of both 300 and 350 micron depths, the final outcome of graft thickness is linked to the initial corneal thickness.

Donor age and pre-processing endothelial cell count were both inversely associated with post-processing graft thickness. While the association of donor age to graft thickness is statistically significant, it may not provide much clinical significance, as a 10 year difference in donor age yields only a 2.4 micron difference in final graft thickness in this model. Endothelial cell density has only a slightly larger clinical significance, with a difference of 1000 cells/mm2 predicting a difference in final graft thickness of 10 microns. Although studies have shown older donor age is correlated with lower endothelial cell count14-17, this correlation was weak and negligible in our dataset. Furthermore, donor age and pre-processing endothelial cell count were found to have no interaction with each other within our regression model.

Analysis of day of the week of tissue processing was only remarkable for Monday, which was associated with thinner grafts ($p=0.0002$) compared to the other days of the week. We speculate that it likely also reflects surgeon preference differences, as tissues processed for each surgeon tend to fall on the same day of the week according to the surgeon's operating schedule. Processing time during normal work hours was also found to be associated with thinner grafts, while those processed after-hours were on average, almost 4 microns thicker ($p<0.0001$). This may be due to having more scheduled tissue processing during the normal workday, compared to having more unscheduled graft processing (e.g. due to failed processing of initial tissue) during after-hours, which may have suboptimal conditions for graft processing such as less time for preparation or less support staff.

Importantly, we found no association of graft thickness to technician experience, death-to-procurement time, or death-to-processing time. Since a number of technicians with varying experience processed graft tissues in our data set, technician experience was a potential confounding factor in the dataset. However, our analysis showed that it was not associated with change in graft thickness. This suggests that technicians employed by this eye bank have sufficient training to enable reliable tissue processing results prior to providing tissue for surgeon distribution. Though two prior studies have shown that death-to-procurement time, or death-to-processing time are not associated with surgical outcome complications 2, 9, there are no published data on their associations with eye bank-prepared graft thickness. Our results reject the hypothesis that time from donor death to tissue procurement and processing time affect the graft thickness.

This study aimed to address factors associated with eye bank-prepared DSAEK graft thickness. The clinical relevance of statistical associations we found with individual factors is not strong, but collectively these factors may contribute to graft thickness. Strengths of this analysis include the large number of graft tissues processed by a single eye bank over the course of five years. However, since all data are from a single eye bank, the associations discussed in this study may not be directly applicable to other eye banks, which may have variations in protocols. We hope that identification of the factors that are and are not associated with DSAEK graft thickness will highlight opportunities to improve consistency of DSAEK tissue thickness in the future.

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