ORIGINAL RESEARCH PAPER

Medical Science

SPECULAR MICROSCOPY: A REVIEW

KEY WORDS: cornea endotheliopathies, endothelial cells imaging, keratoplasty and specular microscopy.

Dr. Vandana Parasar

Ms, Ex- Senior Resident, Rio, Igims, Sheikhpura, Patna

ABSTRACT

Specular microscopy is a non invasive diagnostic tool used to evaluate the corneal endothelial cell morphology, providing better understanding of corneal endothelial cells directly, which were not accessible before without invasive method or post mortem evaluation. Imaging endothelial cells helps in diagnosing various endotheliopathies, helping in pre-operative management and post operative follow-up in various ocular surgeries including cataract and keratoplasties. It helps to evaluate donor corneas and predicts the visual outcome in keratoplasties at eye-bank settings. This article aims at understanding corneal endothelial cells morphology, principle and interpretation of specular microscopy, its clinical application and limitations. A detail PubMed search was done and pertinent references were included.

INTRODUCTION

Specular microscopy is a non invasive diagnostic tool used to evaluate the corneal endothelial cell morphology, used at clinics and at eyebanks routinely. Corneal endothelial cell density (ECD), as determined by specular microscopy, is a traditional measure of corneal health and is one of the factors that predicts the outcome of penetrating keratoplasty[1]. Specular microscopy is important in preoperative evaluation of endothelial cells before high-risk surgeries, comparing various techniques, the impact of lasers during refractive surgery, and the assessment of donor cornea before transplantation[2].

The clinical specular microscopes are all from the laboratory microscope designed by Maurice in 1968[3]. But it was not until computer-assisted image analysis became available in the late 1970 and early 1980s that its use became more widespread[4]. In 1975, Laing first used the specular microscope for clinical use. An year later, Bourne et al used the high magnification specular microscope for photographic evaluation of endothelial cells[5].

Corneal endothelium anatomy

Before discussing and interpreting specular microscopy, it is essential to understand the morphology and functioning of corneal endothelial cells[6]. The corneal endothelial layer is a monolayer of hexagonal cells. The hexagon shape allows for a more efficient and even distribution of membrane surface tension and gives the advantage of a larger surface area than its perimeter[7]. Hence, a perfect cornea should have 100% hexagons[8].

Human corneal endothelial surface area is 130 mm2[9,10]. The endothelial cell density is approximately 6000 cells/mm2 in the first month after birth and slowly declines to 3500–4000 cells/mm2 (i.e., there are 390,000– 520,000 cells per cornea) by 3to 6years of age. This value decreases with age as corneal surface area increases and reaches to 3500 cells/mm2 by 5 years old, 2700 and 2900 cells/mm2 in middle-aged adults (30 years) and 2400 and 2600 cells/mm2 in adults >75 years age[11-14]. These values represent the white race. The cell density decreases in the human cornea from the periphery to the center by 9%[15,16]. The Asian race has greater cell densities at each given age group[17].

The consistent consensus states a gradual decrease in cell density with increasing age[18-21]. The endothelial cell density reduces with aging at an average of 0.6% annually[22]. This has 2 phases: rapid and slow[23]. Studies showed that the rate of cell loss is bimodal, with more rapid cell loss per year in 0–20 years than subjects >20 years old[1]. The younger patient cohort had a 1.1% +_ 0.8% per year loss in endothelial cell density compared to the older patient cohort with 0.6% +_ 0.5% cell loss per year[24]. Racial and geographical differences along with environmental factors are known to influence the rate of decrease in endothelial cell density[17,25,26].

The cells active pump has an integral role in maintaining corneal dehydration, acting as a barrier to aqueous humor, thus, preserving corneal clarity and visual potential. Embryologically, these cells are derived from neural crest cells which migrate from the neural plate to the optic disc around 40 days of gestation. By the eighth week of gestation, these cells form a single layer[27].

Unlike other cells in the body, human corneal endothelial cells are arrested in G1, post-mitotic phase of cell cycle, making them unable to regenerate. In case of any focal endothelial injury/loss of endothelial cell, the healing involves cellular elongation and migration of surviving cells to create a uniform cellular layer over the inner surface of the cornea[2]. This results in increased cellular surface area i.e. polymegathism or coefficient of variation (CV), increased cell shape variation, i.e. pleomorphism measured by reduced hexagonality and reduced endothelial cell count or density. This places greater importance on monitoring their health and integrity to prevent and manage corneal diseases[11].

Principle: Specular light reflex

Specular light reflex is the working principle of specular microscopy. It derives its name from German word; SPIEGEL meaning 'mirror-like' reflection. Specular reflections are normal light reflexes bouncing off a surface. It follows snell's law of optics i.e. the angle of reflection is equal to the angle of incidence **(Figure 1)**[2].

It relies on the difference in refractive index between two media producing reflection at the optical interface between endothelium and aqueous humor. The refractive index of endothelial cells (1.373) is greater than the aqueous humor (1.336). The larger the difference in the index of refraction between the two regions, the more intense will be the reflected light beam. The fraction of light reflected from the lens-saline interface is 0.36%, from the saline-cornea interface is 0.025%, and from the cornea-aqueous humor interface is 0.022%[28]. The light that is reflected from the endothelial surface is collected by the same objective lens and focused onto a film plane or a video monitor screen for examination[2].

The radius of curvature of the reflecting surface dictates the surface area of the specular reflex. A further restriction in the area of the light reflex is caused by the proximity of the 2 concentric surfaces (i.e., the epithelium and the endothelium). The viewable specular area is a compromise between the beam width and the corneal thickness. Because of this restriction, the viewable area of the endothelium is a rectangle, and the radius of corneal curvature dominates the height of the rectangle. The light scatter at corneal stroma is compounded by increasing the width of the beam of light giving better contrast to visualize endothelial cells through stromal reflections[11].

Various specular microscopes are available for documenting endothelial cell details at various magnifications and calibrations[11]. It can be divided into horizontal (clinical use) and upright (used in the eye banks) [2].

Clinically used microscopes are of two types—corneal epithelial cell layer contact and noncontact instruments[2]. The contact instrument has an objective microscope lens that applanates the corneal surface flattening the corneal curvature, hence specular reflex area is enlarged. The noncontact instruments use automatic image focusing technology from curved corneal reflecting surface. Hence, the specular reflex area is smaller than the contact specular microscopes[11].

Analysis Technique

Accurate analysis requires a good quality image to visualize endothelial cells. Accuracy is influenced by a number of factors including cell size, variation in cell area, the total number of cells counted, the method by which the cells are counted, the clarity of the image, the thickness of the cornea, and the position of the cornea when the image is taken[29,30].

Images of automated noncontact specular microscope and contact specular microscope may be used interchangeably to measure ECD, after proper calibration.

There are several methods of obtaining quantitative information about the corneal endothelium such as frame method (fixed or variable) **(Figure 2)**, center to center method, flex center method, corner method, and comparison method[11]. In frame method ECD is determined by computer-assisted manual techniques that count cells within a single or multiple grid pattern of known area (fixed frame) or area outlined by the specific borders of a group of cells (variable frame) and calculating cell density from the mean cell area of this population of cells. The center method, is semi-automated in which the centers of as many cells as possible are selected and image analysis software computes the ECD[31]. In corner method the intersecting sides of cells are located on the endothelial image frame. Comparison method provides a subjective cell density value by a visual comparison of the image to a known set of hexagonal patterns for various cell densities[2].

Erroneous ECD count can occur in automated analysis of frame method due to presence of border cells (cells cut by frame outline). In variable frame method, the corners of each cell can be defined to analyze the image, hence preferred over fixed frame method[31]. Corners and center methods are susceptible for subjective error in determining the cell boundaries. ECD measurements by both the center method and the corners method are reproducible within 1%[32].

The clarity of image is influenced by various corneal hindrances in layers anterior to endothelium. Endothelium visualization is difficult in conditions like corneal scar, opacity or haze, corneal excrescence / guttata, poor ocular surface tear film. Corneal edema due dysfunction endothelium causes optical hindrances. Corneal thickness, is the best indirect measure of endothelial function at the time of image capture[33-36]. Improper centration can capture the peripheral cornea changing the cell count considering the natural zonal variation. If the cell boundaries are not visible, then the manual method should be used to perform a specular count.

An endothelial image quality classification system, categorize images as either analyzable (sub- classified as excellent, good, and fair) or un-analyzable. As the quality of the image decreases, the inter-observer difference between analysis increases. Excellent images had a mean relative difference of 0%-6%, whereas fair images had a difference of 6%-11%[37].

Specular microscopy indices- definitions

After imaging, specular microscope automatically analyzes cells morphology and generates various indices helpful in diagnosis and management of the corneal endothelial diseases **(Figure 3).**

1. Average Cell area ± SD: Area of a single endothelial cell, which increases with age. An average cell size is 150–350 mm2[38]. SD, is standard deviation of cell area from mean cell area of cells within the analysis. Increased cell area or SD is suggestive of endothelial cell under effect of stressors.

2. Cell density (CD): The number of endothelial cells per mm2. It varies with age ranging between 1500–3500 cells/mm2, average number of cells is 2,400 cells/mm2[38]. Count less than 1,000 cells/mm2 suggest higher risk for developing corneal edema post surgery[39]. Minimum count essential to maintain proper hydration of the cornea, is 300 to 600 cells/mm2[40].

It is determined by equation:

$$CD = \frac{10^6}{\text{Average cell area}}$$

3. Coefficient of variation (CV): It represents the variation in cell size derived by the equation:

$$CV = \frac{SD}{\text{Average cell area}}$$

Normal cornea has CV less than 0.30. The increase in variation of corneal endothelial cells is called polymegathism, suggesting endothelial cells remodeling and is an early sign of endotheliopathies. CV >0.40 is significant polymegathism indicating cornea will not tolerate intraocular surgery[41].

4. Hexagonality (HEX): It represents number of cells with 6 angles polygon (hexagonal) shape. A perfect cornea should have 100% hexagonal cells. Under stress or insult to the endothelium, the cell count and hexagonality are reduced. Hence, a normal cornea is expected to have 60% hexagonality[11]. Variation in shape of cells is called Pleomorphism. Higher pleomorphism (i.e. hexagonality less than 50%) do not tolerate intraocular surgery[41]. Increased pleomorphism indicates compromised barrier function of the corneal endothelium.

5. Central corneal thickness (CCT): The most important indices when evaluating for endotheliopathies. As, all indices depicts morphology of endothelial cells but CCT gives indirect measure of endothelial function at the time of image capture[33-36]. Increased CCT is suggestive of corneal edema, meaning compromised functioning of endothelial pump is unable to maintain stromal dehydration.

The most sensitive indices to diagnose corneal endothelial insult and wound healing are CV and its percentage of hexagonality[42].

Specular microscopy in corneal diseases

Other than general endothelial alterations including variable loss of endothelial cells leading to decreased cell density, change in morphology with increased cell area, increased CV and reduced hexagonality **(Figure 4b)**, various specular microscopy patterns are observed at corneal endothelium in various corneal pathologies:

Corneal Guttae (Figure 4a): They are observed as dark 'drop out' areas resembling a hole in the endothelial mosaic due to focal thickening and projections in the otherwise homogenous Descemet's membrane[43]. Guttae morphology can vary from fine to coarse, and confluent to non-confluent, and their number can vary from few to numerous[44]. They are characteristics of Fuch's endothelial corneal dystrophy (FECD) also seen as part of normal aging or in macular dystrophy, and with pseudo-exfoliation[42].

Pseudo-Guttae: They are also seen as dark 'drop out' areas in conditions other than FECD. They are transient and disappear on reversal of primary pathology once treated and does not involve the descemet's membrane. Infection, inflammation, and uveitis and pigments on the endothelial surface as in anterior uveitis can lead to non-imaging of the endothelial cells in that area and may appear as dark lesions similar to guttae as seen in FECD[2].

Dark- light reversal pattern (Figure 4c): Here, the endothelial cell border is seen in white instead of usual black, and inside of the cell seen as black instead of white. It is seen in ICE syndrome. Vesicles, bands, and placoid lesions pattern on endothelial surface are seen in PPCD **(Figure 4d)**.

Indications

The corneal endotheliopathies are divided into primary and secondary (due to an underlying ocular or systemic insult)[2,5].

Examples of Primary Endotheliopathies

- **Fuchs endothelial corneal dystrophy (FECD):** It is characterized by presence of guttae, associated with dysfunctional endothelial cells. Presents as blurry vision early morning, sub-epithelial haze and difference in central vs peripheral corneal pachymetry. Diagnosis is important for preoperative plan if presenting with cataract. Decision regarding functionality of endothelial cells is reliable when correlated clinically along with specular microscopy.
- **Posterior polymorphous endothelial dystrophy (PPCD):** It is characterized by vesicles, bands, and placoid lesions on endothelial surface. The dystrophy can exist in unilateral or bilateral form[45]. ECD typically reduces with increased average cell area. Endothelial cells cannot be visualized in areas of vesicles or bands.
- **Congenital hereditary endothelial dystrophy (CHED):** endothelium is difficult to visualize due to corneal edema.
- **Iridocorneal endothelial syndrome (ICE):** The morphological changes evident on specular microscopy are; rounding of the cell borders, increased intracellular blackout areas, and dark- light reversal pattern[46,47].

Examples of Secondary Endotheliopathies

- **Endothelial changes after surgery:** Intraocular surgeries causes loss of endothelial cells. Hence, ECD is lowered and average cell area is larger. In post keratoplasty eyes, specular microscopy helps to monitor the graft health and its functional survival.
- **Endothelial alterations in uveitis/endotheliitis:** The endothelial pigments and pseudo-guttae is seen in active stages as drop out areas/dark lesions on endothelial imaging. Hence, ECD is lowered and average cell area is increased.
- **Pseudo-exfoliation induced endothelial changes:** it can show guttae with reduced ECD.
- **Endotheliopathy after forceps injury:** During birth trauma, presents with corneal edema at birth which resolves by migration of endothelial cells. Hence, corneas show lower ECD.
- **Contact lens endotheliopathy:** It is characterized by increased polymegathism and pleomorphism, associated with low oxygen permeability contact lens causing hypoxia.
- **Endotheliopathy in macular corneal dystrophy:** It is characterized by the deposition of glycosaminoglycans in the stroma and corneal endothelium. Endothelium shows guttae similar to FECD with low cell density and progressive worsening of endothelial changes.
- **Drug-induced endotheliopathy:** drugs such as memantine such as amantadine, prescribed for movement disorders/Parkinsonism are known to cause endothelial cell loss, which may eventually lead to bilateral corneal edema or failed graft in post keratoplasty eyes.
- **Systemic endotheliopathy:** pre-operative evaluation for endothelial abnormalities in cataract surgery is important in patients with diabetes and chronic kidney disease, especially those on hemodialysis[48,49].

At Eyebanks

Specular microscopy is standard practice at eyebanks for evaluation and grading of donor corneas **(Figure 5)**. It helps to diagnose suitability of the donor cornea in various keratoplasties and achieve better visual success rate in keratoplasties. As per the donor evaluation criteria followed by most eye banks and corneal surgeons, the donor cornea is suitable for penetrating keratoplasty and endothelial keratoplasty if ECD is above 2000 and 2200 cells/mm2, respectively[11].

Limitations

Although specular microscopy is a safe and non-invasive procedure for evaluating the cornea's endothelial layer, it also comes with its limitations. Firstly, it gives information only about the morphological status of cells and not the functional status. Although, corneal thickness is an indirect measure of endothelial function. Additionally, at present available imaging devices can capture only central cornea, extending up-to mid-periphery by changing gaze. But, it is not easily possible to evaluate peripheral cornea.

The image quality can be hampered due to poor corneal clarity. It is challenging to capture image in pediatric patients requiring supine position for examination under anesthesia or uncooperative patients with small micro-movements and nystagmus.

Moreover, interpreting specular images requires trained technician with expertise, for minimal inter-observer variations in reporting, as misinterpretation can lead to error in important clinical decisions.

CONCLUSION

Specular microscopy is an essential tool to provide detailed view of corneal endothelium. Its role cannot be understated in modern day practice of ophthalmology to diagnose and manage various corneal diseases, guiding preoperatively for better surgical outcomes and to assess the endothelial reserve of donor corneas at eyebanks and on follow-ups.

As technology advances, specular microscopy offers improved interpretation with increased automation and better image resolution and user-friendly analysis techniques to enhance accuracy. Smartphone-based endothelial imaging is evolving to overcome limitations of image capture in near future[50,51].

Figure with figure legends

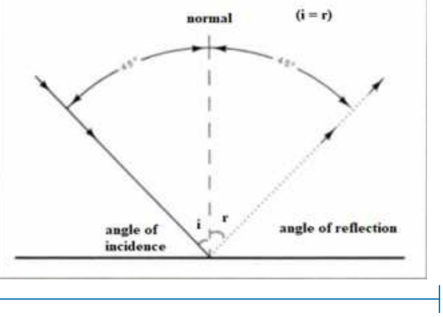

Figure 1: working principle of specular microscopy (schematic diagram): specular light reflex follows snell's law of optics i.e. the angle of reflection is equal to the angle of incidence.

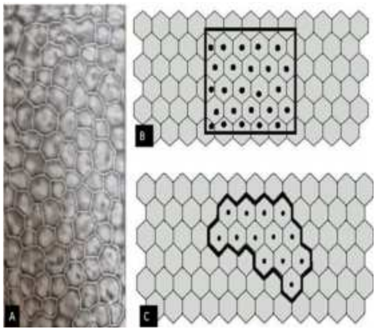

Figure 2: Method of analysis (A) Automated, (B) Fixed frame method, (C) Variable frame method

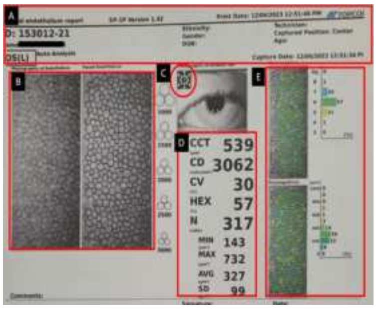

Figure 3: Parts of print-out (A) Patient detail, (B) Endothelial cell image, (C) Method of analysis, (D) Quantitative measures, (E) Histogram of cell area and shape

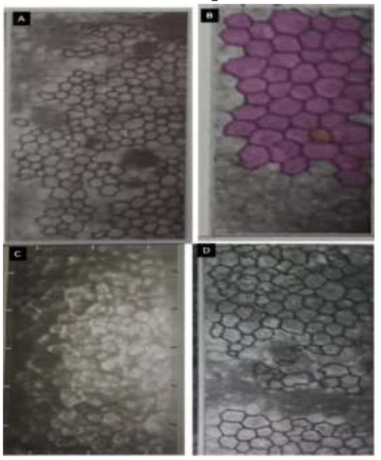

Figure 4: Qualitative assessment of specular microscopy. (A) Presence of guttae in FECD, (B) Polymegathism with reduced cell counts following keratoplasty, (C) Round cell border (black arrow) and a dark and light reversal pattern of normal endothelium in Iridocorneal Endothelial Syndrome, (D band-shaped structures on the endothelium characterizes in Posterior polymorphous endothelial dystrophy.

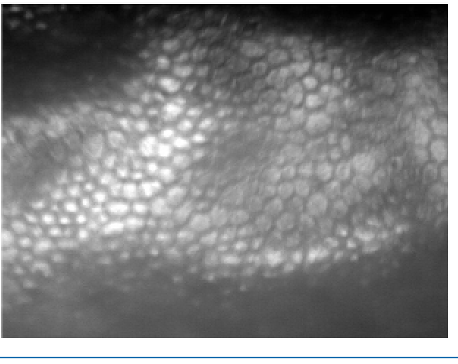

Figure 5: Donor endothelial cells captured on eye bank specular microscope.

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