Objective: To determine if keratocyte populations are different in corneas with Fuchs dystrophy compared with control corneas.

Methods: Eleven corneas excised during penetrating keratoplasty for Fuchs dystrophy and 5 control corneas of eyes enucleated for choroidal melanoma were examined using light microscopy. Twenty control corneas age-matched to the corneas with Fuchs dystrophy were examined using confocal microscopy in vivo. The number of keratocytes in a full-thickness column of central stroma with frontal area of 1 mm², determined using histologic and confocal methods, was compared between corneas with Fuchs dystrophy and controls.

Results: By histology, the mean (SD) number of cells in a full-thickness column of stroma in Fuchs dystrophy (12,215 ± 1,394 cells) was less than in control corneas (15,628 ± 710 cells; P < .001). The mean (SD) number of keratocytes in the anterior 10% of the stroma of corneas with Fuchs dystrophy (682 ± 274 cells) was less than in the control corneas measured using histology (1,858 ± 404 cells; P < .001) and confocal microscopy (1,481 ± 397 cells; P < .001).

Conclusions: Keratocytes are depleted by 54% to 63% in the anterior 10% of the stroma of corneas that require penetrating keratoplasty for Fuchs dystrophy. Keratocyte loss might contribute to anterior stromal changes that persist and degrade vision after endothelial keratoplasty.


UCHS ENDOTHELIAL DYSTROPHY is a bilateral, slowly progressive corneal endothelial disorder characterized by the formation of collagenous excrescences (guttae) on Descemet membrane and corneal edema due to endothelial cell loss or dysfunction. Surgical intervention for Fuchs dystrophy involves endothelial cell replacement by corneal transplantation. Previously, penetrating keratoplasty (PK) was the transplantation procedure of choice for Fuchs dystrophy, with replacement of all layers of the host cornea, but in the last decade, endothelial keratoplasty (EK) has surpassed PK as the preferred transplantation technique. After EK for Fuchs dystrophy, the host’s corneal stroma is retained and scatters more light than the stroma of control corneas, presumably contributing to visual degradation. The scatter, or haze, is highest from the anterior cornea and does not return to normal throughout the first year after EK. The origin of increased scatter has been hypothesized to be cellular or extracellular ultrastructural changes in the stroma in response to chronic edema.

A few studies have examined the stroma of corneas with Fuchs dystrophy and have demonstrated subepithelial fibrosis with alterations in the collagenous extracellular matrix, but little is known about the keratocytes. Keratocytes maintain the stroma and presumably its transparency, and are also involved in wound healing and collagen formation. Activation of kerocytes in response to injury has also been suggested to cause corneal haze, thus, it is possible that keratocyte dysfunction contributes to corneal haze in Fuchs dystrophy. Confocal microscopy in vivo has demonstrated structural alterations in keratocytes in Fuchs dystrophy, but quantification of keratocytes in Fuchs dystrophy by confocal microscopy is potentially inaccurate because of increased image reflectivity caused by corneal haze.

Given the current era of EK, the cells and structure of the retained host cornea are becoming important for understand-
METHODS

SUBJECTS

Eleven corneas from 10 patients scheduled for PK for Fuchs dystrophy were recruited from the cornea service at Mayo Clinic, Rochester, Minnesota; the mean (SD) age was 70 (7) years (range, 62-78 years). Corneas were examined by slitlamp biomicroscopy, and central thickness was measured by an ultrasonic pachymeter (DGH 1000; DGH Technologies, Inc, Frazer, Pennsylvania). Best-corrected visual acuity was measured using the electronic Early Treatment of Diabetic Retinopathy Study protocol15 and expressed as a logarithm of the minimum angle of resolution (logMAR). Patients with a history of contact lens wear, glaucoma, previous ocular trauma, or surgery (except for cataract surgery), diabetes mellitus, or use of medications known to affect the cornea were excluded. Five control corneas from 5 patients who were scheduled for enucleation for choroidal melanoma were also recruited to serve as controls with histologic data; the mean (SD) age was 63 (15) years (range, 40-80 years). Twenty control corneas from 20 subjects enrolled in a previous study16 were used as additional controls for estimates of keratocyte density by confocal microscopy in vivo. This group was age-matched to the subjects with Fuchs dystrophy and had a mean (SD) age of 70 (3) years (range, 61-80 years). This study adhered to the tenets of the Declaration of Helsinki and was approved by the Mayo Clinic Institutional Review Board. Written informed consent was obtained from all subjects after explanation of the study.

CONFOCAL MICROSCOPY IN VIVO

The central cornea was examined in vivo using a Tandem Scanning confocal microscope (Tandem Scanning Corporation, Reston, Virginia); eyes scheduled for surgery were examined prior to surgery (except for 2 control corneas from 2 subjects with choroidal melanoma). The examination procedure has been described in detail previously.14,16,17

HISTOLOGY

After removal of the corneas with Fuchs dystrophy for PK and from enucleated eyes with choroidal melanoma, the central cornea was fixed in 10% buffered formalin. The corneal button was embedded in paraffin and 4-µm-thick serial sagittal sections were cut and stained with 4′,6-diamidino-2-phenylindole. Fifteen serial sections spaced 8 µm apart were photographed at an original magnification of ×20 (Olympus DP70 camera operating on a BX60 transmission light microscope; Olympus America Inc, Center Valley, Pennsylvania). Images of a reticle were captured at the same magnification for calibration of horizontal and vertical dimensions.

Additional sections were stained with hematoxylin-eosin for examination by light microscopy. The diagnosis of Fuchs dystrophy was confirmed by an ophthalmic pathologist based on the presence of guttae, thickening of Descemet membrane, and endothelial cell loss.

KERATOCYTE DENSITY AND STEREOLOGY

From confocal images, cell nuclei, which appeared as bright objects, were identified using a custom automated program.14 From histologic images, 4′,6-diamidino-2-phenylindole–stained nuclei in the corneal stroma were identified by one observer using a point-and-click method (Figure 1). Volumetric cell density was calculated using stereologic methods from the number of cells in a predefined area of the confocal and histologic images, as described previously.16,17 The sum of confocal depth of field and thickness of a keratocyte nucleus was 11.9 µm.18 In histologic images of all corneas, cell densities were calculated using stromal thickness measured by confocal microscopy in vivo16,17 to avoid shrinkage artifacts caused by fixation; the other 2 dimensions were corrected for tissue shrinkage of 5.7%, as described previously in control corneas.16,18 We assumed that the frontal diameter of a keratocyte nucleus was 16 µm in histologic samples.16,20

Keratocyte density was estimated in 5 layers of stroma, the anterior 10%, 10% to 33%, 33% to 66%, 66% to 90%, and the posterior 10% of stromal thickness (Figure 2).16 By confocal microscopy, cell density was estimated from 2 manually selected frames from each layer. Each histologic section was divided into the same layers of stroma based on the stromal thickness in the section.

ABSOLUTE NUMBER OF KERATOCYTES

Keratocyte density decreases in edematous corneas because cells are distributed throughout a larger volume of tissue. To elimi-
were not examined by confocal microscopy preoperatively. In vivo, which was not available for 2 control corneas because they because this did require a knowledge of stromal thickness in vivo, the number of keratocytes could only be estimated in 3 control corneas by histology. In contrast, keratocyte density in corneas with Fuchs dystrophy was determined from the depth of the anterior stromal hypocellular zone in corneas, we used paired multiple comparisons by using the Bonferroni technique, and P < .05 was considered significant.

Keratocyte density and the number of keratocytes determined using confocal microscopy were compared with those determined using histology; for corneas with Fuchs dystrophy, we used generalized estimating equation models to account for possible correlation between fellow eyes of the same subject.22 P values were adjusted for multiple comparisons by using the Bonferroni technique, and P < .05 was considered significant.

Keratocyte density and the number of keratocytes determined using confocal microscopy were compared with those determined using histology; for corneas with Fuchs dystrophy, we used generalized estimating equation models to account for possible correlation between fellow eyes of the same subject.22 P values were adjusted for multiple comparisons by using the Bonferroni technique, and P < .05 was considered significant.

Keratocyte density and the number of keratocytes determined using histology in corneas with Fuchs dystrophy were compared with those determined by histology in the control corneas after enucleation and to those determined by confocal microscopy in vivo in the 20 age-matched control corneas. Densities were compared between groups using generalized estimating equation models to account for possible correlation between fellow eyes of the same subject.22 P values were adjusted for multiple comparisons by using the Bonferroni technique, and P < .05 was considered significant.

Keratocyte density and the number of keratocytes determined using confocal microscopy were compared with those determined using histology; for corneas with Fuchs dystrophy, we used generalized estimating equation models, and for control corneas, we used paired t tests. Correlations were assessed using the Pearson correlation coefficient, with significances calculated by using generalized estimating equation models.

STATISTICAL ANALYSIS

DEPTH OF HYPOCELLULAR ZONE

The thickness of the anterior stromal hypocellular zone in corneas with Fuchs dystrophy was determined from the depth of key structures in confocal scans.16,17 Images were reviewed by 2 experienced observers (J.W.M., S.V.P.) to identify the surface epithelium, subbasal nerve plexus, and first image with normal-appearing countable keratocyte nuclei. In control corneas, the Bowman layer is not visible but the image of the most anterior and highest density of keratocyte nuclei corresponds to the boundary between the Bowman layer and the cellular stroma (Figure 3). In corneas with Fuchs dystrophy, we identified the boundary between the Bowman layer and stroma by the presence of sparse bright objects that were reminiscent of keratocyte nuclei but were morphologically abnormal and possibly represented degenerate keratocytes (Figure 3). This image was always deep to the subbasal nerves, and the distance between this image and the first keratocyte nuclei with normal morphology was defined as the hypocellular zone. Images were also examined to identify activated keratocytes, which morphologically have visible cell bodies and processes.

RESULTS

CLINICAL AND HISTOPATHOLOGIC FINDINGS

By slitlamp examination, all 11 eyes with Fuchs dystrophy had central confluent guttae with variable amounts of stromal edema; epithelial edema was clinically noted in only 1 eye. The mean (SD) best-corrected visual acuity was 0.49 (0.10) logMAR (Snellen equivalent, 20/62; range, 0.34–0.66 logMAR). The mean (SD) central corneal thickness by ultrasonic pachymetry was 642 (54) µm (range, 575 to 766 µm). Seven eyes had a cataract and
FUCHS DYSTROPHY VS CONTROLS

By histology, full-thickness keratocyte density in corneas with Fuchs dystrophy (mean [SD], 23 872 [3340] cells/mm²; n = 11) was lower than that in control corneas (mean [SD], 33 376 [2430] cells/mm²; n = 3; P < .001). Keratocyte densities were lower in all layers of stroma in corneas with Fuchs dystrophy than in corresponding layers of control corneas (P < .001), with the exception of the middle third of the stroma (P = .99; Table; Figure 4). By histology, the mean (SD) number of keratocytes in a full-thickness column of stroma of corneas with Fuchs dystrophy (12 215 [1394] cells; n = 11) was lower than that of control corneas (15 628 [710] cells; n = 5; P < .001; Table). The mean (SD) number of keratocytes in the anterior 10% of the stroma of corneas with Fuchs dystrophy (682 [274] cells; n = 11) was lower than in the control corneas (1858 [404] cells; n = 5; P < .001; Figure 1; Table).

The mean (SD) full-thickness cell density determined by histology in the 11 corneas with Fuchs dystrophy was not different from full-thickness cell density determined by confocal microscopy in the 20 age-matched control corneas (21 463 [4429] cells/mm²; P = .13). In corneas with Fuchs dystrophy, the number of keratocytes was decreased only in the anterior 10% of the stroma compared with the number of keratocytes in the same layer of the age-matched, control corneas (mean [SD], 1481 [397] cells; n = 20; P < .001).

In eyes with Fuchs dystrophy, best-corrected visual acuity correlated with the absolute number of keratocytes in a full-thickness column of stroma (r = −.47; P = .11) or the number of keratocytes in the anterior 10% of the stroma (r = −.47; P = .14; n = 11). There were no correlations between central corneal thickness and the absolute number of keratocytes in a full-thickness column of stroma (r = .28; P = .40; n = 11) or the number of keratocytes in the anterior 10% of the stroma (r = .13; P = .71; n = 11). With a sample size of 11, the minimum detectable correlation was ±0.69 (or $r^2 = 0.48$; α = .05, β = .20).

CONFOCAL MICROSCOPY VS HISTOLOGY

In corneas with Fuchs dystrophy, the weighted mean (SD) keratocyte density for the full-thickness stroma determined by automated assessment of confocal images was 21 507 (3915) cells/mm² and did not differ from that determined by histology (23 872 [3340] cells/mm²; P = .11). By the confocal method, keratocyte density was overestimated in the anterior 10% of the stroma (P < .001) and underestimated in the posterior third of the stroma (P = .02, 66%-90%; P < .001, posterior 10%) compared with the histologic method (Figure 5).

In control corneas, the weighted mean (SD) keratocyte density for the full-thickness stroma determined by automated assessment of confocal images was 29 200 (6534) cells/mm² and did not differ from that determined by histology (33 376 [2430] cells/mm²; P = .25), although our sample size was small (n = 3). There were no differences in keratocyte density between the confocal and histologic methods within the different layers of stroma (P > .50; Figure 5).

<table>
<thead>
<tr>
<th>Stromal Depth</th>
<th>Fuchs (n=11)</th>
<th>Control (n=5)</th>
<th>P Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-thickness stroma</td>
<td>23 872 (3340)</td>
<td>33 376 (2430)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>By stromal layer, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 (anterior)</td>
<td>13 295 (5359)</td>
<td>42 747 (13,555)</td>
<td>.001</td>
</tr>
<tr>
<td>11-33</td>
<td>22 121 (3546)</td>
<td>31 345 (3050)</td>
<td>.99</td>
</tr>
<tr>
<td>67-90</td>
<td>25 611 (4542)</td>
<td>32 906 (1155)</td>
<td>.99</td>
</tr>
<tr>
<td>91-100 (posterior)</td>
<td>27 655 (5608)</td>
<td>37 562 (2735)</td>
<td>.99</td>
</tr>
</tbody>
</table>

aAbsolute number of cells in a section of stroma with frontal area of 1 mm².

bGeneralized estimating equation models.

![Figure 4](https://example.com/figure4.png)

Figure 4. Mean (SD) keratocyte density determined by histologic methods in 5 stromal layers. Keratocyte density was lower in corneas with Fuchs dystrophy (n = 11) than in control corneas (n = 3) in all layers of stroma except for the middle third.
The mean (SD) thickness of the anterior stromal hypocellular zone in corneas with Fuchs dystrophy was 15.6(8.5) µm (range, 4.9-32.0 µm). The first morphologically normal keratocyte nuclei were a mean (SD) of 27.4(9.6) µm (range, 9.6-41.8 µm) deep to the subbasal nerves, which were identified in all eyes. None of the images of corneas with Fuchs dystrophy contained activated keratocytes.

**COMMENT**

The major finding in this study was that keratocytes were depleted from the anterior stroma of corneas in advanced Fuchs endothelial dystrophy, forming an anterior hypocellular zone. Decreased stromal cellularity was strongly associated with worse visual acuity in eyes with Fuchs dystrophy; however, this finding should be interpreted with caution because 7 of the 11 eyes had cataracts, which would confound visual acuity. We also confirmed our suspicion that with the degraded confocal image conditions in corneas with Fuchs dystrophy, the automated estimation of keratocyte density by our software is not accurate.

**KERATOCELLULAR ZONE**

The mean (SD) thickness of the anterior stromal hypocellular zone in corneas with Fuchs dystrophy was 15.6(8.5) µm (range, 4.9-32.0 µm). The first morphologically normal keratocyte nuclei were a mean (SD) of 27.4(9.6) µm (range, 9.6-41.8 µm) deep to the subbasal nerves, which were identified in all eyes. None of the images of corneas with Fuchs dystrophy contained activated keratocytes.

**MECHANISM OF CELL LOSS**

The mechanism of cellular depletion from the anterior stroma of corneas with Fuchs dystrophy is unknown, but it is plausible that the keratocytes undergo apoptosis triggered by cytokine release from the epithelium, a known response to mechanical and viral epithelial injuries. Similarly, in corneas with advanced Fuchs dystrophy, it is conceivable that epithelial cell disruption or bullae formation result in cytokine release causing apoptosis of keratocytes. There is evidence of apoptosis in all cellular layers of the cornea in Fuchs dystrophy. Li et al suggest that keratocytes were hypersensitive to apoptotic stimuli and that changes in keratocytes might precede endothelial and epithelial changes. Szentmary et al found apoptotic cells in all layers of corneas with Fuchs dystrophy and pseudophakic corneal edema, indicating that kera-
endothelial keratoplasty has surpassed PK as the treatment of choice for Fuchs dystrophy, and although EK results in better uncorrected visual acuity compared with PK, many eyes fail to attain a best-corrected visual acuity of 20/20 or have resulting glare and poor contrast.\(^3\,\,^4\,\,^34\) Scatter or aberrations from the host cornea after EK may affect vision\(^1\); thus, understanding the changes and biology of the host cornea might provide insight into improving visual outcomes. Increased scatter in the anterior stroma of corneas with Fuchs dystrophy is not fully understood, although it is clinically recognized as subepithelial haze and contributes to increased forward scatter, which affects vision.\(^4\) Increased scatter after keratorefractive surgery has been attributed to increased reflectivity from activated keratocytes,\(^1\) although our results suggest that increased scatter in Fuchs dystrophy was not directly from keratocytes; we found no evidence of keratocyte activation, and the number of keratocytes in the anterior stroma was decreased. The increased scatter possibly originates from the extracellular matrix and might be related to the deposition of abnormal proteins or changes in the proteoglycan properties of the anterior stroma.\(^7\,\,^33\,\,^36\) Some have also suggested that increased scatter arises from fibril-free regions (lakes) of stroma caused by the death of keratocytes.\(^24\) The temporal relationship of matrix changes to keratocyte loss is unknown, but the loss of keratocytes may also impair the subsequent repair of the abnormal matrix.

The sequence of cellular and extracellular changes in the anterior stroma prior to EK, and whether or not keratocytes repopulate the anterior stroma after restoration of endothelial function, are unknown and warrant further investigation. Correlation of these changes to visual function might provide a better understanding of visual outcomes after EK, and the opportunity to modulate the stromal changes in the future could improve outcomes of this procedure.

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Correspondence: Sanjay V. Patel, MD, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (patel.sanjay@mayo.edu).

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ENDOTHELIAL KERATOPLASTY HAS SURPASSED PK AS THE TREATMENT OF CHOICE FOR FUCHS DYSTROPHY, AND ALTHOUGH EK RESULTS IN BETTER UNCORRECTED VISUAL ACUITY COMPARED WITH PK, MANY EYES FAIL TO ATTAIN A BEST-CORRECTED VISUAL ACUITY OF 20/20 OR HAVE RESULTING GLARE AND POOR CONTRAST.\(^3\,\,^4\,\,^34\) SCATTER OR ABERRATIONS FROM THE HOST CORNEA AFTER EK MAY AFFECT VISION\(^1\); THUS, UNDERSTANDING THE CHANGES AND BIOLOGY OF THE HOST CORNEA MIGHT PROVIDE INSIGHT INTO IMPROVING VISUAL OUTCOMES. INCREASED SCATTER IN THE ANTERIOR STROMA OF CORNEAS WITH FUCHS DYSTROPHY IS NOT FULLY UNDERSTOOD, ALTHOUGH IT IS CLINICALLY RECOGNIZED AS SUBEPITHELIAL HAZE AND CONTRIBUTES TO INCREASED FORWARD SCATTER, WHICH AFFECTS VISION.\(^4\) INCREASED SCATTER AFTER KEROREFRATIVE SURGERY HAS BEEN ATTRIBUTED TO INCREASED REFLECTIVITY FROM ACTIVATED KERATOCE,\(^1\) ALTHOUGH OUR RESULTS SUGGEST THAT INCREASED SCATTER IN FUCHS DYSTROPHY WAS NOT DIRECTLY FROM KERATOCE; WE FOUND NO EVIDENCE OF KERATOCYTE ACTIVATION, AND THE NUMBER OF KERATOCE IN THE ANTERIOR STROMA WAS DECREASED. THE INCREASED SCATTER POSSIBLY ORIGINATES FROM THE EXTRACELLULAR MATRIX AND MIGHT BE RELATED TO THE DEPOSITION OF ABNORMAL PROTEINS OR CHANGES IN THE PROTEOGLYCAN PROPERTIES OF THE ANTERIOR STROMA.\(^7\,\,^33\,\,^36\) SOME HAVE ALSO SUGGESTED THAT INCREASED SCATTER ARISES FROM FIBRIL-FREE REGIONS (LAKES) OF STROMA CAUSED BY THE DEATH OF KERATOCE.\(^24\) THE TEMPORAL RELATIONSHIP OF MATRIX CHANGES TO KERATOCYTE LOSS IS UNKNOWN, BUT THE LOSS OF KERATOCE MAY ALSO IMPAIR THE SUBSEQUENT REPAIR OF THE ABNORMAL MATRIX.

THE SEQUENCE OF CELLULAR AND EXTRACELLULAR CHANGES IN THE ANTERIOR STROMA PRIOR TO EK, AND WHETHER OR NOT KERATOCE REPOPULATE THE ANTERIOR STROMA AFTER RESTORATION OF ENDOTHELIAL FUNCTION, ARE UNKNOWN AND WARRANT FURTHER INVESTIGATION. CORRELATION OF THESE CHANGES TO VISUAL FUNCTION MIGHT PROVIDE A BETTER UNDERSTANDING OF VISUAL OUTCOMES AFTER EK, AND THE OPPORTUNITY TO MODULATE THE STROMAL CHANGES IN THE FUTURE COULD IMPROVE OUTCOMES OF THIS PROCEDURE.

CONFOCAL MICROSCOPY IN FUCHS DYSTROPHY

Confocal microscopy enables visualization of keratocyte and other cell nuclei in vivo as bright objects. Our automated program\(^1\) for estimating keratocyte density is rapid and eliminates the subjectivity introduced by manual analysis but requires keratocyte nuclei to have a consistent appearance and corneas to have normal or subnormal backscatter (haze).\(^25\,\,^33\) In the corneas with advanced Fuchs dystrophy, although automated assessment of confocal images indicated anterior keratocyte loss compared with controls, cell density was overestimated compared with histologic analysis. Manual review of the confocal images indicated that the program erroneously counted small bright objects and other diffuse localized areas of increased reflectivity (Figure 3). In the posterior stroma, the automated program underestimated keratocyte density compared with histologic methods, and we suspect that loss of image contrast caused by increased anterior stromal haze contributed to the underestimate. This program, and most likely other programs designed by using images of control corneas, cannot be used in corneas with advanced Fuchs endothelial dystrophy without modification to overcome these limitations.

Qualitative examination of the confocal images confirmed a sparse population of keratocytes in the anterior stroma of corneas with Fuchs dystrophy; the high density of cell nuclei found in control corneas was not visible in any of the corneas with Fuchs dystrophy (Figure 3), and the mean thickness of the anterior hypocellular stroma was 16 μm. Mustonen et al\(^1\) found a range of appearances of the anterior stroma including normal keratocyte nuclei, activated keratocytes, and diffuse haze in confocal images of corneas with different severities of Fuchs dystrophy. This suggests that the anterior stroma of corneas with Fuchs dystrophy undergoes different stages of cellular changes, or that some corneas behave differently compared with others. Further studies by confocal microscopy are needed to determine if a sequence of events in the anterior stroma is responsible for the changes that degrade vision.

CLINICAL RELEVANCE

Endothelial keratoplasty has surpassed PK as the treatment of choice for Fuchs dystrophy, and although EK...