Summary and conclusions

The cornea is a transparent and colourless tissue that serves as the window of the eye. Together with the pre-corneal tear film it can be considered as a functional unit. In addition to a regular curvature the transparency is essential for a useful vision. The corneal function is compromised by disorders of the epithelium, stroma and endothelium. Transplantation of a donor cornea is the standard surgical procedure in case of irreversible damage to this tissue.

Vital for corneal transparency is its regular structure and a well controlled hydration. The endothelium is the most important layer of the cornea as it plays an essential role in the regulation of this hydration. The morphology of the endothelium reflects its viability, essential for a functional graft (see chapter 2.1).

After the first successful corneal transplantation in 1906, Zirm described that he believed careful handling of the living human donor tissue contributed to the positive outcome of the operation. This more than a century old observation is still relevant. During the first half of the twentieth century ophthalmologists did not have detailed knowledge about the factors responsible for the quality of the donor tissue. Therefore at first clear corneas from living donors were used. Eye banking started when Filatov in 1935 reported the successful use of postmortem tissue. Because of this a larger source of donor tissue became available.

Until the 1970s the corneal surgeon was personally involved in collection and selection and consequentially responsible for the quality of the donor tissue. With the possibility to store corneoscleral discs in tissue culture media and the introduction of these procedures in the 1970s, the function of eye banks changed from small collecting centers to storage facilities. By this change the corneal surgeon had to rely on the professionals in the eye bank for donor tissue quality. At first this was based on personal contacts but today trust alone is no longer acceptable and it is replaced by documented control of the donor tissue quality; quality management systems have been implemented in eye banks (see chapter 2.2).

The risk of disease transmission from donor to recipient became clear over the years. A few cases have been described of communicable systemic diseases transmitted by corneal transplantation: rabies, Creutzfeldt Jacob and hepatitis B. Herpes simplex transmission with subsequent reactivation of donor derived herpes simplex type 1 has also been proven. According to European Union directives and national legislation, that do not discriminate between cornea and vascularised tissue, the donor has to be serologically screened for lues, human immunodeficiency virus, hepatitis B surface antigen, hepatitis C and human T-lymphotropic virus. Because serological screening tests for rabies and Creutzfeldt Jacob disease are lacking, one has to rely on careful screening of the medical history of the donor (see chapter 2.3.1).

6

Compared to the eye in a living person, donor eyes have a high incidence of surface contamination. Eye banks use different procedures for tissue decontamination to prevent donor related infections. The frequency of reported donor related infections, which result in an endophthalmitis after corneal transplantation is low but high compared to the frequency of endophthalmitis after other intraocular surgical interventions (see chapter 2.3.2).

Before the endothelium could be visualised, selection was primarily based on factors that affect the viability of the endothelium, such as donor age and post mortem delay. As functional tests for the cornea cannot be applied in eye bank conditions, one must rely on the morphometric aspects of the endothelium to estimate function and functional reserve. The pre-operative endothelial cell count is an important determinant for the expected postoperative cell count. Two methods are applied for the evaluation of the donor endothelium. Specular microscopy is a non invasive method, generally applied in combination with hypothermic storage. This method allows the observation of a relatively small area of the endothelium. Corneal edema limits the examination of the endothelium which makes this method unsuitable in combination with organ culture. Light microscopy, with the disadvantage of being an invasive technique, allows inspection of the total endothelial surface, independent of corneal swelling. This technique is integrated in the organ culture procedure (see chapter 2.4).

During the years the method for donor tissue storage developed from a simple storage technique of the whole eye on melting ice or in a refrigerator to the storage of excised corneoscleral discs in a tissue culture medium. The storage temperature for corneoscleral discs is either 2-8°C, called hypothermic storage, or 31-37°C, called organ culture. The advantages and disadvantages of each method are described with respect to the technical aspects (storage condition, survival of the corneal cells, storage time), the microbiological testing and tissue evaluation (see chapter 2.5).

Moist chamber is a simple technique; the donor eye is stored in a moistened container on melting ice or in the refrigerator. The storage time is limited to 2-3 days. At present this method is generally used in combination with other preservation methods.

McCarey-Kaufman medium as well as Optisol (GS) belong to the hypothermic storage preservation media. The difference between McCarey-Kaufman medium and Optisol GS is the addition of chondroitine sulphate on top of dextran in Optisol GS and the selection of the added antibiotics. An accepted storage time for McCarey-Kaufman medium is 2-3 days and for Optisol 6-10 days. Microbes hardly grow at a temperature below 80°C. After grafting, microbes are found in many of the corneoscleral rims, consequently the microbiological status is not well defined. This underlines the importance of strict control of the donor selection criteria and decontamination procedures (see chapter 2.3.2) in hypothermic donor corneal storage methods.

In organ culture the corneoscleral discs are stored in a tissue culture medium (modified minimum essential medium). Antibiotics as well as antimycotics are added. The cornea is transferred to a transport medium, shortly before transportation. Endothelial wound healing might occur. Evaluation of the endothelium is mandatory after storage to detect significant changes in the endothelial cell mosaic, reflecting whether the endothelial viability has been affected before or during storage. Organ culture can be considered as an endothelial stress test. The microbiological status of the donor tissue is well defined because microbiological tests are performed during storage (see chapter 2.3.2). Whether the better defined microbiological status of organ culture versus hypothermic storage has consequences for the risk of endophthalmitis has not yet been proven.

The results of corneal transplantation have been improved over the years as a result of better surgical techniques, microsurgical instruments, sutures and pharmaceutical treatment. With this, the indications for corneal transplantation increased. During the last decade the interest in lamellar grafting revived. Besides the known anterior lamellar transplantation technique, the posterior lamellar procedure became possible. The demand for pre-cut tissue delivered by eye banks will grow and therefore control of selection and processing of donor tissue, selection criteria in the bank and validation of these procedures will become even more important (see chapter 2.6).

Follow-up registry of corneal grafts is a requirement to investigate quality. Analysis of the numerous factors influencing the results will provide the opportunity to continuously improve the quality of care by corrective and preventive measures in the chain of transplantation. Graft failure, clear graft, postoperative central endothelial cell density, corneal thickness, graft survival, serious adverse events, serious adverse reactions, visual acuity, visual disability and patient's satisfaction are measurable outcome results . They may be of different interest to the various parties involved, the patient, the surgeon, the eye bank and the national health authorities (see chapter 2.7).

6

Dependent on the registration level of follow-up data, comparison in more or less detail can be made of various factors influencing corneal graft outcome. With the implementation of the EU directives, notification of serious adverse events and serious adverse reactions to the competent authorities is required and collected EU wide. The optimal routing and reporting of serious adverse events, serious adverse reactions and their evaluation, are in the process of development in the different EU member states (see chapter 2.7). In the Netherlands, from 1995 to 2006, the National Follow-up Registry was connected to the eye bank registration. Data in this registration are used for the validation of eye bank procedures.

In chapter 3, the effects of the storage preservation methods on graft outcome have been studied. In a prospective study 14 pairs of human donor corneas were transplanted in patients with keratoconus, matched for age and gender. One cornea of each pair was stored in McCarey-Kaufman medium, the other preserved by the organ culture procedure. After 6 months and 1-2 years post operatively, visual acuity, central corneal thickness and endothelial cell count have been compared. No difference has been found between the two groups in this follow-up period (see chapter 3.1).

The data of the same group of patients , receiving McCarey- Kaufman stored donor tissue in one eye and tissue preserved by the organ culture method in the other eye, have been used for a long term follow-up study. Nine pairs of patients have been recovered after 14 years. Long term follow-up revealed no difference in visual acuity or endothelial cell density. A rapid cell loss rate in the first period (6 months-1.5 years) and a slow cell loss rate in the second period (1.5-14 years)have been observed, fitting well in the model proposed by Armitage and Bourne (see chapter 3.2).
Both prospective studies indicate that no difference exists between the clinical outcomes for corneas stored in McCarey-Kaufman medium or by organ culture procedure, neither on the short, nor on the long term. These studies justify the use of organ culture as preservation method, 26 years after the introduction of organ culture in the Netherlands.

In chapter 4 the effect of donor tissue parameters on the graft outcome are described. Prospective studies on the interaction of tissue parameters and outcome are lacking. Since 1990 donor corneas have been selected in the Cornea Bank Amsterdam especially for emergency procedures. These corneas do not meet the quality criteria judged necessary for elective procedures; they have either a small stromal opacity (selection group 1) or less than optimal endothelial quality (selection group 2) and are made available for immediate use. To validate this selection procedure the following studies are carried out.

In a longitudinal cohort follow-up study 151 emergency grafts are the focus of the attention. Preservation of the globe is the main objective of the emergency procedure and the outcome result of the first study. It is concluded that it is allowed to use well selected donor tissue for emergency procedures when globe survival is the primary goal (see chapter 4.1).

The outcome result for the second study is corneal graft survival. Of the original 151 grafts 115 are studied in a longitudinal cohort follow-up study. Corneal graft survival is not related to the selection group of the donors (see chapter 4.2).
Studies linking endothelial selection criteria with graft outcome as parameter are lacking. Support for cut -off points for morphometric parameters for the endothelium, is not found in the study of chapter 4.2. The graft survival in these emergency cases turned out to be more dependent on other factors than endothelial cell survival.

It is suggested in literature that quality of the endothelium is more important than donor age. The case report described in chapter 4.3 demonstrates that the corneal endothelium can survive more than a life time. It illustrates that corneal buttons can reach a very high age, in this case 136 and 141 years. One case report does not prove that successful grafts for a prolonged period may be obtained from every senior donor. As the endothelium was not evaluated at the time of surgery the report is not yet a support for a minimum acceptable cell density because experiments in this respect are also lacking. Modeling of the long term loss of endothelial cells in corneal transplants may help to provide the rationale for minimum cell densities (see chapter 2.4).

Because of a unique situation in the Netherlands in 2002-2003 it was possible for the first time to validate the processing of shared donor tissue by different eye banks (see chapter 5). Despite the same processing procedures were claimed, results appeared to be in some cases less than expected. At that time this was neither scientifically confirmed, nor investigated. Consequently corneal tissue from certain banks has not been accepted. In this study, performed years later, the risks for primary graft failure, delayed epithelial closure and not clear grafts were significantly increased in the study period compared to those in the historical control group. Relations existed between these risks and specific banks. Primary graft failure is generally accepted as a serious adverse reaction. This study shows that delayed epithelial closure of the graft should be considered as such. With the currently mandatory reporting of serious adverse reactions, deviations of the trends in the results may have been detected.The claimed standard processing in the eye bank was the basis for the trust in outcome results similar to the till so far experienced results. This being not the case, asked for more control.

6

As a first step a standardized procedure in the bank is required in order to deliver a standardized product by that bank. In Europe organ culture is the storage method generally used. Common denominators as well as variations exist to adapt the technique to local circumstances. This is described in chapter 6. Possible effects of these differences on graft outcome are presented for the first time in chapter 4.1 and 4.2. This shows that much more should be done to define the effects of all local differences in an otherwise standardized procedure.

In conclusion:

The corneal surgeon is ultimately responsible for the corneal tissue he or she transplants. Retrieval, preservation and screening for safety and quality, all according to the current state of the art, require professional processing in an eye bank. In most cases nowadays the transplanting surgeon neither has the direct supervision of the eye bank and the donor tissue, nor is directly involved in the eye banking. Therefore it is necessary to rely on a transparent system to be able to carry out the responsibilities. In modern times trust is no longer enough and should be replaced by documented quality control in the eye bank, enhancing the trust. Within an eye bank all procedures must be validated. Clinical outcome data are essential for retrospective and prospective studies regarding the effect of donor tissue on graft outcome. The results of such studies are the basis for continuous improvement in the eye bank.

Although nobody looks forward to the paperwork involved, documentation is vital. Electronic data registration and exchange of these data will certainly facilitate the data collection for the improvement of eye banking and corneal surgery. This will be of great benefit to the patient. Trust will be replaced by control to finally enhance the trust for all parties involved.