ABSTRACT

This current study aimed to review the importance and assessment of corneal endothelium in terms of corneal transparency. The study revealed that the normal metabolic activity is necessary to maintain the temperature of the cornea, to renew its cells, to support the exchange processes of nutrients and to maintain its transparency. The endothelium is the inner layer of the cornea. The main function of the endothelium is to remove fluid from the corneal stroma, thus allowing the cornea to remain transparent. Younger patients recover relatively more easily after endothelial injury because they have a larger number of endothelial cells compared to older patients. Knowledge of the number and condition of endothelial cells are crucial for a number of decisions that the ophthalmic surgeon is called upon to make usually before surgery and Endothelial assessment health is a criterion for choosing a surgical method.

Keywords: Cornea; surgery; transparency.

1. INTRODUCTION

The cornea is a transparent and avascular structure composed mainly of collagen fibers that allows the formation of the visual image in the retina. The high concentration of collagen in the cornea makes it compact and resistant to deformations and injuries and together with the sclera they compose the outer layer of the eye [1]. Together with the tear film, they compose the anterior refractive surface of the eye. Its clarity is the result of both its anatomical structure and its
physiological properties. The human cornea consists of 5 layers, the epithelium, the stroma and the endothelium, and two inner layers, the Bowman membrane and the Descemet [2] membrane. Recently, another layer, the pre-Descemet membrane, was discovered [3]. Harminder S. Dua and his colleagues announced the existence of another layer, which is located between the stroma and the Descemet membrane. This layer was called the Dua layer or pre-Descemet membrane. It is a layer consisting of 5 to 8 types of collagen type I. Small amounts of type V collagen have also been found, as well as type IV and VI [3].

About 70% of the dry weight of the cornea consists of collagen, which is mainly organized in fibers [1]. Collagen types I, II, III, V, VI, XII, XIV have been found in the corneal layer, with type I collagen being the main type. In an average adult, when observing the cornea on its anterior side, its diameter reaches 11.5 to 12 mm on the horizontal axis, while on the vertical axis it is approximately 1 mm smaller [4,5]. Its thickness is smaller in the center (~ 0.5 mm) and gradually increases towards the periphery, in contrast to the thickness of the cornea of many animals which is the same for most of the cornea [6]. Its shape is flatter at the periphery and becomes more curved towards the center, which makes the cornea a non-spherical structure.

2. CORNEAL ENDOTHELIUM

The endothelial surface of human cornea is approximately 130 mm² [7]. The composition of the corneal endothelium consists of single layer of hexagonal cells, rich in mitochondria, which resembles a mosaic and is in contact with the aqueous humor. Endothelial cells have a size of about 20 μm, and their thickness is about 5 to 6 μm [8]. During corneal formation, there is cell migration from the nerve endings that form the hexagonal shape of the endothelium. Endothelial growth occurs around the fourth week of endometrial embryonic development and the endothelial progenitor cells are produced with the participation of mesenchymal cells of the mesoderm [9]. The endothelium does not reproduce after birth and the number of cells decreases from 3500-4000 / mm² at birth to about 2000-2500 / mm² in the adult cornea. In a healthy cornea there are 390,000 to 520,000 [9]. Centrally, the density decreases by 0.6% per year, while at the same time they increase in size to fill the gaps. At the same time, their hexagonal ratio is reduced from 75-80% to 60%.

Endothelial cells play a very important role because they are responsible for regulating of fluid and soluble flow as a pump and leaking barrier between the aqueous humor and the layers of the corneal stoma [10]. Corneal endothelial cells act as a barrier, which allows proteins and molecules to enter the corneal stroma from the anterior chamber. This happens by an active Na⁺/K⁺ATPase pump [11]. The active Na⁺/K⁺ATPase pump takes out water and ions from the corneal stroma into the aqueous humor due to osmosis, which helps to maintain corneal thickness and transparency. Corneal dysfunction is possible in a number of cells Less than 500 / mm² [12].

Although epithelium cells, have the capacity of self-renewing, the endothelium does not renew producing cells. According to this if there is a loss of endothelium cell procure by different pathologies then the undamaged endothelial cells become enlarge and cover any defects by migrating, in order to maintain corneal transparency. Cell density is a good indicator of functionality, which can be supplemented by other parameters such as size variation and cell morphology.

Polymegathism and Pleomorphism of the endothelial cells is a phenomenon of cell changes (increase their shape and size) [13]. Corneal endothelial cells do not have a regenerating capacity, so their loss produce enlargement in size and spread of remaining cells to cover the defective area resulting in altering of their hexagonal shape. This can happen due to age, long-term misuse of contact lens wear, after cataract surgery, inflammation, keratoconus, diabetes and corneal transplant surgery. In addition, endothelial cells, acting as a

Fig. 1. (a) Corneal endothelium of a non-contact lens wearer without blebs, (b) the black spots are endothelial blebs of a contact lens wearer, (c) endothelium of a diabetic patient [14,15]
Fig. 2. Different endothelial cells of (a) 4-year-old child, a person above 70 years old (b) and after cataract surgery (c) [16]

pump that absorbs excess fluid from the layers, are the ones that maintain the transparency of the cornea. They act, even, as a barrier that impedes the uncontrolled entry of aqueous humor into the layers and as a water pump to transport ions, resulting in the hydration and nourishment of the cornea.

The above images show the endothelium of 4-year-old child(a) and of an elder person above 70 years old (b). Figure (a) illustrates the normal healthy cells in size and morphology. The elder person, has larger cells in size and different morphology. The endothelial cells also change in size and morphology after cataract surgery (c).

There are many reasons that the corneal endothelium and its integrity must be assessed.

a. For research reason to understand the pathological changes that occur in certain diseases either of the cornea or systemic diseases that affect it [16].
b. For contact lens users and the hypoxia produced due to overwear [17].
c. To assess the physiology of endothelium before and after refractive, cataract or crosslinking surgery [18], and to qualify the donor’s cornea in transplant surgery.

Corneal endothelium can be assessed by using different imaging techniques. The polygonal endothelium cell shape can be seen with:

i. Slit lamp (Specular reflection set up) 
ii. Confocal microscopy 
iii. Specular microscopy 
iv. Phase contrast light microscopy 
v. Histology: trypan blue and alizarin staining, 
vi. Immunohistochemistry

3. In vivo METHODS

3.1 Slit Lamp (Specular Reflection Set Up)

Specular reflection with slit lamp is an accessible to use technique for optometrist. The beam of the slit lamp is set to 0.1 – 0.5 mm. The illumination system is set at 45° from the optic axis of the eye. The microscope is also set at 45° from the optic axis of the eye from the other side with the maximum magnification 40-50 x. Once the cornea section is focused then at the back side of the section the endothelium will be revealed. If the slit lamp has a camera then the cells can be photographed for observation. This is an in vivo observation technique [19].
3.2 Confocal Microscopy

Confocal Microscopy is a technique for depicting human tissues. It is a type of microscope that allows the display of objects of increased thickness, such as the cornea. In 1957, Marvin Minsky was the inventor of the confocal imaging used by all modern microscopes. Confocal Microscopy is an optical system where the lens and condenser are focused on the same point. It is used for in vivo imaging of the anterior surface of the eye, especially the cornea. It can provide images of the different layers of the cornea in a frontal plane. The image quality is dependent on contrast and resolution.

The operating principle of the confocal microscope is based on the field of view constraint, providing high definition. In reality, only one lens is used for both the lighting and the display of the sample, while mirrors are presented in the visual path. There are two ways for taking pictures of the sample. Minsky is using a fixed light beam (the stage scanning method) in which the object moves around the bond. The light from the targeted specimen tissue plane reaches the detector while light from the above and below specimen tissue plane is suppressed by a pinhole effect [20,21].

The beam scanning advantage of confocal microscopy is that the light beam is the one that moves around the object and that is why the method is used clinically. One of the main advantages is the creation of images in much better contrast and contrast quality compared to the desktop microscope [22,23,24,25]. This is an in vivo method, where three main types of confocal microscopes are clinically used: a) Tandem scanning confocal microscope (TSCM), b) Slit scanning confocal microscope (SSCM) b) Laser scanning confocal microscope Heidelberg Retina TomographHRT I, II & III (LSCM).

The high axial and transverse resolution (x, y axes) as well as the possibility of photo-shooting on the z-axis (perpendicular to the plane of the specimen-object), are the main advantages of the confocal microscope over the conventional microscopes. Objective lenses are selected in a large numerical aperture (e.g. Leitz 50x), because arithmetic aperture determines the resolution, especially the axial one. Airy's rotating disk describes the Point Spread Function (PSF).

Axial analysis can be defined as the minimum distance (d = 2) of two points on the z-axis, so that they are distinct, according to the Rayleigh criterion. In the confocal microscope, the final PSF is three-dimensional and is an assembly of the PSF in the optical observation path and the PSF in the optical illumination path. Also, the confocal microscope reduces the effects of scattering and refraction of light and reduces the blurring and "noise" of the image, thus increasing the bright contrast and the quality of the display. In addition, it provides the ability to electronically adjust the magnification and allows the observation of specimen-objects of increased thickness, such as the cornea. Finally, its clinical value is based on the rapid scanning of the cornea and the fact that it is a fast, transient, in vivo method with high repeatability [27].

The main limitations are related to the quantitative accuracy of the measurements, which depends on the photons that penetrate the object, the size of the minimum doses examined and their location. The depth of field, the working distance and the extent of the observation field are limited by the use of a lens in a large numerical aperture. Thus, a small corneal surface is visible each time, but the image quality is improved. In order to increase the discretion, the wavelength of light (λ) is reduced [27].

3.3 Specular Microscopy

The specular microscope is a device for transient imaging and analysis of the endothelial layer of the cornea. A variety of values can be analyzed such as cell density, size and shape.

The technology is based on projecting light on the back surface of the cornea to capture the image reflected by the visual interface. Between the endothelial layer and the aqueous humor. It is also used as a preoperative imaging device for cataract surgery. The endothelial layer is examined to avoid complications such as corneal ophthalmic opacity. Since its introduction in
1981, endothelial specular microscopy has been fundamental in assessing the progress of surgical procedures. This development has led to greater protection of the endothelial and corneal tissue from surgical damage. New corneal perforation techniques or corneal transplantation would not be possible without the knowledge of specular microscopy.

Evaluation in specular microscopy includes measuring the density of endothelial cells, "size change" (Polymegathism), "hexagonal change" (pleomorphism) and comparing them. Therefore, this technology plays an important role in preoperative evaluation, surgical design and postoperative care [28,29]. Non-contact specular microscopy facilitates early and accurate diagnosis of corneal endothelial pathologies, being the best method for evaluating the integrity and function of the corneal endothelium. It also allows the identification of early keratoconus pathology, helps to develop a treatment plan and allows the long-term evaluation of endothelial function in pathological eyes that may be at risk of endothelial dystrophies, in diseases such as chronic glaucoma, uveitis and any intraocular surgery [30].

In addition, specular microscopy [31] allows the evaluation of chronic changes in the morphology of endothelial cells in contact lens users. However, studies have shown some general limitations of specular microscopy. Restrictions include differences in image quality, resolution, depth, and number of analytical cells that may be sources of inconsistency, bias, limited reproduction, reliability, and validity. It provides multiple images of different areas of the cornea, manual evaluation of abnormal endothelial cells and measurement of as many cells as possible using NAVIS-EX software which can minimize these limitations.

However, although advanced endothelial microscopy is of fundamental significance for clinical and surgical practice, there is no current method for re-measuring the same area of endothelial cells. For this reason, it is recommended that as many cells as possible be evaluated for greater validity and better endothelial evaluation. Evaluation of the central endothelial layer is important for routine examination, however a more detailed examination of the endothelium involves the study of the paracentric and the medial peripheral endothelium. [32] Evaluating more areas of endothelial cells in a single test helps increase the number of cells detected from 250 to 2,500 cells, which is a quantitative advantage of paracentric imaging. The parameters given by

Fig. 5. HRT is a high resolution confocal cornea microscope a) normal b) polymegathism [26]

![HRT Confocal Cornea Microscope](image)

Fig. 6. Specular microscopy. Basic principal [32]

![Specular Microscopy Diagram](image)
Specular Microscopy related to corneal endothelium are numbers of cells present in a unit area, cell density, cell shape variation (round, square, triangular, pentagonal and hexagonal), coefficient variation (CV) of the cell area, standard deviation (S.D.) in cell area/average cell area. Large CV indicate a variety of cells size and polymegathism while low value CV corresponds to normal cornea. Also, cell shape variation percentage of hexagonality (hexagonal cells divided by total cells count) should be at least 60% [33].

Fig. 7. Perseus specular microscope CSO [33]

2.400 cells/mm² 2.400 cells/mm² but with different CV

Fig. 8. Cell shape variation, different CV coefficient variation [33]

Fig. 9. Information given by nidek specular microscope [34]

Fig. 10. Endothelial cell photos where there is a decrease in cell density. a) 2.000 to 2500 cells/mm² b) 1.000 to 1500 cells/mm² c) 500 to 1.000 cells/mm² d) < 500 cells/mm² [34]
The pathology is more obvious peripherally in cases where the paracentric is more prone to stress, such as in eyes with anterior segment intraocular lens, eyes with glaucoma and in cases of polymorphic dystrophies. Measurement of as many cells per image (150 cells or more) is probably the most reliable information on endothelial health in normal or diseased corneas.

Corneal endotheliopathies that specular microscopy can reveal are:

a) Fuch's Dystrophy (Guttata) [35],
b) Posterior polymorphous Dystrophy,
c) Congenital hereditary Endothelial Dystrophy [36],
d) Iridocorneal Endothelial Syndrome,
e) Effects after surgical operations [29],
f) Contact lens wear [17,37],
g) Cytomegalovirus Endotheliopathy,
h) Herpes Simplex Endotheliopathy.

4. In vitro METHODS

4.1 Phase-contrast Microscopy

This is an optical microscopy technique where the brightness of light changes while passing through a transparent media (tissue) so phase shifts occurs. Phase shifts are practically invisible but become visible when there are variations of brightness. This technique is very useful in assessing biological tissues. Cellular structures are becoming visible wherein a simple microscope these structures cannot be observed. In phase-contrast microscopy there is a separation between the illuminating background brightness from the scattered light passing through the investigated tissue. It’s an in vitro method of investigating endothelial cells [38].

Fig. 11. Phase-contrast microscopy of endothelium cells [38]

4.2 Trypan Blue and Alizarin Staining

The integrity of the endothelial cells is evaluated by using trypan blue staining and alizarin red S pigment which stains intercellular the endothelial cells. This technique allows assess the number of cells and the separation of normal and damaged cells. The pH of the alizarin red S is set to 4.2 in order to visualize the optimal color leakage in the intercellular boundaries [39,40,41].

4.3 Immunohistochemistry

This is a technique of immunostaining. It involves the selective identification of proteins in endothelial cells. The visualization of endothelial cells can be accomplished by either Chromogenic immunohistochemistry, where an antibody is conjugated to an enzyme, or Immuno fluorescence, where the antibody is tagged to a fluorophore. It’s a diagnostic tool of abnormal cells [42].

Fig. 12. Staining of corneal endothelium with trypan blue and alizarin red [41]

Fig. 13. Chromogenic immunohistochemistry of endothelial cells [42]
5. CONCLUSION

Normal metabolic activity is necessary to maintain the temperature of the cornea, to renew its cells, to keep up the exchange processes of nutrients and to maintain its transparency. It is also important for the proper functioning of the cornea, which concerns the refraction of light and its response to conditions such as dehydration, invasion of microorganisms and injuries [43,44]. Due to the lack of vessels in the cornea, the necessary nutrients are obtained through the fluids that surround it, and specifically through the aqueous humor, the intermediate fluid that comes from limbal vessels and the lacrimal layer. The main factors are glucose and oxygen, while the products of metabolism that must be removed are mainly lactic acid and carbon dioxide [45].

The main function of the endothelium is to remove fluid from the corneal stroma, thus allowing the cornea to remain transparent [46]. Some diseases that cause damage to the endothelium, such as Fuchs's dystrophy, lead to endothelial lesions and eventually to corneal edema. Endothelial damage can also be caused either by trauma or by the removal of a mature cataract which can lead to endothelial damage and ultimately a reduction in the number of cells [47]. Knowledge of the number and condition of endothelial cells is crucial for a number of decisions that the ophthalmic surgeon is called upon to make usually before surgery and Endothelial assessment health is a criterion for choosing a surgical method.

Concluding endothelioscopy is important in refractive surgery (density measurements of endothelial cells play an important role in refractive surgery), in corneal transplant monitoring (a sudden drop in cell density may indicate the presence of inflammation or subclinical immune rejection), in glaucoma surgery, in cataract surgery and finally monitoring contact lens wearers.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

14. Connor CG, Zagrod ME. Contact lens-induced corneal endothelial polymegathism: Functional significance


32. Craig Thomas OD. Use specular microscopy to diagnose corneal disease. Review of Optometry; 2009.


© 2020 Pateras; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57591