2.4 Donor tissue quality

Introduction

It is generally agreed that the ultimate responsibility for the selection of donor tissue lies with the surgeon. After the transition of whole donor eyes collected and judged by the surgeon to stored corneoscleral discs delivered by the eye bank, the surgeon depends heavily on the eye bank. This emphasizes once more the importance of quality control in eye banking. Methods to judge donor tissue have been developed during the years.

Stocker showed the significance of the endothelium in maintaining corneal transparency.[208] It is generally recognized that an adequate amount of functioning endothelial cells must be present on the donor cornea, to increase the chance of a long lasting successful penetrating kerato-plasty.[209,210,211] In addition, the donor cornea should have a normal curvature and be transparent.

At first only macroscopic inspection and slitlamp examination were available. Careful examination of cornea and anterior chamber is important, particularly in an in situ excision, whereas this may be done in the eye bank in case of enucleation. In the beginning, selection was primarily based on factors that may affect the viability of the endothelium such as age of the donor, post mortem delay, possible trauma before death in case of accidents, homicides, anterior segment surgery and causes of death or medical history such as malignancies, anorexia etc. The slitlamp allows determination of endothelial cell density and estimation of the degree of cell uniformity if the corneal tissue is not too swollen. These parameters gained more impact with the introduction of methods for more detailed inspection of the endothelium in eye banking.

In 1978 Sperling introduced a light microscopy method by artificially induced swelling of the intercellular space to visualize the endothelium.[47,118,212] This invasive evaluation method for the corneal endothelium was generally combined with organ culture as storage method. It was however not the first choice for the hypothermic storage and for this specular microscopy was preferred. The design of the specular microscope has been described in 1968 by Maurice.[213] It was developed for eye banking in the 1980s although its use is precluded by swelling of the cornea. Inspection of the endothelium for hypothermically stored corneas has become mandatory in 2001. The confocal microscope is not yet routinely applied in eye banking.[214,215]

Software programs for morphometric analysis of the endothelial images have been introduced. Although in eye banking general agreement exists about a "well looking endothelial cell mosaic",[216] objective parameters, such as endothelial cell size, cell density, coefficient of variation in cell size, percentage of hexagonal cells, shape factors etc, are needed to support standardization.[217] However, caution is warranted for a unjustified sense of security. The technique needs experts for correct interpretation and interactive correction of the images,[41] correct calibration and the use of sufficient samples. For a

correct application, the technique is time consuming and has to be performed in addition to a good examination. The images should be representative for the endothelial surface. Functional tests for the corneal endothelium exist: corneal hydration control test,[218,219] fluorophotometry,[45] investigational perfusion of the cornea[220] and endothelial pump quantification.[67,221] In experimental conditions, they are very valuable but they cannot be applied in eye bank conditions as they affect the viability of the corneas. In vivo contact pachymetry reflects alterations in both the endothelial barrier and the pump functions by changes in corneal thickness.[223] In vitro maintenance of corneal hydration is disturbed and corneal thickness is meaningless for endothelial function. In addition contact pachymetry in excised corneas is not feasible.[222] Therefore, for the time being, one has to rely on morphometric aspects of the corneal endothelium to estimate the function and the functional reserve as they reflect the stresses and strains that are imposed on the corneal endothelium.

Parameters

Donor age

During life the central endothelial cell density (ECD) decreases and so does the variation in cell size, although the linear correlation is weak (see chapter 2.1). For this reason, age limits have been set for donors. No correlation between donor age and graft clarity however has been shown.[224,225,226,227,228,229] Corneas from donors older than 70 years of age have maintained transparency for long postoperative time intervals, which indicates that they can withstand preservation and surgery (see chapter 4.3).[230,231] This shows that age alone is a poor criterion for selecting healthy donor endothelium. The preoperative cell count appeared to be of greater importance than age.[232] Evaluation of the corneal endothelium therefore may allow safely extending the donor age criterion by identifying older donors with morphologically normal endothelium. Several eye banks have described a significant proportion of corneas judged suitable for transplantation in the higher age groups albeit it a smaller proportion than in the younger age groups.[233,234,235] By setting a maximum age limit, suitable donor corneas may be missed.

In Europe, the mean age of the donor has been higher for OC stored corneas where inspection of the endothelium was included in the storage procedure and lower for the hypothermically stored corneas. With the routine application of the specular microscope this difference is disappearing.[121,236,237] This indicates that to expand the donor supply eye banks prefer a more direct parameter than age alone. In the Netherlands an upper age limit has been introduced in the recent years to obtain a better yield and to balance the supply of donor tissue with the demand.

Although young donor tissue is preferred because of the high ECD, the minimum age limit is generally set between 2-6 years of age. The small diameter, the thinness, the elasticity and pliability of the very young tissue may cause technical problems for the surgeon. A postoperative myopic shift is ascribed to the steep anterior curvature.[238]

Post mortem delay

Based on the knowledge that the viability of the endothelial layer deteriorates with time, a maximum post mortem period is applied. Endothelial cells facing a stagnant aqueous humour show progressive cell death due to the release of hydrolating enzymes from surrounding tissues.[85] The exact time point where cell changes lead to irreversible failure is unknown. In addition, this process depends on the temperature during post-mortem time, which can be discerned in two periods: the first at room temperature where the hydrolysis increases and the second period at low temperature reducing this process. Both periods may vary in time. This is why a definite cut-off point cannot be defined and time periods from 4 to 48 hours have been accepted.[239]

Damaged and necrotic endothelial cells may be observed by inspection of the corneal endothelium. However, the morphologic parameters do not necessarily reveal all functionally affected cells. The process of cell necrosis may progress during storage.

The mean post mortem delay in Europe for hypothermic stored corneas is lower than that for OC stored corneas because during OC wound healing occurs.[121,240,241] Little relation between time after death until enucleation on the one hand and suitability of corneas for grafting on the other hand has been shown; the relation is even weaker when the transport time to the eye bank is taken into account (non published data Cornea Bank Amsterdam).

Morphology corneal endothelium

a. Endothelial cell density

Preoperative endothelial cell count is an important determinant for postoperative endothelial cell count.[232] During keratoplasty, endothelial cells get lost.[209] This loss continues and more than 70% cell loss is observed after 15-20 years.[211] A mathematical model to describe the fall in endothelial cell density allows an estimation of graft longevity based on cell density at the time of transplantation.[35] It provides a rationale for setting a minimum cell density suitable for keratoplasties involving the endothelium. According to EEBA Standards, 2000 cells/mm^2 is considered to be the minimum for long term graft survival. Definite cut-off points however are left to the discretion of the (medical) director of the eye bank as long as studies linking graft outcome with donor cell density are lacking and given the paucity of long-term endothelial cell density data from corneal transplants.

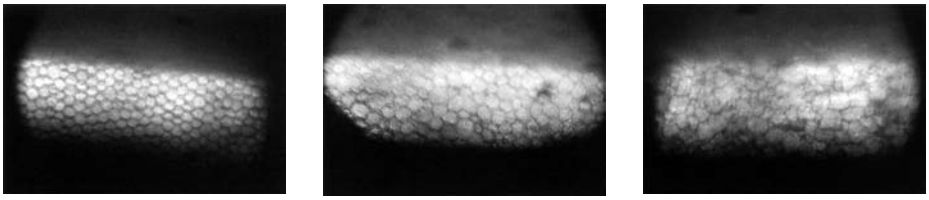

Figure 12: Specular microscopy, from left to right normal endothelium, moderate and severe polymegathism and pleomorphism.

b. Polymegathism and Pleomorphism

It has been reported that corneas with considerable polymegathism or pleomorphism (figure 12) have an increased incidence of postsurgical decompensation and a reduced functional reserve.[33,37,72] This is the reason why they are considered less suitable as donor tissue. The presence of corneal guttata is also known to reduce endothelial function or functional reserve thus contraindicating the use of these corneas for transplantation.[68,242,243]

As studies linking graft outcome with these parameters are still lacking, definite cut off points are not available and generally left to the discretion of the (medical) director of the eye bank. Moderate to severe forms are generally a contraindication for grafting the endothelium. For anterior lamellar keratoplasty, the mosaic of the corneal endothelium only is a contraindication when the stromal morphology is involved. In case of long lasting corneal guttata or severe forms of polymegathism and pleomorphism secondary changes in the stroma cannot be fully excluded.

With software for morphometric analysis polymegathism and pleomorphism can be described with the coefficient of variation in cell size and the percentage of hexagonal cells.

c. Endothelial cell loss during preservation

This parameter is exclusively used with organ cultured corneas. Organ culture may be considered as a stress test. The endothelium of corneas affected pre- or post mortem will significantly loose more cells.[244,245] Also the presence of HSV virus or other microbes may lead to significant endothelial cell death.[166] At the physiological temperature during organ culture endothelial wound healing (see chapter 2.1) takes place and cell loss will be visible either by the presence of reformation figures[113] resulting in a less regular cell mosaic or by a reduced cell density compared to pre storage.[119] The cell loss during preservation is less in the older age donors than in the younger ones.[234]

Endothelial evaluation methods, application and reliability

a. Specular microscopy

Non contact specular microscopy enables to see unstained endothelial cells and epithelial cells in an eye bank environment,[246,247,248] either in the enucleated eye or in the excised cornea in a storage solution, as long as there is no significant corneal edema.[249,250] To exclude artefacts associated with the appearance of the endothelium the observation should take place at room temperature. A disadvantage of this method is the limited sample size not allowing an accurate quantitative judgment of the endothelium.[41,239] An advantage is the fact that the images are well recognizable for corneal surgeons because they are used to the endothelial images of their patients (figure 13).

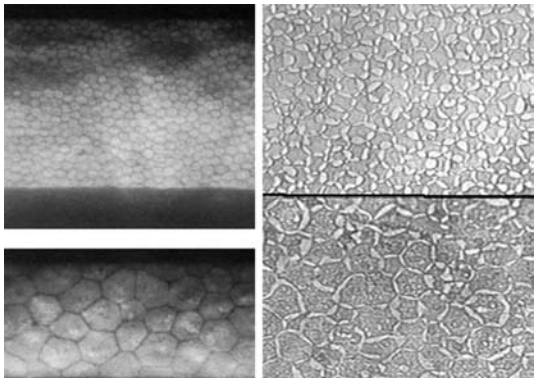

Figure 13: Microscopical evaluation of the endothelium. Specular microscopical image (left: light microscopical immage, right: after swelling of the intercellular borders, top = high density, bottom = lower density).

b. Light microscopy

The endothelial cells are visualised by swelling the intercellular space with a hypotonic solution. This invasive technique takes place under aseptic conditions. It allows the inspection of the entire endothelial surface,[251,252] irrespective of the hydration status of the cornea. The swelling is transient and disappears after a couple of minutes. As it is dependent on the integrity of the cellular membranes, the incidence of no swelling may indicate membrane damage and necrotic cells. Induction of the swelling and the swelling pattern are dependent on storage conditions, such as storage time and storage medium used.[253] The interpretation of swelling pattern and images requires experience. For the corneal surgeon not used to work in an eye bank these images might be very difficult to understand. They differ from the patient's images obtained by specular microscopy or confocal microscopy because the swelling of the intercellular space interferes with the endothelial cell mosaic. The application of a vital stain such as trypan blue preceding the

artificial swelling of the intercellular space may help to discriminate dead or necrotic cells (figure 12).[254,255]

c. Morphometric analysis

Nowadays the specular microscope is often equipped with software for morphometric image analysis. To achieve correct and interchangeable endothelial cell densities, the magnification of the specular microscope has to be calibrated.[256,257] In addition interactive manual correction of the image is needed.[256] The inter-observer reproducibility is good for ECD and Coefficient of variation in cell size but moderate for percentage of hexagonals.[41]

Morphometric analysis programs are available for the light microscope systems (Rheintec, Samba). All programs aim for automated cell analysis that is independent of the observer and of his experience, but reliable results require also interactive manipulation to correct the automatically assumed cell borders. Correct calibration of the microscopes is as important for the light microscope as it is for specular microscopy.[41]

Manual counting by Gunderson's method, although time consuming[119,258] provides reliable cell counts.[259] For parameters such as variation in cell size and the percentage of hexagonal cells, image analysis is necessary. The cell counts should be similar to those obtained with Gundersen's method. Only after fulfilling that condition these parameters can be considered reliable.

Corneal curvature

Concern exists that donor corneas with an abnormal curvature (keratoconus, after photo refractive keratectomy (PRK) or laser- assisted in situ keratomileusis (LASIK) escape detection in the eye bank and become available for grafting. Methods are advocated for eye bank use [260,261,262] but they are nowhere routinely applied because reliable results for post-mortem tissue are still lacking.

Selection for lamellar grafting

Tissue judged not suitable for penetrating keratoplasty may be designated for anterior or posterior lamellar grafting.[263] For donor tissue to be designated for anterior lamellar grafts an abnormal endothelial morphology may be accepted as long there are no indications for secondary changes in the stromal structure. For posterior lamellar grafting, an abnormal curvature and stromal opacities are no contra-indications. Some authors suppose a higher cell density is needed to compensate for the surgical trauma, others however have demonstrated that experience is more important.[264,265] Follow-up results will help to define the cut-off points.