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Comparison of Corneal Endothelial Mosaic According to the Age: The CorImMo 3D Project

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Abstract

Aim: The human corneal endothelium is a monolayer of flat hexagonal cells. It is a nearly regular hexagonal tessellation during the first years of life, but with age, becomes less regular in shape and size. The aim is to evaluate geometrically the age of an endothelial mosaic.
Material and methods: Segmented endothelial mosaics of healthy subjects of different age groups are compared by morphological criteria. The mosaics are studied according to their age group (decades), their age and their location (center or mid-periphery of the cornea). The measures used are: the cell density, the Ripley's L function and the cell area and perimeter density.
Results: These measures point out the endothelial cell density decrease, the cell area, perimeter and diameter increase, the cell heterogeneity increase, and the differences between central and mid-peripheral cells increases with age.
Conclusion: These measures are able to characterize healthy mosaics.

Keywords: Corneal endothelium; Cell morphology; Ripley's function; Area density; Perimeter density

1. Introduction

The human corneal endothelium is a monolayer of flat hexagonal cells, which do not regenerate and are responsible for the maintenance of the cornea transparency. When the number of endothelial cells (ECs) is too low, the cornea becomes edematous, causing irreversible loss of vision that can only be treated by a corneal graft. The donor cornea brings numerous new functioning ECs into the recipient eye. Because of their location at the most posterior layer of this transparent tissue, ECs can be visualized in vivo using a specular microscope using the light reflected by the interface between ECs and the liquid that fills the anterior chamber of the eye. Similarly, they can be observed ex vivo during corneal storage using a transmitted light microscope or a specular microscope. The morphologic characteristics of ECs have been studied since the 1950s. Three parameters are universally used to describe the endothelium: the EC density (ECD, by convention expressed in cells/mm^2), the coefficient of variation of cell area indicative of the pleomorphism (CV is the standard deviation divided by the mean cell area), and percentage of cells with 6 neighbors, indicative of polymorphism (hexagonality).

During the first years of life, the endothelial mosaic is a nearly regular hexagonal tessellation. With aging, endothelial cells (ECs) become less regular in shape and size and their number slowly decreases, at a rate of 0.6% per year during adulthood [1]. Nevertheless, in healthy corneas, the number of ECs remains always high enough to maintain corneal clarity even in

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centenarians. This important notion of endothelial reserve disappears when diseases or traumatisms alter the endothelium. In these situations, decrease of ECD and changes in pleomorphism (i.e. shape variability) and polymorphism (i.e. size variability) can be dramatically accelerated, ultimately leading to corneal opacification requiring corneal graft.

In eye banks, donor corneas are stored and strictly controlled in order to verify if they are suitable for corneal graft. Quality of the endothelium is the main criterion to decide whether a cornea can be grafted or must be destroyed. At present, ECD is the only quantitative parameter used. A threshold under which a cornea is unsuitable for graft determines the fate of each donor cornea. It is usually of 2000 cells/mm^2 for corneas destined to penetrating keratoplasty (replacement of the whole thickness of the central cornea, constituting the gold standard and the most frequent technique worldwide) and 2400 cells/mm^2 for corneas destined to posterior endothelial graft (selective replacement of the endothelium, requiring preparation of a thin posterior lamellae that can be slightly harmful to the ECs, explaining the higher threshold). For CV and hexagonality that can be measured with image analysis [2], their influence on the post graft endothelial survival has never been studied. They are at present used as additional criteria to help qualifying corneas with ECD near the threshold.

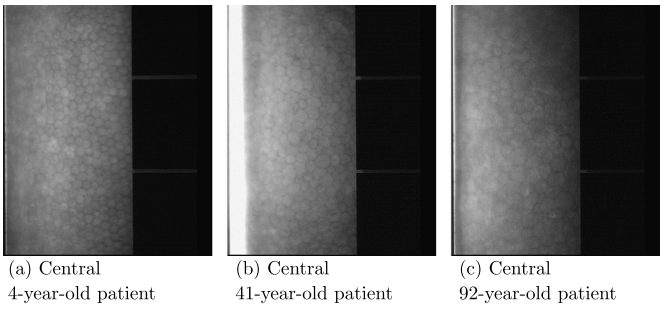
In order to better explain endothelial aging and some of the most frequent clinical situations (ECD decrease in Fuchs corneal endothelial dystrophy, the most frequent primary endothelial dystrophy, and after corneal grafts), new methods to qualify the endothelial mosaic, using geometrical and morphological criteria, are studied. The aim is to establish an original mathematical model of the human corneal endothelium. In the present work, three measures of the cell size variability are presented: the Ripley's L function and the area and perimeter cells densities. These mathematical parameters are used to assess the age of an endothelial mosaic of healthy corneas.

2. Material and methods

2.1. Source of endothelial images

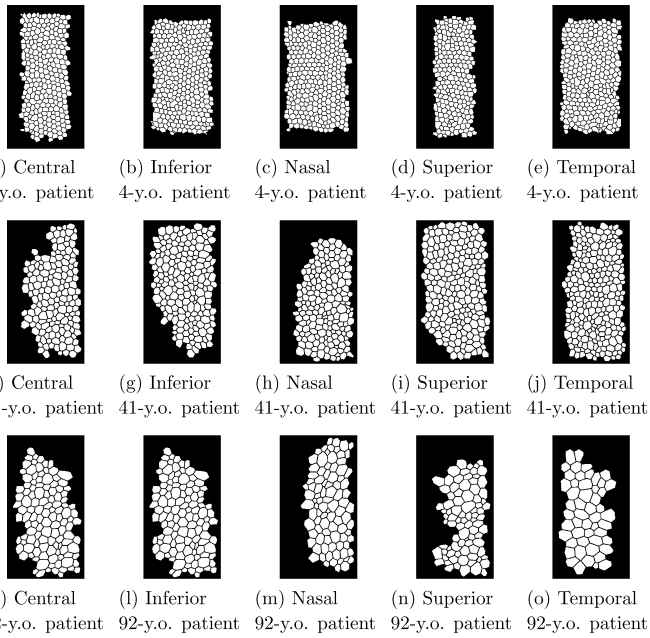
Images were taken using a small field non-contact specular microscope (SP 3000, Topcon, Tokyo, Japan) (Fig. 1). In 10 age groups (from 0 to 10 years old, 11 to 20, 21 to 30, ..., and 91 to 100), images of healthy eyes of 5 subjects that were taken during routine examination, were selected. Images were anonymised and patients could not be recognized from the pictures.

ECD is not homogeneous on the whole endothelium, it progressively decreases toward center [4,3]. For each eye, five images were therefore taken in the central, temporal, nasal, superior and the inferior zones of the endothelium, by asking the patient to focus on each of the 5 LEDs placed on the microscope to orientate the eyeball. The 4 non-central positions were localized 3 to 4 millimeter from the center, that is to say not in the extreme periphery of the cornea. As non-contact specular microcope have a narrow field of view, the acquisition of 5 images distributed on the corneal surface is the usual protocol used in routine to increase the sampling and obtain a more representative analysis. Each image was manually segmented by an expert using ImageJ (Fig. 2).



(a) Central 4-year-old patient (b) Central 41-year-old patient (c) Central 92-year-old patient

Fig. 1. Representative images of the endothelial mosaic taken using a small field non-contact specular microscope.



(a) Central 4-y.o. patient (b) Inferior 4-y.o. patient (c) Nasal 4-y.o. patient (d) Superior 4-y.o. patient (e) Temporal 4-y.o. patient
(f) Central 41-y.o. patient (g) Inferior 41-y.o. patient (h) Nasal 41-y.o. patient (i) Superior 41-y.o. patient (j) Temporal 41-y.o. patient
(k) Central 92-y.o. patient (l) Inferior 92-y.o. patient (m) Nasal 92-y.o. patient (n) Superior 92-y.o. patient (o) Temporal 92-y.o. patient

Fig. 2. Representative segmented endothelial mosaics of the central, inferior, nasal, superior and temporal zones of the right eye of three patients. They illustrate that cell area, the polymorphism and pleomorphism increase with age.

2.2. Ripley's L function

The Ripley's L function (RLF) is used to analyze the spatial distribution of a collection of points. The RLF counts the mean number of mass centers at a given distance from another mass center [5,6].

Let $P = \{p_1, p_2, \ldots, p_N\}$ be a collection of N points in the image I, considered as a bounded region of $\mathbb{R}^2$, and let A be the area of I.

An estimator of the RLF is given, for all $r \geq 0$, by:

$$\hat{L}(r) = \sqrt{\frac{A}{\pi N^2} \sum_{i=1}^{N} \sum_{j \neq i} \delta_{ij}(r)}, \tag{1}$$

where $\delta_{ij}(r)$ is equal to 1 if the distance between the points p_i and p_j is less than r, and 0 otherwise.