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Imaging the Corneal Endothelium in Fuchs Corneal Endothelial Dystrophy

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Abstract

Fuchs endothelial corneal dystrophy (FECD) is characterized by the progressive degeneration of the corneal endothelium (CE). The purpose of this article is to review the diagnostic tools available to image and assess the CE in FECD. Slit-lamp biomicroscopy with specular reflection and retroillumination are important techniques to assess the CE. Objective diagnostic tests, such as retroillumination photographic analysis, specular microscopy, *in vivo* confocal microscopy (IVCM), and anterior segment optical coherence tomography, are valuable tools to evaluate the CE in FECD. Specular microscopy can be performed rapidly without touching the eye but requires a clear cornea with a smooth CE. In contrast, IVCM can image all layers of the cornea, even in advanced FECD. However, IVCM is contact-based and more technically challenging. It is important to select the appropriate objective diagnostic test to image and assess the CE in managing patients with FECD.

Keywords

confocal; cornea; endothelium; Fuchs; specular

Introduction

The corneal endothelium (CE) is the innermost layer of the cornea composed of interdigitated endothelial cells that form a mosaic pattern of mostly hexagonal shapes. The CE plays an essential role in maintaining the clarity of the cornea by acting as a barrier to the aqueous humour and by providing a metabolic pump. Corneal endothelial cells are arrested in a post-mitotic state and have a limited ability to proliferate *in vivo*.¹ There are approximately 4000 cells/mm² at birth, which decreases with age as the cornea grows and corneal endothelial cells undergo apoptosis.² While the progressive loss of corneal endothelial cells is considered part of the normal aging process, in certain conditions there is an expedited loss of corneal endothelial cells leading to corneal edema and vision loss. The most common disease affecting the CE is Fuchs endothelial corneal dystrophy (FECD), which affects approximately 4% of Caucasians over the age of 40 years old in the United

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States.³ FECD is a late onset, autosomal dominant disease that is characterized by the slow, progressive degeneration of the CE, resulting in corneal edema and vision loss.⁴ As FECD progresses, there are morphological changes in the endothelial cells' hexagonal shape and size, as well as the concomitant formation of extracellular deposits called guttae.^{5, 6} Guttae are thought to be excrescences of abnormal collagen deposited by the CE, and the accumulation of guttae is the first clinical sign of FECD since they can occur in corneas without edema or vision loss.^{6, 7} Guttae typically originate in the central cornea and radiate out toward the periphery, which leads to reduced endothelial cell density (ECD), loss of normal endothelial cell morphology, and endothelial cell death.⁸ FECD typically progresses through well-documented clinical stages, whereby in early stage disease, there are non-confluent central guttae without significant corneal opacification and edema.⁴ In advanced FECD there is a significant decrease in ECD, guttae become confluent, Descemet membrane (DM) is thickened and there is ensuing corneal edema, subepithelial fibrosis and opacification.⁴ The ability to visualize the CE is essential in the diagnosis of FECD and for monitoring the clinical course for endothelial cell loss. The purpose of this article is to review the diagnostic tools available to image and assess the CE in FECD.

Slit-lamp biomicroscopy

The slit-lamp biomicroscope allows for the direct illumination (diffuse illumination, focal illumination, and specular reflection) and indirect illumination (proximal illumination, sclerotic scatter, and retroillumination) of the cornea. Slit-beam illumination with a beam width of about 1 mm or less produces an optical cross section of the cornea that allows for visualization of the CE and any abnormalities including guttae. Specular reflections are normal light reflexes bouncing off a surface, and in the cornea a faint reflection comes from the posterior corneal surface. The specular reflection from the posterior corneal surface can be enhanced by setting the slit-beam arm at an angle of 60° from the viewing arm and using a short slit, and superimposing the corneal endothelial light reflex onto the lightbulb's filament's mirror image. Specular reflection can allow the clinician to assess the CE morphology at the slit-lamp. Retroillumination of the cornea is another important technique to assess and document the distribution and number of guttae.^{9, 10} After pupillary dilation, the cornea is examined with reflected light from the fundus using a small angle between the illumination and biomicroscope.¹⁰ This retroillumination technique results in the visualization of individual and confluent guttae from light scattering (Figure 1).¹⁰ Retroillumination photography analysis is an effective way to document the number and distribution of guttae, and to demonstrate the formation of new guttae and progression over time.^{9, 10} To address the resource-intensive nature of manually counting guttae, an automated method for retroillumination photography analysis has been developed and has shown to be highly correlated with both manual and Krachmer grading of guttae.⁹

While the slit-lamp examination including specular reflection and retroillumination is an essential part of the clinical examination, additional objective diagnostic testing such as specular microscopy and confocal microscopy are often needed for a more comprehensive evaluation of the CE for initial diagnosis or ongoing management.

Specular microscopy

Specular microscopy is a non-invasive technique used to assess the structure of the CE and is now the most widely used imaging modality to study FECD.^{11, 12} In 1920, Vogt first described the *in vivo* visualization of the CE.¹³ In 1968, Maurice developed specular microscopy to study the CE *in vivo*.¹¹ His techniques were further developed into the specular microscope, which could be used clinically to evaluate and photograph the CE in patients.^{14, 15} Specular microscopy allows for the *in vivo* visualization of the CE using specular reflection with slit-lamp biomicroscopy (Figure 2A). Since the refractive index of the endothelial cells is greater than that of the 1.336 value of aqueous humour, the CE reflects 0.022 % of the projected light and thus can be visualized as a high-magnification image of the specular-reflected light.^{11, 16, 17} The specular microscope was initially designed to contact the corneal surface with a coupling gel, but a non-contact interface is primarily used in clinical practice since it is easier to use and has been shown to be equivalent in determining ECD in normal corneas.¹⁸ Some commercially available non-contact specular microscopes included Konan Noncon Robo (Konan Medical, Japan), CEM-530 (Nidek, Japan), Tomey EM-3000 (Tomey, United States), Topcon SP-2000P and Topcon SP-3000P (Topcon Corp, United States). Specular microscopy has numerous advantages including its non-contact image acquisition technique, rapid image acquisition time, automated focusing technology and analysis of the CE.¹⁷ It can determine ECD, polymegethism or coefficient of variation (CV), pleomorphism, central corneal thickness (CCT), and also allows for the visualization of guttae. ECD is the average number of endothelial cells per mm². Polymegethism or CV describes the variation in cell area and is calculated by dividing the standard deviation of the cell area by the mean cell area (µm²). Pleomorphism is the percentage of hexagonal cells in the CE, and a healthy cornea is expected to have 60% of cells as hexagons.¹⁷ These outcomes are important for the diagnosis, monitoring and surgical planning in patients with FECD. For example, ECD <1000 cells/mm², polymegethism or CV >0.40, and/or pleomorphism <50% might not tolerate intraocular surgery.¹⁹

In FECD, guttae appear as dark hyporeflective round bodies with an occasional central white reflex (Figure 2C, 2E).^{6, 20, 21} This central white reflex corresponds to the umbilicated top of the guttae, where there is an abrupt change in the index of refraction between the surface of the guttae and the aqueous humour.²¹ Specular microscopy has also revealed the progressive morphological changes of corneal guttae in FECD, which can be described in five specific stages based on size, abnormality of endothelial cells, and coalescence of excrescences.⁶ Stage 1, the earliest form of corneal guttae, is a dark structure with a single sharply defined bright spot at its center that is smaller than the size of an individual endothelial cell.⁶ In stage 2, the excrescence is larger and is approximately the size of an individual endothelial cell, with adjacent endothelial cells forming a rosette pattern (Figure 2E).⁶ In stage 3, the excrescence is significantly larger and affects many endothelial cells, and adjacent endothelial cells are distinctly abnormal (Figure 2C, 2E). There are two types of excrescences that are described in this stage: a smooth round excrescence and a rough excrescence. In stage 4, there are many coalesced excrescences and non-adjacent endothelial cells tend to be larger in size than normal. In stage 5, there are no recognizable cells or cell

boundaries and there is a reversal of the typical pattern of light gray cells outlined by dark boundaries, where there is a dark interior surrounded by a bright boundary.⁶ All 5 stages of guttae can be observed in the same patient without any clinically significant corneal edema. While it has been suggested that more advanced cases of FECD are correlated with later stages of guttae, late stages of guttae can be observed in early FECD and early stages of guttae in late FECD.⁶ However, limited conclusions can be made using specular microscopy in advanced FECD with significant corneal edema since no reliable images can be acquired in these patients.⁶ The major limitation of specular microscopy is that image acquisition is limited to transparent corneas that have a smooth corneal endothelial layer, since corneal pathology such as scarring or edema can increase light scattering in the stroma from collagen lamellae and keratocytes.²² Therefore, specular microscopy has a limited application in patients with advanced FECD with significant corneal edema and endothelial cell loss but is an important diagnostic instrument in patients with early FECD.

While a specular microscope is a valuable diagnostic tool to quantify ECD, its high operation costs may limit accessibility in rural areas or underdeveloped countries. Smartphone-based specular microscopy of the CE has been described utilizing the specular reflection from the endothelial layer.²³ Using this technique, sub-cellular resolution images were obtained and ECD could be determined.²³ While this smartphone-based specular microscopy seems promising, especially if it can be applied in rural and underdeveloped countries in a cost-effective manner, it still has not been validated or compared to commercially available specular microscopes.

Confocal microscopy

The confocal microscope was first used to examine the human eye *ex vivo* in 1985.²⁴ In 1990, confocal microscopy was further developed into a safe and rapid contact based imaging technique that allowed the visualization of all corneal layers *in vivo*.²⁵ *In vivo* confocal microscopy (IVCM) provides a clear picture of the endothelial mosaic with discernible cell borders that allows for identification and visualization of corneal endothelial cells and guttae (Figure 2B, 2D, 2F).^{26, 27} The principle of confocal microscopy is that the illumination and detection paths share the same focal plane. This optical arrangement is called confocal and overcomes the problem of defocused light and avoids the limitations in image quality achieved with conventional light microscopy.^{28, 29} However, IVCM requires a coupling gel to reduce light scattering at the corneal epithelium. This allows for clearer images in diseased corneas such as advanced FECD with significant corneal edema.^{20, 26, 30} In 1998, IVCM was first utilized in FECD patients to show the structural changes in the CE and other corneal layers.³⁰ Different technologies exist with various specifications such scan acquisition time and Z resolutions including the tandem scanning confocal microscope (30 frames/sec; 9–12 μm), slit scanning microscope (25 frames/sec; 8–25 μm), and laser scanning confocal microscope (30 frames/sec; 4 μm).^{28, 29} Some commercially available confocal microscopes are Confoscan P4 (Tomey Corporation, USA), Confoscan 3 or 4 (Nidek Technologies, Japan), and the Heidelberg Retina Tomograph II Rostock Corneal Module (HRT II RCM) (Heidelberg, Germany).²⁹ There is limited data comparing the different types of IVCM but some studies have found good correlation between devices, while other studies have found poor correlation.^{29, 31, 32} This has been attributed to

differences in methods used to calculate ECD, different endothelial areas used for assessment, and the lack of repeatability between measurements.³¹

IVCM has several advantages including its non-invasive image acquisition technique, its high magnification and resolution of corneal structures, its ability to provide images of all layers of the cornea, and offers the ability to analyze structures through corneal opacities and corneal edema.^{26, 27, 29, 33} Kaufman and colleagues first described the confocal microscopic findings in FECD in 1993.³⁴ In FECD, guttae appear as dark round bodies (20–400 μm) with occasional central white reflex (5–10 μm) (Figure 2D, 2F).^{20, 30, 33, 34} Confocal microscopy also allows for the monitoring of pathological changes in FECD in all corneal layers including the epithelium, Bowman's layer, anterior and posterior stroma, DM and the CE.³⁰ In FECD, while more anterior changes such as epithelial bullae and cystic lesions are observed, most changes occur in the posterior layers including a thickened DM, which appears as an abnormal diffuse acellular reflection between the posterior stroma and CE, and dark bands in the thickened DM.^{30, 35} Furthermore, it has been recently demonstrated through IVCM that there is a profound diminishment of sub-basal corneal nerves and increased immune dendritiform cell density in FECD.^{36, 37}

Since IVCM can image the entire cornea, it is also helpful in assessing corneal ectasias, dystrophies, degenerations, limbal stem cell deficiency, iridocorneal endothelial syndrome, sub-basal nerve architecture, diabetic neuropathy, corneal deposits, infective keratitis (especially *Acanthamoeba* and fungal keratitis) and post-surgical corneas.^{29, 37-39}

Specular Microscopy vs Confocal Microscopy

Both specular microscopy and confocal microscopy produce endothelial images easily in normal eyes without significant corneal scarring or edema, and no difference in ECD measured by either technique is observed.^{26, 30, 31, 40-45} A prospective study by Salvétat and colleagues comparing confocal microscopy (HRT II RCM) and non-contact specular microscope (Tomey EM-3000) showed an overall good intermethod agreement in determining ECD in normal corneas.³¹ Similarly, Kitzmann and colleagues compared confocal microscopy (ConfoScan 3) with non-contact specular microscopy (Konan) and showed no difference in ECD in normal patients when the ECDs were manually corrected.⁴¹ Scarpa and Ruggeri used a fully automated method of determining ECD and found no differences between confocal microscopy (Confoscan 4) and non-contact specular microscopy (SP-3000P).⁴⁴ A comparative study by Hara and colleagues investigating the clinical efficacy of confocal microscopy (ConfoScan) with non-contact specular microscopy (SP-2000P) showed that both imaging techniques generated similar images of the CE in all normal patients, but that confocal microscopy was superior to non-contact specular microscopy in FECD.²⁶ Overall, these studies show that in normal patients and patients with early stage FECD with minimal corneal edema, central ECD as determined by either non-contact specular microscopy or confocal microscopy is highly correlated.^{26, 31, 41, 44, 45} However, in cases of late FECD, where corneal edema prevents adequate specular imaging, confocal microscopy is superior to non-contact specular microscopy for imaging the CE and results in a larger percentage of high quality images of the CE.^{20, 26, 45} In a study of 7 FECD eyes, specular microscopy was precluded in 1 eye due to significant corneal edema, while all

7 eyes were imaged with confocal microscopy.²⁰ Similarly, in another study, specular microscopy was precluded in 7 eyes due to significant corneal edema, while all 11 eyes were imaged with confocal microscopy.²⁶ We have also recently demonstrated similar findings, where specular microscopy was precluded in 88 eyes out of 115 eyes with FECD, while all 115 eyes were imaged with confocal microscopy.⁴⁵ Furthermore, high quality specular images were captured in only 4 out 33 patients with late stage FECD.⁴⁵

FECD is a disease that typically first manifests as central corneal guttae followed by peripheral corneal involvement.⁸ Furthermore, it has been demonstrated through non-contact specular microscopy (CEM-530) that there are regional differences in ECD between the central, paracentral and peripheral zones in FECD, where the CE is damaged more in the central zone than peripheral zones.⁴⁶ The areas in between guttae have also been shown to have a lower ECD than the normal endothelial mosaic.⁴⁷ The decrease in ECD surrounding guttae has been shown to involve apoptosis of adjacent endothelial cells in FECD.⁴⁸⁻⁵⁰ Moreover, we have recently demonstrated in FECD patients that there is a 32% decrease in mean ECD in areas surrounding guttae compared to non-guttae areas as determined by confocal microscopy (HRT II RCM).⁴⁵ These findings strongly support the association between guttae and endothelial apoptosis and highlight the importance of acquiring high quality images of the CE. In advanced FECD, confocal microscopy is superior to specular microscopy since it can provide high quality images of the endothelial mosaic and is capable of imaging both the central and peripheral cornea.⁵¹ This is important in advanced FECD where peripheral ECD has been shown to be the best predictor of disease severity and has the highest number of correlations with other clinical markers (central ECD, logMAR best-corrected visual acuity, clinical disease grade, CCT).⁵¹ The ability to assess the CE with objective imaging is important for the evaluation, monitoring and guidance of management in FECD.

Optical Coherence Tomography of the Anterior Segment and Ultrasound Biomicroscopy

Optical coherence tomography (OCT) allows for non-contact *in vivo* imaging of the anterior segment through low-coherence interferometry. Anterior segment OCT (AS OCT) has many clinical applications and is extremely useful in studying anterior segment pathology since it allows for the imaging of all corneal layers and structures in great detail.^{52, 53} AS OCT plays an important role in the pre-operative, intra-operative and post-operative evaluation of patients requiring corneal surgery.⁵² In FECD, AS OCT can detect an early graft detachment after Descemet membrane endothelial keratoplasty and can help guide the clinician to determine if a secondary surgical intervention is required, especially in the presence of significant corneal edema.⁵³ However, existing commercially available AS OCT systems are limited in their ability to image individual corneal endothelial cells *in vivo* and can not produce images to determine ECD or detailed endothelial cell morphology.⁵² AS OCT may have a potential role in monitoring disease progression and predicting the need for surgical intervention. The corneal central-to-peripheral thickness ratio (CPTR) has been reported to be an objective, repeatable and possibly functional metric of the severity of FECD.⁵⁴ The central-to-peripheral thickness at 4 mm from the center ratio (CPTR₄) has been shown to be

higher in advanced FECD than mild or moderate FECD, which in turn is higher than in normal corneas.⁵⁴ The CPTR₄ is also highly correlated with the clinical grade of FECD and can discriminate between normal and FECD corneas.⁵⁴ While CCT and peripheral corneal thickness (PCT) were determined by scanning-slit pachymetry in this study, AS OCT has the capability of measuring both CCT and PCT, therefore CPTR could be calculated.^{54, 55} Future studies are needed to determine the utility of AS OCT-determined CPTR as a metric for monitoring disease progression and its role in determining surgical intervention.

Ultrasound biomicroscopy (UBM) allows for *in vivo* imaging of the anterior segment through high frequency ultrasound transducers with an immersion technique.⁵⁶ While UBM can provide valuable diagnostic information about the anatomy and pathology involving the anterior segment, even in the presence of optically opaque structures, its spatial resolution limits its applicability in assessing corneal ECD or individual morphology.⁵⁶

Conclusion

In summary, slit-lamp biomicroscopy with specular reflection and retroillumination are important techniques to assess the CE in FECD. Objective diagnostics tests to image the CE, such as retroillumination photographic analysis, specular microscopy, IVCN, and AS OCT, are valuable tools to evaluate the CE in FECD. Non-contact specular microscopy can be performed rapidly without touching the eye. However, specular microscopy requires a clear cornea with a smooth endothelium, thereby limiting its utility in advanced FECD. In contrast, even in advanced FECD with corneal edema, IVCN can image all the layers of the cornea from epithelium to endothelium with high resolution. IVCN is also capable of imaging both the central and peripheral cornea in advanced FECD, which can provide a better assessment of the regional variability in these corneas. Despite the advantages of IVCN it has not become a routine imaging device in most clinical practices. IVCN is contact-based, more technically challenging, user-dependent, and requires a coupling gel. It is important to select the appropriate objective diagnostic test to image and assess the CE in managing patients with FECD.

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Figure 1. Slit-lamp photograph using retroillumination in a Fuchs endothelial corneal dystrophy subject.
Confluent guttae (solid arrow head) and individual guttae (double arrow head) are visualized.

Figure 2. Non-contact specular microscopy and *in vivo* confocal microscopy (IVCM) in healthy and Fuchs endothelial corneal dystrophy (FECD) subjects.

(A) Non-contact specular microscopy in a healthy subject. (B) IVCM in a healthy subject. (C) Non-contact specular microscopy in a FECD subject with stage 3 guttae (solid arrowhead) (D) IVCM in a FECD subject with stage 1 guttae (arrow) (E) Non-contact specular microscopy in a FECD subject with stage 2 guttae (solid arrowhead) and stage 3 guttae (double arrowhead) (F) IVCM in a FECD subject with stage 1 guttae (arrow) and stage 2 guttae (solid arrowhead).