Corneal Endothelial Regeneration and Examination Overview

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Abstract

Background: The corneal endothelium is the innermost layer of the cornea, 4 to 6 µm thick which is essential for maintenance of normal corneal hydration, thickness and transparency by the function of its metabolic pump. The human corneal endothelium has little or no ability to divide after birth. Cataract is the leading cause of blindness worldwide. The majority of these cases are in developing countries. With advances in microsurgical techniques, small-incision techniques have become increasingly popular. Phacoemulsification with a foldable lens is considered to be the Gold Standard of the cataract surgical techniques available today. Although corneal endothelial cells are arrested in the G1 phase of cell cycle, there is no current evidence of in vivo regeneration of cells. It may be due to Low levels of stimulating growth factors in the aqueous humor such as E2F2 transcription factor Or Presence of anti-mitotic growth factors in the aqueous humor such as TGFβ2.

Background

Anatomy of Corneal Endothelium

Cornea is the anterior part of outer fibrous coat of the eye. Anteriorly, the cornea is elliptical with the horizontal diameter measuring about 11.7 mm, and the vertical diameter measuring about 10.6 mm. posteriorly, the cornea is circular with equal diameter in all meridians (1). The radius of curvature of the convex anterior surface is 7.7 mm and that of the concave posterior surface is 6.9 mm hence the cornea is thicker in the periphery than the center attaining a prolate shape (2).

Regarding thickness, the central corneal thickness is 0.5 mm, while the peripheral portion is 0.7 mm thick.

The cornea is transparent and avascular coat thus permitting light transmission. It provides a proper refractive surface together with the overlying tear film (Total refractive power of the cornea is about 43D, 70% of that of the eye). Also, it helps to protect the contents of the globe from infection and structural damage (1, 2).

The human cornea consists of six layers (Fig. 1): The multilayered epithelium with its basement membrane, Bowman’s membrane, substantia propria, Dua layer, Descemet’s membrane and the endothelium (3).
Corneal endothelial regeneration

Although corneal endothelial cells are arrested in the G1 phase of cell cycle, there is no current evidence of in vivo regeneration of cells (5). It may be due to Low levels of stimulating growth factors in the aqueous humor such as E2F2 transcription factor Or Presence of anti-mitotic growth factors in the aqueous humor such as TGFβ2 (6).

Progenitor (stem-like) cells have been identified in the transitional zone between the periphery of the corneal endothelium and the anterior non-functioning part of the trabecular meshwork. Recent studies have suggested that these cells might be able to divide and supply new cells for the corneal endothelium and/or trabecular meshwork. (7)

Examination of Corneal Endothelium in vivo

Detailed clinical assessment of the corneal endothelium include: slit lamp examination, contact and noncontact specular microscopes and confocal microscope. Routine slit lamp examination by specular reflection technique has difficulties as low magnification, difficulty of visualization due to scattering of light and small area of examination confined to the size of the slit beam. Despite being rapid, it is a rough method. It can be used to visualize corneal guttae (8, 9).
Specular Microscopy:

Specular microscopy (SM) is the study of the layers of the cornea under very high magnification (100 times greater than that of the slit lamp). It's mainly used to analyze corneal endothelium for size, count, shape and distribution, variation in cell size as measured by the coefficient of variation of cell area (polymegethism) and cell shape (the percentage of hexagonal cells–pleomorphism) (10). Specular microscopy is mainly used before intraocular surgeries to assess the endothelial functional reserve or for evaluation of the donor cornea in penetrating keratoplasty. (11).

Optics and types of specular microscopes:

When a beam of light passes through the cornea, a small portion of it is reflected specularly, like a mirror, at a series of optically distinct interfaces, air-cornea and endothelium-aqueous interfaces. Only 0.02% of the incident light on the cornea is reflected at the endothelium-aqueous interface due to the small difference in refractive indices between the cornea (1.376) and the aqueous (1.336). Any interface that does not give out a specular reflection will give a dark appearance for example, edges between cells. The reflected light forms an image of the endothelium which can be photographed and analyzed (12).

The presence of corneal opacity or edema can degrade the quality of the reflected image. Clear media is needed for acquisition of reliable specular images (10).

Types of specular microscope:

- **Contact specular microscope:**
  Using a contact lens with a coupling fluid whose refractive index is similar to that of the cornea. So that reflections from the front of the cornea are eliminated. Though it has the advantage of higher magnification and inhibition of eye movement, patient intolerance is an obstacle.

- **Non-contact specular microscope:**
  Reflected light rays from the corneal endothelium are collected, isolated from rays reflected back from another interface.

When comparing contact and non-contact methods, Szalai et al (2011) found that contact specular microscopes overestimate the average cell area and coefficient of variation of the cell. Thus both methods should not be used interchangeably in the same study and the examiners must be consistent to the same technique in their analysis (14)

A minimum of 50 cells per image should be detected by the device in order to obtain maximum accuracy of endothelial cell density (ECD) results. (14)
Evaluation criteria:
The analysis of endothelial cells includes:
1. Endothelial cell area by μm².
2. Endothelial cell density (ECD) by cells/mm².
3. Coefficient of variation of cell area (CV).
4. Percentage of hexagonal cells (HEX).
5. Degree of pleomorphism and polymegathism

- **Endothelial Cell Density (ECD):**
  Cell density=106/average cell area with cell density (cell per mm²), average cell area (μm²), and the value 106 is used to convert units of measure.
  Normal average of ECD ≥ 2500 cells/mm², finding an endothelial count of less than 2000 cell/mm² in unoperated eye is considered abnormal (15).

- **Coefficient of variation (CV):**
  The CV value is a parameter describing the variation in endothelial cell area.
  CV=SD of cell area /mean cell area μm²
  With CV as coefficient of variation and SD as standard deviation of the mean cell area.
  Normally CV ranges from 22 to 31 with an average of 27 for young adults. Values from 32 to 40 are elevated and values above 40 are abnormal (15).

- **Percentage of hexagonal cells:**
  Presence of six-sided cells constitutes an indicator of an even distribution of membrane surface tension and of normal cells and their percentage is expected to be more than 60% in adult healthy corneas (44). Theoretically, a perfect cornea has 100% hexagonal endothelial cells. The average percentage of hexagonal cells in a normal cornea is expected to be higher than 60%. A percentage of hexagonal cells below 50% are considered abnormal (16).
4-Degree of pleomorphism and polymegathism.
Polymegathism is a reflection of normal endothelial movement as a part of the endothelial healing mechanism. It is represented by the coefficient of variation. Normally CV ranges from 22% to 30%, Values from 31% to 40% are elevated and values above 40% are abnormal (17).
Polymegathism is considered the first sign of endothelial stress and overactive wound healing. Those corneas with polymegathism are vulnerable to more damage during intraocular surgery or other stresses as contact lenses and diabetes. (17)
Polymegathism and pleomorphism are positively correlated to each other as when the endothelial cell enlarges, it also exhibits a variable shape. (18).

Corneal confocal microscopy:
New technologies such as corneal confocal microscopy are being developed to examine in detail each individual corneal layer, including the endothelium. Confocal microscopy offers several advantages, including the capacity to focus on a single depth to obtain clear images, and the ability to collect images from serial sections within a given thickness of tissue (19).
In confocal microscopy measurement of the cornea, light is sent from an illumination source and then passes through a slit and is focused on a spot within the cornea by an objective lens. Light reflected back from the cornea is filtered by another slit which blocks the out-of-focus light. A detector then receives the focused light. Because of this optical design, the image taken by confocal microscopy has a superior resolution than specular microscopy (19).
However, confocal microscopes measurement of the corneal endothelial cell requires contact between the instrument and the cornea; therefore, topical anesthesia is required. Also, it is unlikely to replace specular microscopy given its relatively inexpensive method to yield a large amount of useful data (19).
There are different image analysis methods, including manual photo digitized, manual, semi-automatic, automatic, or manual digital. Information about the corneal endothelial cell density, cell size area, shape, and variation of cell size can be gathered after corneal endothelial image capture and analysis.

Fig. (2): Laser-scanning in vivo confocal microscopy of the cornea (HRT3-RCM system) (A) the laser-scanning interface. (B) Magnified view of the scanning head with plastic cap and transparent tear gel applied (20).
**Fig. (3):** Confocal microscopy images of a normal cornea: (a) flat superficial epithelial cells; (b) intermediate "wing" epithelial cells; (c) basal epithelial cells; (d) sub-epithelial nerve plexus; (e) corneal stroma – keratocytes; and (f) endothelial cell layer (21).

**References**


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