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Post DSAEK Optical Changes: A Comprehensive Prospective Analysis on the Role of Ocular Wavefront Aberrations, Haze, and Corneal Thickness

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Abstract

Purpose—To assess the visual impact of ocular wavefront aberrations, corneal thickness, and corneal light scatter prospectively after Descemet’s Stripping Automated Endothelial Keratoplasty (DSAEK) in humans.

Methods—Data were obtained prospectively from 20 eyes pre-operatively and at 1, 3, 6, and 12 months post- DSAEK. At each visit, best spectacle corrected visual acuity (BSCVA) and visual acuity with glare (Brightness Acuity Testing - BAT) were recorded and ocular wavefront measurements and corneal Optical Coherence Tomography (OCT) performed. Magnitude and sign of individual Zernike terms (higher order aberrations HOA) were determined. Epithelial, host stromal, donor stromal, and total corneal thickness were quantified. Brightness, intensity profiles of OCT images were generated to quantify light scatter in the whole cornea, subepithelial region, anterior and posterior host stroma, interface, and donor stroma.

Results—Mean BSCVA and glare disability at low light levels improved from 1 to 12 months post-DSAEK. All corneal thicknesses and ocular lower- and HOAs were stable from 1 through 12 months, whereas total corneal, host stromal, and interface brightness intensities decreased significantly over the same period. A repeated measures ANOVA across the follow up period found that the change in scatter, but not the change in higher order aberrations, could account for the variability occurring in acuity from 1 to 12 months post-DSAEK.

Conclusions—While ocular HOAs and scatter are both elevated over normal post-DSAEK, our results demonstrate that improvements in visual performance occurring over the first year post-DSAEK are associated with decreasing light scatter. In contrast, there were no significant changes in ocular HOAs during this time. Because corneal light scatter decreased between 1 and 12 months despite stable corneal thicknesses over the same period, we conclude that factors that induced light

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scatter, other than tissue thickness or swelling (corneal edema), significantly impacted the visual improvements that occurred over time post-DSAEK. A better understanding of the cellular and extracellular matrix changes of the subepithelial region and interface, incurred by the surgical creation of a lamellar host-graft interface, and the subsequent healing of these tissues, is warranted.

Endothelial keratoplasty is now the surgical treatment of choice for endothelial failure. Currently, 89% of patients with Fuchs' endothelial dystrophy and 55% of patients with post-cataract corneal edema are treated with endothelial keratoplasty.¹ Yet, visual performance is often sub-optimal following Descemet's stripping with automated endothelial keratoplasty (DSAEK), and efforts aimed at understanding the optical causes of these limitations is ongoing.²

The main causes of optical degradation in the cornea are optical aberrations and light scatter. Because of the change in the corneal contour associated with the addition of the donor lenticule in DSAEK it is not surprising that corneal wavefront aberrations, particularly from those arising from the posterior corneal surface, are increased.³⁻⁷ While corneal videokeratography/topography measurements allow for quantification of the anterior and posterior corneal aberrations, any potential aberrations induced by the graft/host interface are missed. Furthermore, the quality of vision ultimately depends not only on the cornea, but on the entire optical system (including the lens and media). As such, when assessing the impact of aberrations on retinal image quality and visual performance, it is more appropriate to study whole eye aberrations.

Corneal light scatter has also been reported within anterior stroma and interface post-DSAEK and has thus been implicated as a factor limiting visual performance.⁸⁻¹⁵ Additionally, there has been great interest in whether corneal thickness plays a role in post-DSAEK visual performance; to date, this remains a point of contention.^{14, 16-19}

Although we know that visual acuity outcomes continue to improve over the first three years post-DSAEK,²⁰ the relative role of aberrations and light scatter in this improvement is poorly understood. We sought to conduct a prospective study, in which operative variables (e.g. surgical technique and graft size) and follow-up time points were controlled, to quantify ocular aberrations, corneal light scatter, and corneal thickness, and to assess their contributions to changing visual performance with time post-DSAEK.

METHODS

Patient Population

Twenty pseudophakic patients were recruited prior to undergoing DSAEK for endothelial failure. All subjects provided informed consent and were enrolled in the University of Rochester's HIPAA-compliant Institutional Review Board (IRB)/Ethics Committee approved protocol. The research adhered to the tenets of the Declaration of Helsinki. Patients were excluded if they had any other ocular pathology that might limit post-operative visual performance.

Surgical Technique

Standardized DSAEK surgeries were performed. The donor tissue was cut with a Moria microkeratome (Moria Inc, Doylestown, PA), cut with an 8.25 mm diameter trephine, folded into a 60/40 fold, and inserted through a temporal 5.2 mm scleral-tunnel incision using forceps. Surgical inferotemporal peripheral iridotomies were performed through separate incisions and four draining keratotomies were made.

Clinical Assessments

Preoperatively, and at each post-operative time-point (1, 3, 6, and 12 months post-DSAEK), best spectacle-corrected visual acuity (BSCVA) was recorded using Snellen charts. Glare disability testing was performed using the Marco Brightness Acuity Tester (BAT, Star Ophthalmic Instruments, Willowbrook, IL) at each of three, standard brightness settings, with best spectacle refractive correction in place.

Wavefront Measurements

Wavefront measurements were obtained using a customized Shack Hartmann wavefront sensor (the characteristics and accuracy have been previously described)²¹ at 1, 3, 6, and 12 months post-DSAEK following dilation and paralysis of accommodation with combination 2.5% phenylephrine and 1% tropicamide drops. Data were collected over the largest pupil diameter possible (> 4.0 mm). Ten patterns were analysed per eye and time-point, and wavefront errors were calculated using Zernike polynomial expansion up to the 10th order and reported in standard format.²² For illustration purposes, an eye of a representative patient was selected and higher-order wavefront maps were generated for each post-operative time-point using MATLAB.

Optical Coherence Tomography

A custom-built, 1310 nm anterior segment optical coherence tomographer (OCT) was used to image corneas pre-operatively and at 1, 3, 6, and 12 months post-DSAEK. The OCT was aligned to the apex of each cornea, a video stream of the central 10mm recorded, and fifteen corneal images extracted, analysed as described below, and averaged per eye and time-point.

Thickness analysis—Thickness measurements were obtained using Image J software. Measurements were taken as close to the central pixel as possible while remaining outside the specular reflection (within a distance of ± 94 microns (25 pixels) of the central pixel). A perpendicular line from the anterior aspect of epithelial layer to the posterior surface of the donor tissue was drawn in each eye (total thickness). Individual corneal layers (epithelial, host stroma, and donor stroma) were measured as demonstrated in Figure 1A. The length of each line was recorded, and multiplied by the pixel scale of 3.75 (conversion factor established for our OCT) to obtain a thickness value in microns.

Intensity Analysis—To quantify corneal light scatter, Image J software was used to measure pixel intensity according to established methods.²³⁻²⁵ The profiles of the pixel intensities were normalized to the background intensity and the mean brightness at each time-point was calculated for each eye. Two types of intensity profiles were generated:

1. Transverse intensity profiles were generated across each corneal image along a 20 pixels (75 microns) wide area running transversely across the full thickness of the total cornea from the anterior surface to the posterior surface (see colored lines in Figure 1B and 1C). From this the magnitude of backscatter reflectivity, an index of scattering, was computed across the entire corneal thickness (Figure 1 B) and in the different regions of the cornea (Figure 1C: subepithelial region (anterior 7% of stroma), anterior 50% of host stroma, posterior 50% of host stroma, interface, and donor stromal tissue). Areas of analysis were located 100 and 200 pixels (375 and 750 microns) from the central pixel in order to avoid the effect of the specular reflection.
2. Arc intensity profiles were generated by computing average pixel intensity along the length of an arc with a thickness of 10 pixels (37.5 microns) that extended along the central 400 microns of the host/donor interface (Figure 1D).

Statistical Analyses

For statistical analysis of changing visual acuity, BAT, ocular aberrations, corneal thickness measures, and brightness intensity over time, Kruskal-Wallis tests (nonparametric ANOVAs) were performed with Dunn's multiple comparisons post-tests. Correlations between variables were illustrated by calculating Spearman correlation coefficients. Data analysis was performed using GraphPad InStat Software (GraphPad Software, Inc., San Diego, California), which corrects for multiple comparisons. To understand the role of scatter and aberration on the visual acuity improvement between 1 and 12 months, a repeated measures ANOVA was performed with SPSS 15 (SPSS, Inc) using total corneal scatter and higher-order aberrations (HORMS) as covariates.

RESULTS

Baseline Patient Characteristics

Baseline characteristics of the patients in this study are provided in Supplemental Table 1. Preoperatively, mean BSCVA was 0.64 ± 0.34 (mean $\pm$ SD, Snellen = 20/87) and mean total corneal thickness was 717 ± 66 μ m.

Visual Acuity and Glare Disability Improved After DSAEK

Compared to pre-operative measures, the mean BSCVA significantly improved at most time-points post-DSAEK (Figure 2 A). Mean BSCVA also improved significantly during the post-operative follow-up period from 1 month (0.47 ± 0.05 , Snellen 20/60) to 12 months (0.22 ± 0.03 , Snellen 20/33, $D=36.5$, $p<0.001$). Glare disability under low glare (dim) conditions improved significantly with visual acuity improving from 0.49 ± 0.29 (Snellen 20/62) at 1 month to 0.34 ± 0.18 (Snellen 20/44) at 12 months, whereas visual acuity under moderate glare conditions showed a non-significant improvement, and under high glare (bright) conditions remained constant and poor at 0.8 (Snellen 20/125) before surgery and through 12 months of follow up (Figure 2B).

Lower and Higher-Order Ocular Wavefront Aberrations Did Not Change Significantly During the First Year After DSAEK

Because of corneal edema, wavefront sensing could not be performed pre-operatively in DSAEK eyes. After DSAEK, mean defocus shifted non-significantly in the negative direction (Figure 3A), equating to a non-significant mean hyperopic shift of .04 D from 1 to 12 months post-DSAEK, which corresponded to the clinically measured spherical equivalent change (0.28 D). At 12 months-post DSAEK, the astigmatism, as estimated from the Zernike modes J3 and J5 had decreased 0.29 D from the 1 month time-point and equated to a refractive cylindrical correction of 1.57 ± 0.30 D and corresponding to the clinically-measured refractive astigmatism (1.26 ± 0.22 D) and keratometric astigmatism (1.25 ± 0.30 D). Higher-order root mean square (HORMS) (Figure 3B) and the magnitude of individual higher-order aberration terms (J6-20, Figure 3C) did not change significantly post-DSAEK. There was considerable variability in the amplitude of individual HOA terms at each time point (Figure 3C). The largest contributors to the ocular higher-order aberrations were the 3rd order aberrations [comas (J7 & J8, ~ 31%) and trefoils (J6 & J9, ~ 27%)] and spherical aberration (J12, ~8%). Positive spherical aberration was consistently noted across post-operative time points.

Host Corneal Stromal Thickness Decreases Initially Post-DSAEK, and All Corneal Thicknesses Remained Stable from 1 to 12 Months

Mean epithelial and donor stromal thicknesses were stable across all time points (Figure 4). Mean host stromal thickness decreased significantly from pre-operative levels to 1 month post-DSAEK ($D=44.55$, $p<0.001$), then stabilized at $\sim 500\mu\text{m}$ through 12 months.

Backscatter Reflectivity Decreased Post-DSAEK

Overall, light scatter across the whole cornea decreased significantly from pre to post-operative time points ($KW=20.024$, $p<0.0005$) (Figure 5A,B,C). The decrease in scatter from preoperative levels was significant at both the 6 and 12 months time-points (Mean rank difference (D) = 25.851, $p<0.05$ and $D = 31.508$, $p < 0.01$, respectively). A significant decrease in reflectivity also occurred between the 1 and 12 months ($D = 25.199$, $p < 0.05$). Decreasing interface intensity was also observed; the arc analysis revealed the backscatter reflectivity at the interface to be significantly lower at 6 ($D = 20.801$, $p < 0.05$) and 12 months ($D = 26.292$, $p < 0.001$) than at 1 month post-operatively (Figure 5D,E). Scatter from the subepithelial ($D=28.4$, $p < 0.01$), anterior ($D = 26.368$, $p < 0.05$) and posterior ($D = 26.232$, $p < 0.05$) aspects of the host stroma, as well as from the interface ($D = 29.923$, $p < 0.001$) decreased significantly between 1 and 12 months post DSAEK (Figure 6). The mean subepithelial intensity decreased by 15%, the host stromal intensity by 13%, and interface intensity by 20% over that period. A change in donor tissue reflectivity was not identified over these time periods (Figure 6C).

BSCVA Correlated with HORMS and Scatter Post-DSAEK but not with Thicknesses

BSCVA correlated with HORMS (Spearman $r = 0.2453$ (corrected for ties) 95% confidence interval (CI): 0.009134 to 0.4555, $p < 0.05$) and with scatter throughout the whole thickness of the cornea (Spearman $r = 0.2698$, 95% CI: 0.02231 to 0.4861 $p < 0.05$). In post hoc analyses, BSCVA correlated with scatter from the subepithelial region (Spearman $r = 0.4221$, 95% CI: 0.1916 to 0.6086, $p < 0.0005$), the anterior host stroma (Spearman $r = 0.4142$, 95% CI: 0.1822 to 0.6024, $p < 0.0006$), and the interface (Spearman $r = 0.4462$, 95% CI: 0.2199 to 0.6269, $p < 0.0002$). BSCVA did not correlate with either total corneal or donor stromal thickness.

Improving Visual Acuity Post-DSAEK is Most Likely Due to Decreasing Light Scatter in the Cornea

The repeated measures ANOVA demonstrated significantly improved visual acuity between 1 and 12 months, $F(3,33) = 9.70$, $p < 0.0005$, partial $\eta^2=0.469$. When the HORMS difference over this time period was included as a covariate, the difference in acuity was still significant, $F(3,30) = 8.532$, $p < 0.0005$, partial $\eta^2 = 0.46$, and HORMS was not a significant predictor of acuity ($F(1,10) = 0.218$, $p = 0.651$, partial $\eta^2 = 0.021$), indicating that aberration does not account for the difference in acuity. However, when the difference in total corneal scatter was used as a covariate, the change in acuity was no longer significant, $F(3,30) = 2.267$, $p = 0.101$, partial $\eta^2 = 0.185$, and scatter was also not a significant predictor, $F(1,10) = 0.001$, $p = 0.983$, partial $\eta^2 < 0.005$, indicating that change in scatter and the change in acuity account for largely overlapping variance in acuity.

DISCUSSION

Endothelial keratoplasty represents more than 1/3 of all keratoplasty procedures currently performed in the United States.¹ In order to further improve upon this procedure and its outcomes, we must understand the optical changes occurring with time. The present prospective study, examines the role of changing ocular aberrations, scatter, and thickness

simultaneously, assessing their relative role in visual performance. Our study was limited by a small sample size, and absence of contrast sensitivity, corneal topographic analysis, and peripheral corneal thickness data. Additionally, while it is possible that intraocular lens glistenings could have also impacted upon our results,^{26, 27} we did not observe any notable IOL glistenings nor is there data to suggest glistenings induce aberrations. Despite these potential shortcomings, we were able to identify important factors in visual performance improvements post-DSAEK.

Visual Acuity Improved During the First Year Post-DSAEK

Although this is a relatively small study, the one year visual acuities (20/33) are consistent with those reported in larger studies by Guerra et al (20/32)²⁸ and Li et al. (20/30),²⁰ suggesting that our results may be generalized to the larger DSAEK population. The lower percentage (90%) of patients in our study who achieved BSCVA of 20/40 as compared to Li. et al's (93%), this may be explained by our worse mean pre-operative vision (20/87 as compared to 20/51)), which may reflect baseline corneal edema and associated changes.

Post-DSAEK Visual Performance Under Low and High Glare Conditions

The impact of Brightness Acuity Testing (BAT) on post-DSAEK patients' vision has not been assessed. Yet glare complaints are frequent among patients with corneal edema or opacity. BAT has been used to identify patients who might benefit from keratoplasty.²⁹ Hence, we felt that BAT could represent an important functional visual assessment in post-DSAEK patients. Visual dysfunction associated with glare was worse in post-DSAEK patients than previously reported in normals. Whereas normals lose 0.6 lines of acuity on average with increasing brightness,³⁰ the post-DSAEK patients lost 3 lines in response to the same change at 1 month. With time post-DSAEK, glare disability at the low intensity setting improved, while it persisted in bright conditions. Similarly, McLaren and Patel modeled the effect of forward scatter on visual acuity after DSAEK and found that under adverse glare conditions, extreme forward scatter was induced that had a greater impact on visual acuity than normal lighting conditions.³¹

Glare disability has been correlated with light scatter caused by corneal edema³² and opacity along the visual axis.³³ Because corneal thickness remained unchanged between 1 and 12 months, it suggests that the noted improvement in BAT visual acuity at dim setting was related to changes in light scattering properties of the cornea unrelated to corneal edema, such as opacification and scarring.³⁴ However, the role of aberrations on brightness acuity testing performance has not been studied, and it is possible that this could also impact glare disability.

While we did not assess contrast sensitivity in this study, we previously showed that DSAEK eyes have better contrast sensitivity at lower spatial-frequencies (4 cycles / degree) than at higher ones (12 cycles / degree).³⁵ Others have noted improvement in contrast sensitivity with time post-DSAEK.⁸ Straylight is another measure used to study glare disability, which has shown improvement at all prospective time points through 1 year ($P < .001$).¹⁵

Astigmatism is Greater than Normal After DSAEK

Although decreasing slightly from 1 month levels, the 12 months post-DSAEK astigmatism terms equated to a refractive cylindrical correction of 1.57 ± 0.30 D of astigmatism, which may have been related to the 5.2 mm incision. Nonetheless, regular astigmatism can be corrected with spectacles and should not account for limitations in BSCVA observed in our patients.

Ocular Aberrations are Greater than Normal in Post-DSAEK Eyes

While other studies have looked at anterior and posterior corneal aberrations post-DSAEK,^{4, 5, 7, 36} we prospectively characterized the total ocular wavefront aberrations in order to predict retinal image quality and understand the impact on visual function. Although advanced corneal edema prevented us from measuring pre-operative aberrations, we identified greater *ocular* HORMS and magnitudes HOAs (J6-J20) at all post-operative time points compared to normal eyes.³⁷

Relative Contribution of Ocular vs. Corneal Aberrations in Understanding Post-DSAEK Optical Changes

The greater amount of HOAs in our post-DSAEK eyes as compared to pseudophakes with the same pupil diameter³⁸ may be explained by the corneal aberrations induced by chronic edema affecting the anterior surface regularity and the disruption of the posterior surface regularity by the addition of donor tissue. The changes in HOAs with time likely reflect changes in regularity of these surfaces with healing; however, as anterior and posterior corneal aberrations were not measured, we must refer to the literature for insight.^{4, 5, 36}

Nonetheless, the combined impact of the anterior and posterior corneal aberrations is important, because unlike in normals³⁹ and other post-keratoplasty populations, the posterior surface after DSAEK does not compensate for the anterior aberrations. Instead, it contributes *more* to the total corneal aberrations -- negatively impacting the modulation transfer function.⁴⁰ In measuring ocular aberrations, we assessed how the two corneal surfaces, in combination, impacted the wavefront aberration and the retinal image quality achieved. Additionally, measuring total ocular aberrations accounted for factors such as graft decentration, stromal edema, and irregularity of the donor/host interface, which may impact retinal image quality.

Ocular Aberrations Do Not Change Significantly Post-DSAEK

No significant changes in the magnitude of any HOAs were identified with time post-DSAEK. However, considerable variability in the amplitude of some terms across time points was observed (especially trefoil). The source of these fluctuations is unclear but could be associated with corneal shape changes occurring with removal of the scleral wound suture or healing of the draining keratotomies.

In our study, we identified relative stability in mean *ocular* HORMS with a non-significant trend toward decreased ocular HORMS with time between 1 and 12 months post-DSAEK. Our study was not designed to provide insight on how the different corneal layers contribute to the total ocular aberration. But in a prospective study, Patel et al found that the total anterior *corneal* aberrations were elevated in Fuchs' endothelial dystrophy patients above normal controls and remained stable across time points through two years post-DSAEK.³⁶ Thus it seems that the damage to the anterior surface regularity induced by chronic swelling is permanent. Based on these findings, if indeed a change in ocular HORMS does occur, it would most likely be attributed to the posterior surface. Prospective studies looking at posterior corneal irregularity with time post-DSAEK might shed additional light on this question.

Yet, the important question is whether the aberrations, and their changes over time, impact on visual outcomes. We demonstrated with the repeated measures ANOVA that *change* in ocular HORMS did not account for the *change* in the BSCVA between 1 and 12 months post-DSAEK. Based on the results of previous studies showing that anterior corneal aberrations have correlated with BSCVA post-DSAEK^{5, 36} while posterior corneal aberrations have not,^{3, 5, 11} even if the posterior corneal aberrations were to change

significantly over the first year postoperatively, they are unlikely to impact significantly on measures of visual acuity.

Corneal Thickness Does Not Change Between 1 and 12 Months Post-DSAEK

There continues to be debate regarding the roles of graft and total corneal thicknesses on post-DSAEK visual acuity.^{17-19, 41-46} We did not find a correlation between BSCVA and donor, host, or total stromal thicknesses. Prospective decrease in central corneal thickness from 2 weeks to 3 months post-DSAEK has been reported, although statistical analyses were not performed to determine if these were significant.¹⁴ Such thinning is likely related to a combination of the host stromal and donor tissue thinning; the latter having been found to stabilize by 6 months.¹⁶ In our study, the majority of the deturgescence had occurred by 1 month with no subsequent significant changes.

Corneal Light Scatter Improves Post-DSAEK, Accounts for the Variability in BSCVA, and Occurs Most Dramatically at the Interface

In this study, we systematically quantified corneal haze by measuring light reflectance from high-resolution, cross-sectional *in vivo* OCT corneal images. The primary benefit of using OCT as a surrogate for haze is that it allows for the localization of areas of increased reflectivity due to changes in refractive index over a relatively large spatial area of the cornea, providing an indication of the optical uniformity of these areas. Since the infrared light source utilized in our OCT has a longer wavelength (1310 nm), our findings are an underestimation of the light scatter that would be induced by light in the visible spectrum.^{47, 48} With repeated prospective images and analyses, we quantified the change in reflectivity and optical quality of regions of interest with time after surgery. Various other methods (confocal microscopy, scatterometry, and Scheimpflug cameras)^{9, 11, 13} have been used to assess scatter post-DSAEK. Confocal microscopy utilizes a point illumination light source and a pinhole to minimize out-of-focus signal. Increased resolution comes at the cost of decreased signal intensity requiring longer exposures. As a result of this design principle, the impact of light scatter could be underestimated. Moreover, confocal microscopy can provide information on the cellular level, but only in a very small spatial area of the cornea (typically 400 × 400 μm). The scatterometer is effective in quantifying corneal light backscatter, but provides no information regarding the source or location of the scattering features. Similar to OCT, the rotating Scheimpflug topographers provide a 3 dimensional image output which can be used to identify sources of light scatter within the cornea. And while the OCT lacks the resolution required for cellular detail, we were able to do a similar analysis of the scatter induced by the subepithelial region by quantifying the scatter from the anterior 7% of the host stroma as measured from the peak generated by the epithelial/stromal junction so as to compare our results to those previously reported utilizing confocal imaging.⁸

All of the instruments, including OCT, detect light reflectance or back scatter; however, the relationship between backscattered light and forward light scatter is complex. Although it is forward light scatter and not back-scattered light that ultimately affects visual performance, the relationship between the two is complex. Although they are likely derived from the same sources within the cornea,^{49, 50} a direct relationship between backscattered light and visual function associated with forward scatter cannot be drawn. Nonetheless, as we have no direct way of imaging forward light scatter in the cornea *in vivo*, each of these methods provides insights that help us better understand optical imperfections within the cornea.

Presently, we found significantly decreased corneal light scatter from 1 to 12 months post-DSAEK throughout the host stroma (including subepithelial, and anterior and posterior stromal regions) as well as at the host/graft interface; yet, the greatest decline in intensity

occurred at the interface. Decreasing haze post-DSAEK has also been identified by Scheimpflug camera¹⁴ and *in-vivo* confocal microscopy,^{8, 10} with Baratz et al identifying two peaks in reflectivity: one which corresponded to the subepithelial layer and one occurring at the interface. However, in none of these other studies were ocular aberrations simultaneously measured. This puts us in a unique position to assess the relative contributions of light scatter and higher-order aberrations to limited visual acuity post-DSAEK. One study of 6 month post-DSAEK eyes simultaneously assessed measures of scatter (large angle domain of the point spread function by measuring stray light) and aberrations (small angle domain (50% width) of the point spread function by measuring retinal image quality) and found high contrast visual acuity correlated with higher-order aberrations (50% width), but not with straylight.⁵¹ However, this study, did not describe the progression of these measures over time post-DSAEK. Another study by the same group used modeling techniques to determine the effect of forward scatter and aberrations on visual acuity after endothelial keratoplasty – concluding that under typical testing conditions, higher-order aberrations were more likely to result in the limited visual acuity.³¹ Yet, importantly, our repeated measures ANOVAs demonstrated that the post-operative changes in scatter rather than whole eye aberrations accounted for the time course of changes in BSCVA during the first year post-DSAEK. For conducting this analysis we used the total corneal scatter across the thickness of the cornea, as we felt that it was likely the combined impacts of the haze in the subepithelial region, interface, as well as all other regions that could contribute to the visual outcomes. We also analyzed how local reflectivities changed with time. Indeed our findings are consistent with those of others in that the interface reflectivity decreased more than the subepithelial surface reflectivity. This supports the conclusion of others that persistent subepithelial haze may limit vision long-term post-DSAEK.⁸ It also suggests that the improvements in visual acuity with time may be linked to the improvements in the optical properties of the interface with time. However, our study was underpowered to study the impact of scatter from multiple regions of the cornea and on BSCVA with time post-DSAEK – for that, larger studies will be required.

To our knowledge, our study is the first to measure ocular aberrations and scatter in a prospective fashion post-DSAEK so as to determine the *relative* impact of changing aberrations and scatter on post-operative vision. Consistent with the present findings, we previously reported that eyes >1 year post-DSAEK still do not perform as well as other keratoplasty populations when lower and HOAs were fully corrected using adaptive optics techniques³⁵ -- suggesting that factors other than wavefront aberrations impact visual performance long-term post-DSAEK.

Causes of post-DSAEK light scatter

While it has been demonstrated that visual acuity continues to improve through 3 years post-DSAEK,²⁰ the explanation for these continued improvements in visual acuity is unknown. In the present study, we found that decreased light scatter, especially at the host/graft interface, played a more prominent role than changing aberrations in improving visual acuity during the first year post-DSAEK. This decrease in light scatter was not associated with a change in corneal thicknesses (host stroma, donor stroma, or total). Hence, it is unlikely that deturgescence of the tissue was responsible for changes in light scatter over this period, leaving factors such as cellular activity and extracellular matrix alterations as more likely candidates. Our knowledge of the biological changes occurring at the levels of the anterior stroma and interface post-DSAEK is limited. Using confocal microscopy in post-DSAEK eyes, the anterior host stroma has demonstrated keratocyte activation and reflectivity.^{8, 10, 13} It is believed that the anterior stromal changes are secondary to chronic changes in the host tissue associated with endothelial dysfunction, which lead to chronic corneal edema, extracellular matrix changes, subepithelial fibrosis, keratocyte loss, and changes in corneal

nerve architecture.⁵²⁻⁵⁵ The interface has sometimes been characterized by the presence of reflective particles;¹³ yet, the source of these particles is not understood. Additionally, reflective “needle shaped materials” have occasionally been noted in the deep host stroma,¹⁰ similar to areas of acute corneal inflammation.⁵⁶ A thorough understanding of the cellular and extracellular matrix changes that occur prior to, and as a result of, DSAEK is integral to our ability to modify these processes in order to achieve rapid and full visual recovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Methods for Analyzing Corneal Optical Coherence Tomography (OCT) Images
A. Corneal Thickness Measurements. Using Image J software, the (A) whole corneal, (B) host stromal, (C) donor tissue, and (D) epithelial thicknesses were measured. **B. Transverse Intensity Analyses Across Corneal Stromal Thickness.** Pixel intensity profiles were generated across the full thickness of the host and donor stroma at 4 locations central corneal locations to provide a measure of backscatter reflectivity. **C. Transverse Intensity Analyses of Corneal Layers.** After generating pixel intensity profiles from the base of the epithelium to the endothelium, the profiles were divided into layers of interest: subepithelial region, anterior host stroma, posterior host stroma, interface, and donor tissue to assess backscatter reflectivity from these different layers. **D. Arc Intensity Analyses.** A curved arc of 10 pixels in thickness was drawn end to end along the host/donor interface to quantify the backscatter reflectivity along the central interface.

Figure 2. Visual Function After Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK)

A. Best Spectacle-Corrected Visual Acuity (BSCVA) Improves After DSAEK. Mean BSCVA improved significantly from preoperative levels (~ 20/87) to 3, 6, and 12 months post-DSAEK. Additionally, there was significant improvement between 1 month (~ 20/59) and 12 months (~20/33) post-DSAEK. KW $p < 0.0001$. B. Glare Disability Decreases After DSAEK But Only Under Dim Lighting Conditions. Glare disability under low glare (dim) conditions improved significantly with visual acuity improving from 20/63 at 1 month to 20/40 at 12 months, whereas visual acuity under moderate and bright glare conditions

remained stable and poor. KW $p < 0.005$ for dim setting. ** = $p < 0.01$ and *** = $p < 0.001$ for Dunn's post-tests for 1 to 12 months. Error bars = SEM.

Figure 3. Ocular Aberrations After Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK) Over a 4 mm Pupil

A. Lower-Order Aberrations (LOA) After DSAEK. Mean LOAs are relatively stable at 1, 3, 6, and 12 months post-DSAEK. **B. Higher-Order Root Mean Square (HORMS) After DSAEK.** HORMS trended downwards post-DSAEK; however this trend was not significant. Representative higher-order wavefront maps are provided for each time-point. **C. Higher-Order Aberrations (HOAs) After DSAEK.** Mean HOAs fluctuate non-significantly at 1, 3, 6, and 12 months post-DSAEK. Error bars = SEM.

Figure 4. Thicknesses of Corneal Layers Post-Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK)

A. Representative Optical Coherence Tomography (OCT) Image Demonstrating How Corneal Layers Were Identified. B. Thicknesses of the Different Corneal Layers with Time Post-DSAEK. The host stromal thickness significantly decreased from pre-operative levels to the one month time point and then remained stable through 12 months. Donor stromal thickness was stable across all post-operative time points. Error bars = SEM. KW $p < 0.0001$ for host stromal thickness. *** = $p < 0.001$ for Dunn's post-tests for 1 to 12 months.

Figure 5. Mean Corneal Light Scatter Measured Transversely Through the Entire Stroma and Along the Central Interface Decreased During the First Year Post-Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK)

A. Plot of Normalized Brightness Across Corneal Depth. Transverse intensity profiles were generated across each corneal image to quantify light scattering with peaks in brightness intensity representing the anterior epithelial surface, anterior stroma, interface, and corneal endothelium. **B. Location of Generated Brightness Profiles.** For each corneal image, transverse intensity profiles were generated along a 20 pixels (75 microns) wide area running transversely across the full thickness of the total cornea and were located 100 and 200 pixels from the central pixel. **C. Mean Normalized Brightness Through the Entire**

Stroma Decreased with Time Post-DSAEK. The decrease in scatter from preoperative levels was significant at both 6 and 12 months. A significant decrease in reflectivity also occurred between 1 month and 12 months. KW $p = 0.0005$. **D. Location of Interface Arc Intensity Analyses.** A curved arc of 10 pixels in thickness was drawn end to end along the host/donor interface to quantify the backscatter reflectivity generated along the graft/host interface. E. Mean Interface Intensity Measured Along the Curve Decreased Significantly Post-DSAEK. The intensity measured along the interface was significantly lower at 6 and 12 months than at 1 month. Error bars = SEM. KW $p < 0.0005$. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$ for Dunn's post-tests for 1 to 12 months.

Figure 6. Corneal Light Scatter in Host Cornea and Interface as Measured by Optical Coherence Tomography (OCT) Decreased During the First Year Post-Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK)

A. Plot of Normalized Brightness Across Corneal Depth. Transverse intensity profiles were generated across each corneal image to quantify light scattering in different corneal layers (purple = subepithelial, maroon = anterior host stroma, pink = posterior host stroma, yellow = interface, and orange = donor stroma). B. Location of Generated Brightness Profiles. These were generated as described in Figure 5. C. Mean Normalized Brightness Through Regions of the Host Stroma and Interface Decreased with Time Post-DSAEK. Between 1 and 12 months, the subepithelial, anterior host stroma, interface, and posterior host stroma

decreased significantly in pixel intensity, while the donor stroma pixel intensity remained unchanged. Error bars = SEM. KW $p < 0.005$ for subepithelial, anterior, and posterior host stromal, and $p < 0.0001$ for interface. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$ for Dunn's post-tests for 1 to 12 months.