Scanning Electron Microscope Analysis of Stromal Surface Regularity in DSAEK Using Manual or Automated Microkeratomes or DMEK

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Purpose: To analyze differences in the stromal bed according to the method used to obtain the disc for endothelial transplant with 1) an automated microkeratome for Descemet stripping automated endothelial keratoplasty (DSAEK), 2) homogeneous dissection of Descemet membrane (Descemet membrane endothelial keratoplasty [DMEK]), or 3) a manual microkeratome used for DSAEK.

Methods: We analyzed the stromal bed of 25 consecutive corneas used for endothelial transplantation, divided into 3 groups: 1) 11 samples cut with an automated microkeratome, 2) 5 samples used for DMEK, 3) 9 samples cut with a manual microkeratome. A scanning electron microscope was used to obtain an image of the center of the stromal bed. The irregularity index of the surface complementary to the stromal surface of the disc obtained for grafting was calculated with the Canny algorithm (0: completely smooth; 1: completely irregular).

Results: At all thresholds studied (20, 30, 50, and 70), the mean irregularity index for group C (35.2, 24.4, 13.7, and 8.8, respectively) was higher than that of group A (26.2, 14.8, 6.7, and 4.0, respectively), which in turn was higher than that of group B (7.0, 4.2, 2.4, and 1.8, respectively). Differences were statistically significant among all groups and for all thresholds.

Conclusions: Irregularity of the stromal bed after any dissection can be quantified using the Canny method. The use of an automated microkeratome for DSAEK reduces irregularity and helps obtain a surface more similar to that obtained with DMEK than with a manual microkeratome.

Key Words: Descemet stripping automated endothelial keratoplasty, Descemet membrane endothelial keratoplasty, microkeratome, visual quality

(*Cornea* 2017;36:588–593)

Corneal endothelial transplantation is the most widely used surgical option for treating corneal edema due to endothelial dysfunction. The 2 most common procedures used at present to achieve this objective are Descemet membrane endothelial keratoplasty (DMEK) and Descemet stripping automated endothelial keratoplasty (DSAEK),[1] although there is still some controversy regarding the risks and benefits of both techniques. In the DSAEK technique, Descemet membrane (DM), endothelium, and a thin layer of donor stroma are transplanted, whereas in DMEK, only DM and endothelium are transplanted. Best-corrected visual acuity (BCVA) after DSAEK is generally lower than that obtained with DMEK,[2–5] probably as a result of problems in the interface, folds in the donor disc due to poor fitting to the recipient stroma, disc decentration, and problems related to its thickness.[4,6,7]

According to the literature, numerous factors can cause this variability in DSAEK outcomes: postoperative corneal thickness of the transplanted disc, characteristics of the patient and tissue, surgical technique, correct disc centration, disparity in curvature between the posterior surface of the recipient and the transplanted disc or postoperative inflammation and scarring.[7,8] High-order aberrations in the anterior and posterior surfaces can also negatively affect the final visual acuity (VA), as can the presence of an interface between donor and recipient.[9–14]

Several authors suggest that the quality of the interface is a determining factor in final VA after DSAEK,[15–17] and it has been well established that its regularity affects the forward light scatter or glare that can occur after DSAEK, which would effectively result in a poorer BCVA.[10]

Given that the recipient bed is the same for DSAEK and DMEK, because a descemetorhexis is performed in both techniques,[4] one component that could affect the quality of the interface is the contact surface of the transplanted tissue. This study developed from the hypothesis that the less homogeneous the contact surface of the transplanted tissue

Received for publication October 6, 2016; revision received November 21, 2016; accepted November 22, 2016. Published online ahead of print January 11, 2017.

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The authors have no funding or conflicts of interest to disclose.

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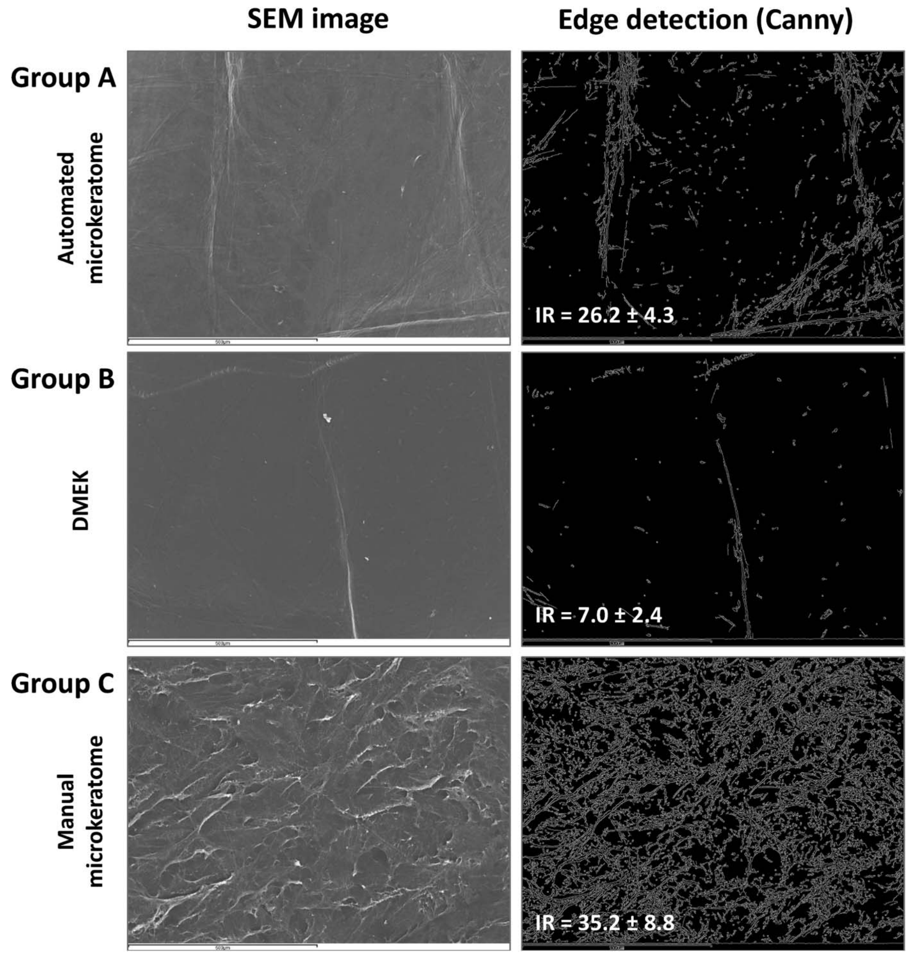

FIGURE 1. Images of 3 samples from the 3 groups. Photographs obtained by SEM are shown on the left. The right-hand column shows its conversion to a digital image with the Canny method using the 20 threshold, the lowest used in this study to detect irregularities. For informative purposes, the irregularity indices (IR) of these groups are included as mean and SD.

is, the more refractive the interface might be and more light scatter would be produced, which has implications for the BCVA.[10,18–20] Thus, the cutting method in DSAEK could be crucial, and differences in the homogeneity of the interface resulting from the use of different methods for obtaining the graft could have contributed to the variability in DSAEK outcomes reported in the literature. The aim of this study was therefore to determine differences in the regularity of the graft stromal surface according to whether it had been obtained using a manual or automated microkeratome for DSAEK, and, furthermore, to compare it with the surface obtained using DMEK.

MATERIALS AND METHODS

The stromal beds of 25 corneal discs that were obtained consecutively and used for endothelial transplantation (DSAEK or DMEK) were analyzed. The grafts had been obtained using 1 of 3 techniques, subdividing the sample into the following groups: 1) cut with an automated linear microkeratome with a constant translation speed (Amadeus II; Ziemer Ophthalmic Systems AG, Port, Switzerland) (11 samples); 2) manual dissection of DM to perform DMEK (5 samples); 3) cut with a manual rotating microkeratome (ALTK system; Moria, Antony, France) (9 samples).

Obtaining and Processing the Stromal Bed Samples

Once each corneal graft had been obtained for the intervention (dissection of DM in DMEK or cut with a microkeratome in DSAEK), the stromal bed was trephined with a 3-mm dermatological punch (Stiefel biopsy punch, SmithKline Beecham Ltd, Slough, Berks, United Kingdom) and the tissue was immersed in cold 2% glutaraldehyde (4°C) for fixation and preservation. Sample preparation for scanning electron microscopy (SEM) consisted of dehydration in a graded acetone series, mounting on copper stubs with conductive carbon tape, and coating with colloidal gold (BAL-TEC SCD 005 gold sputter coater, Fürstentum, Liechtenstein).

Image Acquisition and Analysis

An optimal image of the center of the stromal bed on the reverse of the epithelial side was obtained with a scanning electron microscope (JEOL JSM 6300, Semicon, Tokyo, Japan). This consisted of a 1- × 1-mm specular image of the transplanted tissue, which was magnified 100 times. All the images were taken in the same brightness and contrast conditions (230 and 225, respectively, according to the manufacturer's units), and we ensured that the image was grayscale.

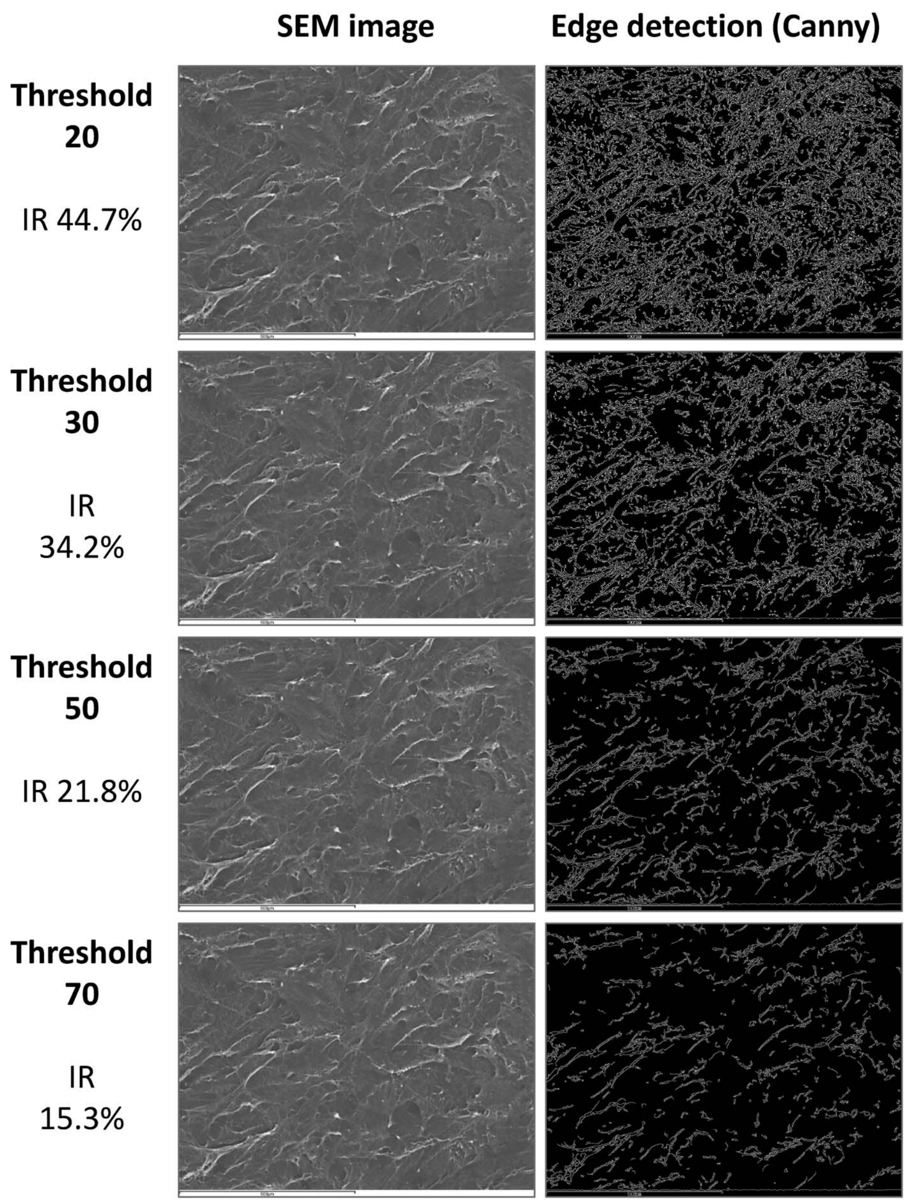

FIGURE 2. Images of the same sample (left) in which the Canny algorithm (right) was applied using the different sensitivity thresholds for the detection of edges included in the analysis in this study. The lower the threshold, the more irregularity can be detected, so the same sample will have a lower IR at a higher threshold.

For digital image processing, we used the basic rule that an edge is the boundary between an object and the background. By identifying this edge, the whole object can be located and its basic properties can be measured.[21] Global edge detection was performed using the Canny method.[22,23] This is subdivided into: 1) the filtering step, in which the magnitude and direction of the gradient vector in each pixel are calculated, to remove noise from the original image; 2) taking decisions about the possible edges of the image according to the brightness gradient, achieving thinning of the width of the edges; and 3) thresholding with hysteresis: application of detection thresholds to determine the definite edge pixels or background pixels. Two thresholds were used in the algorithm: the minimum threshold and the maximum threshold. Implementation of the Canny algorithm used in this study was based on the OpenCV library system,[24] so the minimum threshold and a 3:1 optimized ratio or relationship with the maximum threshold was used, that is the maximum threshold was 3 times the minimum threshold. The aim was to mathematically detect the percentage roughness or irregularity index (IR) of the original image. To that end, a fourth step was added to the Canny algorithm, and the IR of the image was calculated as the proportion of pixels other than the background detected in the third step, with respect to the total number of pixels in the image. Values were assigned on a scale between 0 and 1, where 0 (or 0%) is a completely smooth image (all pixels are black) and 1 (or 100%) is a completely irregular image (all pixels are white) (Fig. 1). The IR was assessed using 4 different detection thresholds (Fig. 2).

Statistical Analysis

A descriptive analysis of the IR or irregularity percentages was performed. Results are shown as minimums, maximums, mean and SD, and medians at the 20, 30, 50, and 70 thresholds for the whole sample, and for the 3 groups (A, B, and C). Sample groups were compared according to the method used for obtaining the graft with nonparametric tests (Kruskal–Wallis test for the 3 groups and the Mann–Whitney *U* test for post hoc comparisons), and the significance level was set at 0.05. Analyses were performed in the R environment.

RESULTS

Table 1 summarizes the overall sample characteristics. Group B (corneas dissected by DMEK) showed the lowest percentage of irregularity at all thresholds with respect to the other 2 groups, whereas group C (corneas dissected with a manual microkeratome) presented the highest IR at all sensitivity thresholds for edge detection. Comparison of irregularity medians between the 3 groups revealed statistically significant differences (Table 1). Statistically significant differences were also found between the 3 groups compared 2 to 2 at all the thresholds (Table 2).

Figure 3 depicts the degree of irregularity of each group with respect to the mean of the whole sample (corresponding to the 100%) considering the different thresholds measured. The maximum level of regularity was observed in the DMEK samples in group B.

DISCUSSION

This image analysis study using SEM and validated image analysis techniques conclusively shows significant differences, depending on the cutting method used, in the stromal surface of the cornea obtained to form part of the interface between the recipient cornea and the graft. The safety and efficacy of DSAEK is already well established in the literature, although DMEK seems to be preferred over DSAEK, as it provides complete, more rapid visual rehabilitation.[2,5,25] The significant improvement in visual function with DSAEK, which provides good uncorrected visual acuities, gives it a major advantage over penetrating keratoplasty, but the outcome can be suboptimal in some patients. Vajaranant et al[26] reported a mean VA of 20/40 one year after DSAEK in more than 300 eyes. Outcomes in the literature with this technique tend to vary widely,[5,13,25,27] whereas those achieved with DMEK are relatively homogeneous. Although multiple factors can cause this variability, according to the literature, the reason for a lower VA in patients with partial thickness corneal transplant (as in DSAEK) is not clear.

Forward light scatter affects the visual outcome and is caused by imperfections in the optical elements of the eye, and some of the components of this scatter are related to the incapacitating glare.[14,28] Because light scatter is a phenomenon by which light is diverted from its straight path because of lack of uniformity of the medium through which it passes,[19] irregularities in the contact interface of 2 optical elements of the eye will affect the visual quality, as occurs in the case of DSAEK and DMEK.[15–17] Barrajón-Rodríguez et al[10] evaluated changes in BCVA in a group of patients who had undergone DSAEK, and correlated this value with the value of the donor–recipient interface measured by the Scheimpflug camera in corneal topography. They found that when a lower optical density value was obtained in the

TABLE 1. Descriptive Analysis of the Percentage Irregularity of the Corneas Included in the Study, Measured at the Different Image Analysis Thresholds

	N	Minimum	Maximum	Mean	SD	Variance	Median	*P*
Threshold 20								
Total	25	3.5	44.7	25.6	12.8	163.5	24.8	
Group A	11	14.7	39.8	26.2	8.4	71.1	24.1	
Group B	5	3.5	9.6	7.0	2.4	5.6	7.0	0.001
Group C	9	17.1	44.7	35.2	8.8	78.1	37.6	
Threshold 30								
Total	25	2.1	34.2	16.1	9.3	87.3	15.6	
Group A	11	6.8	25.3	14.8	6.1	37.3	12.9	
Group B	5	2.1	5.5	4.2	1.4	2.1	4.7	<0.001
Group C	9	11.3	34.2	24.4	6.9	47.1	24.9	
Threshold 50								
Total	25	1.3	21.8	8.4	5.3	28.6	7.0	
Group A	11	2.9	12.1	6.7	3.0	9.1	6.3	
Group B	5	1.3	3.0	2.4	0.7	0.5	2.7	<0.001
Group C	9	7.0	21.8	13.7	4.1	16.6	13.6	
Threshold 70								
Total	25	1.2	15.3	5.3	3.4	11.7	4.4	
Group A	11	1.9	7.3	4.0	1.7	2.9	4.0	
Group B	5	1.2	2.3	1.8	0.5	0.2	1.9	<0.001
Group C	9	4.9	15.3	8.8	2.8	8.1	9.0	

Comparison between groups using the Kruskal–Wallis test.

TABLE 2. Paired Comparisons of the Irregularities of Each Group or Technique at the Different Thresholds Used

	Threshold	Group B (n = 5)	Group C (n = 9)
Group A (n = 11)	20	<0.001	0.03
	30	<0.001	0.011
	50	0.002	0.001
	70	0.005	0.001
Group B (n = 5)	20	—	0.001
	30	—	0.001
	50	—	0.001
	70	—	0.001

Comparisons made using the Mann–Whitney *U* test.

interface, the change in the BCVA was greater. Patients who had a better BCVA at 3 months were also found to have a lower donor–recipient interface value, or in other words, the higher the refraction of the resulting interface, the lower the BCVA.

Our group previously showed that discs <130 μm thick obtained a mean postoperative corrected distance VA (0.80 ± 0.16; range, 0.55–1.2) that was 0.7 or better in 78.1% of patients.[27] Our conclusion was that corneal thickness could be one of the main factors that might explain the variability in corrected distance VA after DSAEK, despite other contradictory findings in the literature.[29–31] We also considered that variability with other studies might be partly explained using different techniques for obtaining tissue. Our study was performed with an automated linear microkeratome (the one used in this study), whereas the other studies referenced used a manual rotating microkeratome.[13,17,32,33]

This study found that a manual microkeratome provides more irregular stromal beds than does an automated one. We cannot ascertain the reasons underlying this difference, although nonuniform advancement speed in the former (depending on the operator's ability) compared with the constant and uniform speed of the latter may play a role. This irregularity is presumably the specular reflection of the complementary surface of the graft that was implanted. To avoid subjectivity in describing these irregularities,[20] we used the Canny method, an objective quantification method that uses image analysis algorithms.[22,23] Analyses were performed with different thresholds to avoid possible problems in thresholding the sensitivity of detection, because if a very high sensitivity threshold is established, part of the edges (part of the irregularities) is lost. Conversely, if a very low threshold is entered, all the edges are detected, but a lot of "noise" also appears in the image. It was therefore considered advisable to perform a comparison study between different values. The results obtained after transforming the image of each cornea to an IR clearly show that the bed after dissection of DM with DMEK is much more regular than the one obtained after cutting a cornea with a microkeratome. Because of the issues of light scatter discussed above, we believe that these differences in the IR, along with the scarring processes, could contribute to the better visual outcomes obtained after DMEK compared with DSAEK.[9–12,14]

It is interesting to note in this study that the mean IR with the automated microkeratome in group A is significantly better than the mean IR of the tissues cut manually (group C) at all thresholds, and closer to the mean of the tissues analyzed for DMEK surgery. As expected, the samples from group B (manual dissection of DM) presented an IR that was much lower than the mean.

The main limitation of this experimental study is that we were unable to compare the IR of each sample with the clinical outcomes, because most of our patients operated by DSAEK had some associated comorbidity that prevented the necessary measurements from being performed (age-related macular degeneration, high myopia, stromal fibrosis, irregular astigmatism, lens decentration, multifocal lens, etc).

We consider that the Canny method in SEM images enables irregularity of a corneal stromal bed to be objectively quantified after any cut or dissection. The results suggest that an automated linear, uniform-speed microkeratome provides

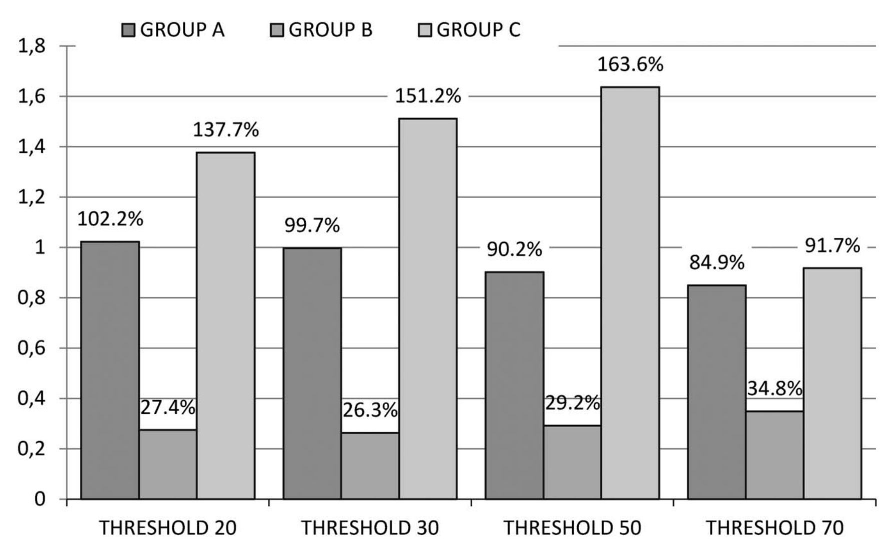

FIGURE 3. Irregularity percentages of each group with respect to the IR of the total sample of 25 stromal beds (taken as 100%).

a homogeneous interface that could significantly help to improve the visual quality resulting from a DSAEK intervention. The fact that most published studies on DSAEK are performed using a manual microkeratome with a nonuniform speed could help explain the great variability in DSAEK outcomes described by various authors,[5,9,32] whereas the homogeneity of the surface obtained by dissection of DM by DMEK could have contributed to the fact that the results obtained with DMEK in the different series are very similar to each other.[5,9,30] The cause of the variability in outcomes with DSAEK may be multifactorial, but the variability of irregularities in the stromal surface of the grafts obtained with different instruments could be a very important factor.

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