Biology of the corneal endothelium in health and disease

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Introduction

Our knowledge of the human corneal endothelium and our understanding of its role in maintaining vision have gradually increased. I will review here our current clinical concepts of the corneal endothelium. Owing to the space and time limitations, this must be a superficial review. You will notice perhaps an undue attention to studies conducted at the Mayo Clinic over the past 25 years. This is not intended to imply an increased importance or superiority over similar investigations, but rather it is because of my familiarity with the Mayo Clinic studies.

Endothelium in health

The monolayer of cells called the corneal endothelium that lines the posterior corneal surface is derived from the neural crest during embryologic development.¹ Human endothelial cell density is approximately 6000 cells/mm² during the first month of life,² but decreases to about 3500 cells/mm² by age 5 years.³ Growth of the cornea accounts for some of this decrease in density, but a decrease in the number of cells also occurs.² There is no evidence that human endothelial cells divide under normal circumstances, although they can be induced to divide in cultured corneas.^{4,5} They are arrested in the G1 phase of the cell cycle.⁶ The reason for the lack of progression into the cell cycle is still unknown, but it may involve cellular contact

inhibition.⁷ Central endothelial cell density decreases throughout life at an average rate of about 0.6%/year⁸ so that the mean cell density was found to decrease from 3400 cells/mm² at age 15 to 2300 cells/mm² at age 85 years.⁹ Racial or geographic differences appear to exist; higher cell densities were found in Japanese than in American subjects.¹⁰

The corneal endothelium secretes collagen that forms a basement membrane called Descemet's membrane. At the time of birth, Descemet's membrane is approximately 3 µm thick and consists of collagen in a banded pattern with a periodicity of approximately 110 nm.¹¹ Throughout life, endothelial cells continue to secrete Descemet's membrane, but the collagen added after birth is not banded. By age 70 years, the average Descemet's membrane is 13 µm thick, including the original 3 µm of anterior banded (foetal) Descemet's membrane and an additional 10 µm of posterior nonbanded Descemet's membrane. After age 70 years, the posterior nonbanded layer is thicker in females than in males.¹¹ When the endothelial cells are stressed by damage or disease, they may secrete banded Descemet's membrane to form a posterior banded layer.¹² This layer of abnormal Descemet's membrane has also been termed a posterior collagenous layer.¹³

The cornea is an exquisite example of natural engineering; the requirement for a living, optically clear lens on the surface of the eye was solved by packing collagen and cells in an orderly lamellar arrangement without blood vessels. The crystalline organization and critical spacing of collagen fibrils makes this tissue optically transparent. Any accumulation of fluid would disrupt this spacing and degrade the transparency. The endothelium must serve two functions to maintain the health and clarity of the stroma: it must control hydration (maintain stromal deturgescence) and it must be permeable to nutrients and other molecules

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from the aqueous humor because these are not supplied by blood vessels as they are in other tissues. This problem was solved by the development of an endothelial layer that behaves as a partial, or leaky, barrier to the movement of fluid and metabolic substrates. The endothelium maintains stromal deturgescence by functioning both as a barrier to fluid movement into the cornea and an active pump that moves ions, and draws water osmotically, from the stroma into the aqueous humor. These cells are metabolically very active, with large numbers of mitochondria, consistent with their need to move water efficiently. Endothelial cells have an incomplete zonula occludens, a leaky tight junction between adjacent cells, and this accounts for the weak endothelial barrier function that allows nutrients and other molecules to enter the stroma. The combined leaky barrier and fluid pump has sometimes been called a pump-leak mechanism.¹⁴ The barrier and pump functions can be measured clinically by fluorophotometry and pachometry. The barrier function can be estimated from the endothelial permeability to fluorescein.¹⁵ The efficiency of the pump can be studied by measuring the corneal deswelling rate from a 10% increase in thickness induced by 2 h of closed-eye aphakic soft contact lens wear.¹⁶ The endothelial pump rate can be calculated from the deswelling rate and the endothelial permeability.¹⁷

Endothelium in disease

Primary corneal endotheliopathies

There are relatively few primary corneal endothelial diseases, and a classification of primary corneal endotheliopathies devised 20 years ago¹⁸ is still appropriate (Table 1). The most common is Fuchs' dystrophy, an inherited bilateral disease in which the endothelial cells gradually malfunction. This condition becomes evident in middle age or later. The endothelial cell density is decreased, and the enlarged endothelial cells secrete excess amounts of abnormal banded Descemet's membrane. This abnormal tissue appears as drop-like warts (guttatae) that project from the posterior surface of Descemet's membrane, and create the condition known as cornea guttata (*guttate cornea*). There is evidence that the pump function of the endothelium

decreases first and is followed by a decrease in barrier function.¹⁹ When the endothelium is unable to maintain fluid balance, the cornea soon swells and loses its transparency. The only effective treatment at present is corneal transplantation. Recent evidence links the disorder to a mutation in the gene for collagen VIII.²⁰

The second endothelial disease is a spectrum of inherited bilateral disorders called posterior polymorphous dystrophy (PPD). In these conditions, the endothelium contains epithelial-like cells. The abnormal cells may appear in isolated areas, across entire endothelium, or there may be intermediate involvement between these two extremes.²¹ The third primary corneal endotheliopathy, congenital hereditary endothelial dystrophy (CHED), is a bilateral disorder of the corneal endothelium like PPD, except that the entire endothelium is involved at birth so that the cornea is congenitally cloudy. There is some overlap between PPD and CHED, both pathologically,^{22,23} and genetically.^{24–27} A recent study found two families with Fuchs' dystrophy and a family with PPD with the same mutation in the gene for collagen VIII.²⁰ Therefore, all three endothelial dystrophies may have genetic abnormalities in common.

A fourth primary endothelial disease is the iridocorneal endothelial (ICE) syndrome. This disorder is comprised of three syndromes that are now recognized to be varied manifestations of the same primary endothelial disease: essential iris atrophy, Chandler's syndrome, and Cogan–Reese iris nevus syndrome. The ICE syndrome is usually unilateral (although occasionally bilateral) and appears to be acquired. It is most common between 30 and 50 years of age and is more common in women.²⁸ The endothelium has a beaten metal or cobblestone appearance and the cells have many characteristics of epithelial cells such as microvilli and cytokeratin markers^{29,30} and decreased permeability to fluorescein.³¹ On specular microscopy, these 'ICE cells' appear as the 'negative image' of normal endothelium; the cell junctions appear light and the cell bodies appear dark, a characteristic of epithelial cells³² (Figure 1). Although the endothelium is diffusely involved in most cases, occasionally the abnormal cells only occupy a portion of the cornea so that there is 'partial'³³ or 'subtotal'³⁴ involvement. In these cases, there is a sharp line of demarcation between the abnormal and normal endothelium (Figure 2) with the 'normal' cells being smaller than they are in an uninvolved cornea, creating a higher cell density in that area. The abnormal 'ICE-cells' spread peripherally onto the iris and form a membrane across the anterior chamber angle. This membrane can gradually contract and pull peripheral iris towards Schwalbe's line, forming small bridging synechiae that gradually enlarge. ICE

Table 1 Primary corneal endotheliopathies

Fuchs' dystrophy
Posterior polymorphous dystrophy (PPD)
Congenital hereditary endothelial dystrophy (CHED)
Iridocorneal endothelial syndrome (ICE)
Intermediate forms

Figure 1 Appearance of abnormal endothelial cells in the iridocorneal endothelial (ICE) syndrome. These 'ICE cells' are enlarged and appear as the 'negative image' of normal endothelial cells; the cell junctions appear light and the cell bodies appear dark.

Figure 2 Partial corneal involvement in the iridocorneal endothelial (ICE) syndrome. A sharp junction is present between the abnormal 'ICE cells' on the right and the more normal endothelial mosaic on the left. The normal-appearing endothelial cells on the left are smaller than normal, and their density is 3634 cells/mm². Small bridging peripheral anterior synechiae were present.

cells can remain stable, increase and gradually spread over the entire cornea, or, in rare cases, they can gradually regress.³⁵ The endothelium of the fellow eye is generally considered normal, but Lucas–Glass found polymegethism and pleomorphism with an unchanged cell density in the fellow eye.³⁶ Others have noted similar changes in the fellow eyes.^{37,38}

If one examines the endothelium of all patients carefully, occasional primary endothelial abnormalities are seen that do not fit the first four well-defined entities. I have called these primary corneal endotheliopathies of

Figure 3 Intermediate form of primary corneal endotheliopathy in a 75-year-old man who was examined several times over the course of 13 years without detectable change. There is a sharp line of junction between the abnormal, cobblestone-appearing region with enlarged cells, and the more normal endothelial mosaic in which the cells are smaller than normal (cell density = 5607 cells/mm²). This patient had no iris abnormalities or peripheral anterior synechiae.

indeterminate type or intermediate forms (Table 1), since they often have features of both PPD and partial ICE syndrome, with no peripheral anterior synechiae (Figure 3). In the future, genetic testing of individuals with these intermediate forms may be diagnostic. It is possible that all primary corneal endotheliopathies share a similar genetic abnormality, since PPD, which shares genetic abnormalities with Fuchs' dystrophy and CHED (see above), has been linked to the ICE syndrome by some investigators.^{39–41} Alternatively, a nongenetic developmental anomaly may be at fault. Epithelialization may represent a single endothelial response to many different stimuli.⁴²

Secondary corneal endotheliopathies

Contact lens wear Contact lens wear causes the endothelial cells to become more varied in size (polymegethism, an increase in the coefficient of variation of cell area), and in shape (pleomorphism a decreased percentage of hexagonal cells, Figure 4). These changes are thought to arise from hypoxia since they are not associated with oxygen-permeable silicone lenses.⁴³ Extensive studies have failed to detect an effect of long-term contact lens wear on mean endothelial cell size or on endothelial barrier or pump function, as measured by fluorophotometry and deswelling.⁴⁴

Endothelial effects of surgical procedures The effects of two surgical procedures, cataract extraction, and corneal

Figure 4 Polymegethism and pleomorphism induced by long-term contact lens wear. Top: endothelium of a 59-year-old 'normal' individual (the author) who has never worn contact lenses. Endothelial cell density (ECD) = 2131 cells/mm², coefficient of variation of cell area (CV) = 22%, percentage of hexagonal cells (HEX) = 73%. Bottom: Polymegethism (increased CV) and pleomorphism (decreased HEX) in the endothelium of a 54-year-old woman who has worn polymethylmethacrylate contact lenses for 34 years. ECD = 3081 cells/mm², CV = 44%. HEX = 43%.

transplantation, on corneal endothelial cells have been extensively studied. At 1–5 days after cataract extraction, a variable decrease in endothelial cell density was noted depending upon surgical trauma.⁴⁵ After these initial cell losses, the endothelial cell density decreases at an average rate of 2.5% per year for at least 10 years after surgery, with or without a lens implant.⁴⁶ This is four times the rate in unoperated eyes.⁸ The causes of this accelerated decrease in endothelial cell density after

cataract extraction are unknown, although they may include decreased nutrition from aqueous humor that can bypass the pupil through a peripheral iridectomy, decreased innervation, increased subclinical inflammation, and exposure to vitreous humor.

In corneal transplantation (penetrating keratoplasty), the central recipient endothelium is replaced with the endothelium of an allogeneic donor cornea removed post mortem. After transplantation, endothelial cell density on these donor corneas is decreased by an amount that depends on the methods and duration of corneal storage and surgical trauma. For corneas preserved at 4°C, the method used most commonly in the United States, there is a significant correlation between storage time and the decrease in cell density from the time before storage to the time after surgery, with corneas stored for longer periods having more cell loss.^{47,48} This relation between storage time and endothelial cell loss has also been found for corneas preserved by organ culture, the method most commonly used today in Europe.⁴⁹ A major portion of cell loss can be attributed to apoptosis for both methods of storage.^{50,51}

After transplantation, the endothelial cell density on the donor cornea decreases at a fairly rapid, but gradually decreasing, rate for several years. By 3 years 53% of the preoperative endothelial cell density is lost.⁵² The endothelial monolayer on the graft seems to spread during this time to cover the wound and the areas of peripheral damage. Studies have shown that endothelial cells can spread both from recipient to donor⁵³ and from donor to recipient.⁵⁴ Episodes of endothelial allograft rejection, although successfully aborted, cause a decrease in endothelial cell density.⁵⁵ In our study of 500 consecutive penetrating keratoplasties performed between 1976 and 1986, transplants that had no known rejection episodes or reoperations lost central endothelial cells at average rates of 7.8%/year between 3 and 5 years postkeratoplasty, and 4.2%/year between 5 and 10 years.⁵⁶ Our most recent data on this long-term prospective study indicate that the rate of cell loss gradually decreases, but remains more than three times the normal loss of 0.6%/year⁸ for 20 years postkeratoplasty.⁵⁷

Figure 5 shows two examples of transplanted corneal endothelium observed for 20 years after keratoplasty for keratoconus. Endothelial cell density in one of the grafts gradually decreased at a rate similar to the average losses in corneal transplants.⁵⁷ In the other graft, most of the donor endothelial cells did not survive. The graft was oedematous for months after keratoplasty, but it had cleared enough by 2 months for adequate specular microscopy, which showed a cell loss of 88% from the preoperative donor cell density. We know that the donor cornea was of poor quality because its mate failed after

transplantation and was replaced (few endothelial cells remained when it was examined histologically). The central endothelial cell density of this graft from a poor-quality donor (Figure 5) increased for 5 years to a density that was similar to the mean density in the other graft in Figure 5. Presumably, healthy recipient cells had spread onto the donor cornea. The endothelial cell density of both grafts decreased gradually for the subsequent 15 years. This is certainly atypical, since grafts that have low initial endothelial cell densities usually fail before those that do not (see below).

As the transplanted corneas age and the number of endothelial cells decreases, the corneas thicken.⁵⁷ This is the result of decreased endothelial cell function. The pump activity of the enlarged endothelial cells on corneal transplants is markedly diminished despite an increased barrier function (decreased endothelial permeability to

fluorescein).^{58,59} This change in both the pump and permeability can be explained by the loss of total intercellular space.⁶⁰ As endothelial function continues to deteriorate, the grafts can develop a condition, called late endothelial failure, characterized by persistent graft swelling and haze that is unresponsive to corticosteroids.⁶¹ Late endothelial failure accounts for more than 90% of graft failures after the first five postoperative years.⁵⁶

Is a lower initial postoperative cell density or an accelerated loss of cells responsible for the extremely low cell densities that lead to late endothelial failure? In a series of 21 corneal transplants that developed late endothelial failure, we found that the rate of cell loss after the first 2 months was no different from that of the grafts that did not develop late endothelial failure. The initial endothelial cell density, however, both preoperatively and two months postoperatively, was significantly less than it was in the grafts that did not develop late endothelial failure.⁶¹ This suggests that improved corneal storage techniques or interventions that increase the postoperative endothelial cell density may decrease the rate of late endothelial failure and increase graft longevity. Three strategies in particular might be tried to improve endothelial cell survival. First, a molecule that inhibits apoptosis might be included in the preservative medium. Such a strategy improved the cell survival in cold-stored human livers.⁶² If post-transplant inhibition of apoptosis is needed, the transduction of cell survival genes such as *bcl-2* or *p35* into the corneal endothelial cells could be accomplished during corneal storage.^{63,64} Second, the number of endothelial cells on the donor cornea might be increased by the use of growth factors and the disruption of cellular contact inhibition. This strategy has been used to induce mitosis in human donor endothelial cells.⁵ Both of these strategies could be tested with human corneas in a xenograft model of transplantation⁶⁵ and, if successful, could be easily incorporated into current methods of corneal preservation. Finally, improved methods of corneal preservation at low temperatures (cryopreservation) could eliminate the time-dependent deterioration of donor tissue during storage.⁶⁶

Figure 5 Sequential endothelial photographs for 20 years after transplantation of donor corneas of good and poor quality. The endothelial cell densities at each examination are shown on the right. Only 12% of the central endothelial cells of the poor-quality donor remained 2 months postkeratoplasty. The central endothelial cell density of this graft gradually increased for 5 years, presumably from the spread of cells from the recipient cornea, until it became equal to that of the more normal graft on the left. The cell densities of the two grafts then decreased at similar rates for the next 15 years. This is atypical; grafts with fewer endothelial cells early after transplantation usually develop late endothelial failure earlier.⁶¹

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