Evaluation of Corneal Endothelial Cell Density and Morphology in High Myopic Eyes in Patients in the Third and Fourth Decades of Life

Thesis

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Introduction

The human corneal endothelial cell is a non-regenerating predominantly hexagonal cell which covers the posterior surface of descemet's membrane and faces the anterior chamber of the eye. Corneal endothelium is metabolically active and plays an imperative role in maintaining the corneal transparency by pumping water from stroma to the aqueous humor and keeping the stroma in the hydration level of 70% of water.

Corneal endothelial cell density and morphology can be analyzed using specular microscope. The specular microscope has been shown to be reliable and reproducible with appropriate calibration of the instrument ***(Benetz et al., 1999; Módis et al., 2002; Van Schaick et al., 2005)***.

Non-contact Specular microscope provides a non-invasive method of morphological analysis of the corneal endothelial cell layer. It makes the measurement of mean cell density (MCD), measurement of coefficient of variation (CV) in the cell size as well as hexagonal appearance of the cell. These parameters provide an index of the functional status of corneal endothelial layer ***(Sheng and Bullimore, 2007)***.

Myopia is a common pathologic change of the eye. High degrees of myopia are associated with increased risk of cataract, posterior vitreous detachment, retinal tears and retinal detachment, increased risk of choroidal neovascularization, and myopic macular degeneration ***(Ogawa and Tanaka, 1988; Grossniklaus et al., 1994; Lim et al., 1999; Younan et al., 2002; Vongphanit et al., 2002).***

In spite of an impressive body of research, little is known about the effect of myopia on the cornea, particularly the corneal endothelium.

Aim of the Work

This study aims to describe the corneal endothelial cell density and morphology in high myopic adults and to compare the corneal endothelial characteristics with emmetropic subjects.

Structure and Function of the Cornea

Gross Anatomy

The eye is composed of three concentric tunics: outer fibrous tunic, a middle uveal tract, and an inner neuroretina. The cornea makes up one-sixth of the outer tunic; the sclera makes up the other five-sixths. The cornea is a clear transparent tissue that joins the opaque sclera at the corneoscleral limbus. Although the cornea is circular when viewed from the posterior surface, it is oval when viewed from the anterior surface because of a more prominent limbus superiorly and inferiorly. The average diameters of the cornea are 12.6 mm horizontally and 11.7 mm vertically. The anterior surface of the cornea is not uniformly curved ***(Kuwabara, 1978).***

The central one-third of the cornea is called the optical zone and is approximately spherical. The average radius of curvature of the anterior surface centrally is 7.8 mm. As the major refractive surface of the eye, the anterior surface of the cornea provides approximately 48 diopters. The peripheral cornea is flattened, more so nasally than temporally. The posterior surface of the cornea is more spherical than the anterior surface. Therefore, the central cornea is thinner (520 micrometers) than the peripheral cornea (650 micrometers or more) ***(Kuwabara, 1978).***

Microscopic Anatomy

The cornea can be divided into six layers: the epithelium, Bowman's layer, stroma, Dua's layer (pre-descemet), Descemet's membrane, and the endothelium **(Fig.1).**

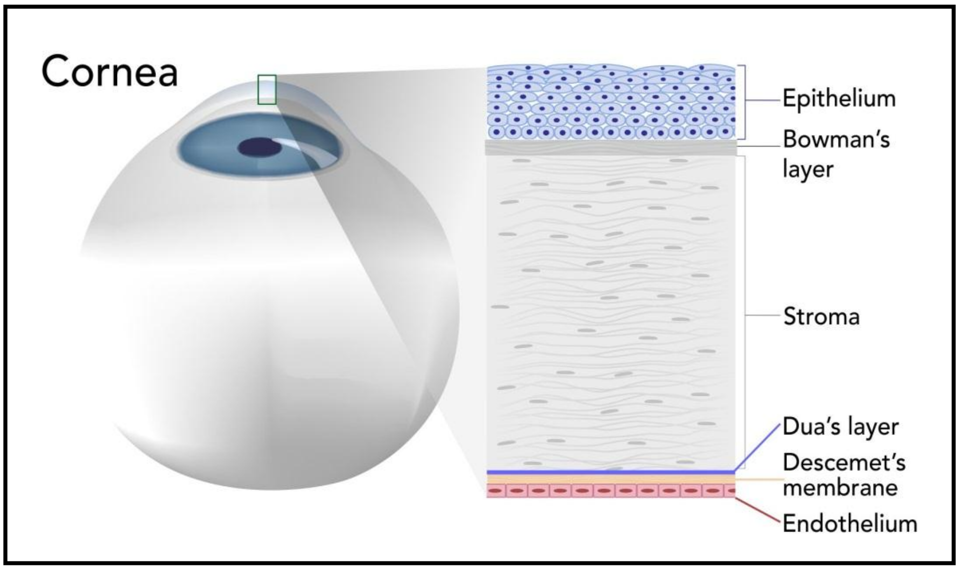

Fig. (1): Microscopic structure of the cornea
(Harminder and Lana, 2013).

Epithelium:

The non keratinized, non secretory, stratified squamous epithelium of the cornea consists of four to six layers of cells and represents 10 percent of the corneal thickness (40-50 um). The epithelium is divided morphologically into three layers: the superficial or squamous cell layer, the middle or wing cell layer, and the deep or basal cell layer **(Fig.2).**

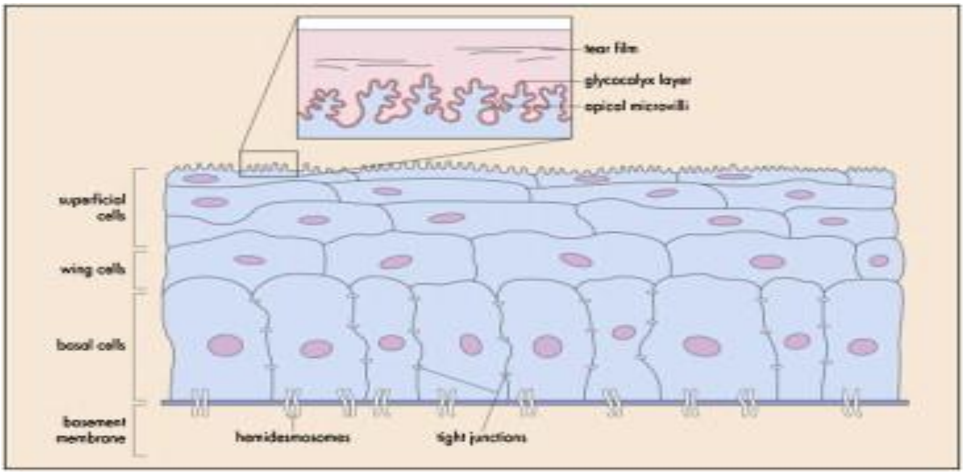

Fig. (2): Cross sectional view of the corneal epithelial cell layer ***(Derek and TerryKim, 2011).***

The epithelium is covered with a tear film of 7 um thickness, which is optically important in smoothing out microirregularities of the anterior epithelial surface. The tear-air interface, together with the underlying cornea, provides roughly two thirds of the total refractive power of the eye. Corneal epithelial cells undergo orderly involution, apoptosis (programmed cell death), and desquamation. Complete turn over of corneal epithelial cells occurs in about 7-10 days, with the deeper cells eventually replacing the desquamating superficial cells ***(Ayad and Mark, 2008)***.

The most superficial cells of the corneal epithelium form an average of 2-3 layers of flat cells. Extensive apical microvilli and microplicae characterize their cell membranes, which in turn are covered by a fine, closely apposed, charged glycocalyceal layer ***(Jay et al., 2011)***. The apical membrane projections increase the surface area of contact and adherence between the tear film mucinous undercoat and the cell membrane. Laterally, adjacent superficial cells are joined by barrier tight-junctional complexes, which restrict entry of tears into the intercellular spaces.

Beneath the superficial cell layer, there are 2-3 cell layers; the suprabasal or wing cells, so named for their cross-sectional alar shapes. Cells are less flat than the overlying superficial cells, but possess similar tight, lateral intercellular junctions ***(Jay et al., 2011)***.

Beneath the wing cells are the basal cells. The basal cell layer is composed of a single-cell layer of columnar epithelium approximately 20 um tall. The basal cells are the only epithelial cells that undergo mitosis. They are the source of both wing and superficial cells, and possess lateral intercellular junctions characterized by gap junctions and zonulae adherens. The basal cells are attached to the underlying basement membrane by an extensive basal hemidesmosomal system. This attachment is of pivotal importance in preventing the detachment of the multilayer epithelial sheet from the cornea ***(Jay et al., 2011).***

Bowman’s layer

A wide band of 8-12 microns of amorphous material along the anterior surface of the cornea. Bowman's layer is a layer of randomly arrayed collagen fibrils that merge into the more organized anterior stroma. Bowman's layer is attached to the stroma by collagen fibrils that insert into Bowman's layer and become part of the anterior stromal lamellae ***(Binder et al., 1991).***

Stroma

Stacked lamellae of collagen fibrils are the most obvious morphologic characteristic of the stroma, which constitutes about 90 percent of the corneal thickness. The layered arrangement is more regular in the posterior stroma than in the anterior stroma, where the lamellae are narrow and interleaved. The cellular density in the stroma is low, with many keratocytes, scattered throughout, primarily between lamellae. Nerve axons and their associated Schwann cells are found in the anterior and middle third of the stroma. Collagen constitutes about 71 percent of the dry weight of the cornea and is the structural macromolecule providing tissue transparency and mechanical resistance to intraocular pressure ***(Newsome et al., 1981).***

Although there is some disagreement about which collagen types are found in the corneas of various species, there is agreement that type I collagen predominates. Additionally, there is strong evidence that types III and V collagen are found in the human cornea. The collagen fibrils, which are packed in parallel arrays, make up the 300 to 500 lamellae of the stroma. The lamellae extend from limbus to limbus and are oriented at various angles to one another, less than 90 degrees in the anterior stroma but nearly orthogonal in the posterior stroma ***(Maurice, 1984).***

Dua's layer

It was discovered in 2013 by prof. H. Dua and his team, as the 6th layer of the cornea which redefined the known corneal anatomy, on basis of Big Bubble (BB) technique used in Deep Anterior Lamellar Keratoplasty (DALK). It is also called Pre-Descemet layer. It is around 10 um thickness lying between the stroma and Descemet's membrane ***(Harminder and Lana, 2013)*** according to their research.

Histologically the DL is made of 5 to 8 thin lamellae of tightly packed collagen bundles running in longitudinal, transverse and oblique directions. The DL, like corneal stroma, is primarily composed of collagen I. Collagen V is weak immunohistologically positive in DL and stroma. Collagens IV and VI are more positive for DL compared with the corneal stroma. The intensity of staining for pro-teoglycans lumican, mimecan and decorin was similar in the DL and corneal stroma. CD34 was negative in the DL, confirming the lack of keratocytes in DL ***(Harminder and Lana, 2013).***

Descemet's Membrane

Descemet's membrane is the thick basal lamina secreted by the endothelium. It is composed of an anterior banded layer and a posterior non banded layer. Secretion of Descemet's membrane begins at approximately 4 months of gestation, the banding appears in the anterior layer before birth. Descemet's membrane increases in thickness during life, but this accumulation appears to be limited to the non banded posterior layer. Descemet's membrane is loosely attached to the stroma. Obvious mechanical attachment sites have not been seen by conventional electron microscopy, suggesting that intraocular pressure may play a role in attachment ***(Johnson et al., 1982).***

Rupture of Descemet's membrane result in the penetration of aqueous humor into the corneal stroma and consequent stromal edema. Descemet's membrane does not regenerate. Descemet's membrane becomes continuous and uniform, fusing peripherally with the trabecular beams ***(Watsky et al., 1989).*** The fusion site, known as Schwalbe line, is a gonioscopic landmark that defines the end of Descemet's membrane and the start of the trabecular meshwork.

Endothelium

The corneal endothelium, forms a single layer of approximately 400,000 cells, 4 to 6 microns thick, on the posterior corneal surface. Viewed from their posterior surface, these cells are predominantly hexagonal in shape and approximately 20 microns wide **(Fig.3).** Their outline at the level of Descemet's membrane is irregular because of the marked infolding and interdigitation between adjacent cells. The posterior cell membrane is thought by some to be coated with a viscous substance possibly of endothelial origin, which, like the huffy coat on the epithelial surface, may reduce lipid membrane surface tension to promote wetability. A single primary cilium has been demonstrated in many endothelial cells, but its function is unclear. The cilium is apparently not motile and is structurally associated with the cell's centriole pair ***(Sperling and Jacobsen, 1980)***.

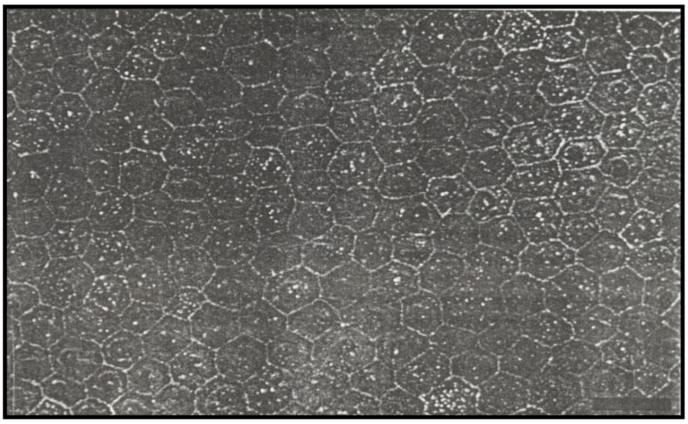

Fig. (3): Scanning electron micrograph of the central endothelial surface showing a regular cellular mosaic. Irregular projections and an occasional cilium mark the cell surface between the lateral boundaries ***(Rao et al., 1979).***

This association may be related to the inability of these cells to undergo mitosis in the adult. However, the cells have the ability to enlarge and to maintain tight apposition with neighboring cells, preventing excessive seepage of aqueous humour into the stroma. Additional resistance to paracellular flow is provided by specific junctional complexes located between endothelial cell membranes near the apical membranes close to the anterior chamber ***(Kreutziger, 1976).***

The cytoplasm has many mitochondria and elaborate smooth and rough endoplasmic reticulum, as well as a Golgi complex, all of which are characteristic of cells actively involved in metabolic energy production and molecular synthetic processes. Pinocytotic vesicles near the posterior cell membrane have been identified and may be related to the maintenance of the layer of viscous substance noted above. At the light and transmission electron microscopic level, endothelial cell cross sections suggest a relatively homo-geneous population of cells, even though certain metabolic stresses, such as anterior corneal hypoxia, appear to alter the status of some cells before others. From a clinical point of view, it is important to emphasize that the corneal endothelium is functionally essential to the cornea. Normally, the endothelium enjoys a privileged and protected environment in the anterior chamber, but it remains a fragile cell layer whose integrity and viability must be guarded to ensure the success of any intraocular procedure ***(Zantos and Holden, 1977).***

Similar to the central nervous tissue, the cells of the human corneal endothelium are essentially amitotic after birth. However, corneal endothelial cells have a remarkable ability to enlarge and to maintain normal function in the face of cellular inadequacies or deficiencies, as are seen during the postnatal growth of the cornea, during normal cell loss in the aging process, and after cell loss caused by intraocular surgery and trauma. At birth, cell densities range from 3,500 to 4,000 cell/mm^2, whereas the adult cornea normally has densities of 1,400 to 2,500 cell/ mm² **(Fig.4).** Corneal transplants may have fewer than 1,000 cell/mm² and remain clear. Also the percentage of hexagonal cells declines throughout life from approximately 75% to approximately 60% ***(Yee et al., 1985).*** The central endothelial cell density decreases at an average rate of 0.6% per year in normal corneas ***(Bourne et al., 1997).*** It would appear that as long as endothelial cells can enlarge to provide a confluent monolayer on Descemet's membrane, normal corneal function is maintained ***(Zantos and Holden, 1977).***

A lower limit to this ability occurs at densities of 400 to 700 cells/mm², below which endothelial function fails and deterioration of vision occurs due to corneal edema ***(Zantos and Holden, 1977).***

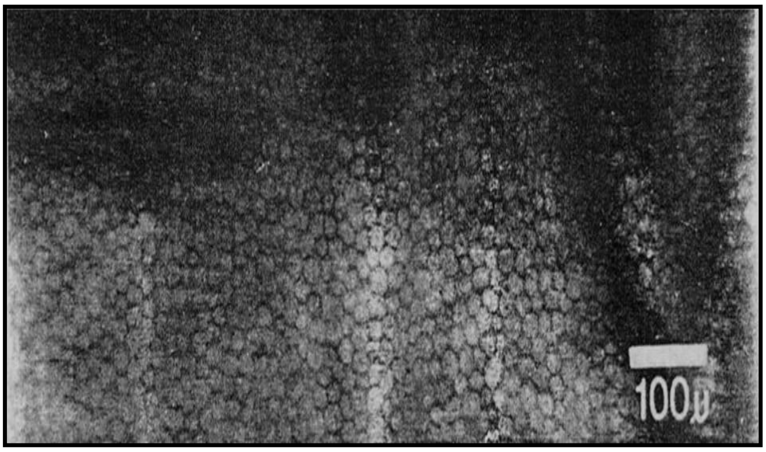

Fig. (4): Specular photomicrograph of the corneal endothelium of a 28-year-old patient. The cell density is approximately 2,800 cells/mm2. The dark bands are artifacts from applanation ***(Zantos and Holden, 1977).***

In summary, there is a critical density of endothelial cells (400 to 700 cells/mm^2) below which endothelial decompensation occurs, with progressive stromal edema and eventual epithelial edema. Stromal edema, which is associated with endothelial pathologic states, can occur with higher cell densities and is the probable result of disruption of the paracellular pathways, as in early Fuchs' dystrophy. When endothelial cells are subjected to stress, and especially when some cells are lost, the remaining cells may lose their regular hexagonal shape and become irregular in shape (pleomorphism) and size (polymegethism) ***(Fig.5)***, ***(Fig.6)***, ***(Fig.7)***. These changes can occur with age, after trauma, after intra ocular inflamation and in long-term contact lens wearers. The significance of pleomorphism and polymegethism is unclear, but there is evidence that a cornea with these changes cannot withstand additional trauma as normal cornea. Contact lens wearers can develop small blebs in the endothelium shortly after a contact lens is inserted. These blebs are transient and are probably caused by the osmotic effects or low pH resulting from lactic acid accumulation ***(McCartney et al., 1987).***