

2.5 Methods of corneal preservation

The objective of corneal preservation is to store the corneal tissue while maintaining its function and cellular integrity after retrieval of the tissue from the donor until its use for grafting. General agreement exists that the vitality of the corneal endothelium in particular has to be preserved. Over the years, various methods have been developed and introduced, on the one hand to increase the duration of storage with the aim of a more efficient use of the donor tissue and the possibility of safe transport to transplantation sites and on the other hand to increase tissue quality by microbiological testing and tissue evaluation procedures (see chapters 2.3.2. and 2.4).²⁶⁶

The current preservation methods may be discerned in various ways:

- whole globe preservation versus the preservation of the corneoscleral disc,
- hypothermic versus organ culture storage,
- short term versus intermediate and long term.

In addition, the methods differ concerning the technical aspects of the method, storage condition, survival of the corneal cells, storage time and the possibilities for microbiological testing and tissue evaluation. Each method has its pros and cons.

Moist chamber storage

Technical aspects

a. Storage condition

Directly after enucleation the donor eye is placed in an airtight container with a gauze moistened with saline. The air in the container becomes saturated with water which prevents desiccation. The eye is stored on melting ice or in the refrigerator (4°-8°C).²⁶⁶ Antibiotics may be applied to decrease contamination of the epithelial surface. Mechanical rinsing with saline solution, treatment with povidone iodine and excision of the conjunctival tissue may help to reduce the microbial load.²³⁹ However, the effectiveness of the antibiotics at this storage temperature is low and the usefulness of this addition is debated.

b. Survival of the corneal cells

The cadaver time affects the survival of the cells. Usually two periods can be discerned:

- 1) For some time after death, the cadaver is exposed to room temperature. A prolonged duration of this situation may be critical for the survival of the cells.
- 2) During the cadaver storage time between 4°C and 8°C, the cells have a better chance to survive.

A direct relation has been shown between the concentration of hydrolytic enzymes in the chamber fluid, cadaver time, and the number of death and damaged cells in the endothelium.⁸⁵ The exact point in time from which cell changes lead to irreversible failure, in particular of the endothelial cells, is difficult to determine. Amongst others, it is dependent on the relation in duration of the first and the second phase of the total cadaver time, a relation that is rarely known in detail. Donor corneas have been described with functioning endothelium after 41 hours cadaver time.²³⁹ Others described a 44-55% decrease in viability after 2 to 5 days.²⁶⁷

c. Storage time

The described storage time is limited to 2-3 days, but most surgeons prefer transplantation within 48 hours after death.¹²⁵

Microbiological testing

Taking a bacterial culture of the eye by means of a swab is thought not be warranted because there has been no proven relation with postoperative keratitis or endophthalmitis.^{125,237}

Tissue evaluation method

For tissue evaluation one has to rely on slitlamp biomicroscopy. Specular microscopy can be performed upon whole donor globes supported in special fixation devices²⁶⁸ until about 6 hours post mortem folds in Descemet's membrane begin to occur and the corneal thickness is increasing (see chapter 2.4).²⁶⁹ In addition there may be a significant time of re-warming necessary to allow viewing of the endothelium.²⁷⁰

Pros and cons of the moist chamber storage

The major advantage of this method is its simplicity, since it requires little expertise and a minimum of manipulation. The surgeon has all the means to judge the quality of the tissue. Therefore the technique is still commonly used throughout the world as a sole storage technique in case surgeons prefer fresh donor tissue for limbal cell transplantation or in case they like to modify the shape and excision technique or in developing countries where the means are not available or whether they do not prefer to do it otherwise.

The major disadvantage is the very limited storage time. In most cases in modern eye banking or eye banking in Western countries the time period is not sufficient to allow donor screening or HLA matching, thought necessary to be of benefit for corneal grafting. In addition grafting has to be performed as an emergency procedure to prevent loss of donor tissue.

Hypothermic storage

Technical aspects

a. Storage condition

After thorough decontamination (see chapter 2.3.2) the corneoscleral disc is placed with the epithelial surface down in a container filled with storage solution after dissection in situ or after dissection from the globe in the eye bank. The container is refrigerated at 2° - 8°C without change of the medium, until the tissue is needed for surgery.^{78,266}

b. Survival of the corneal cells

b1. M-K medium

The original medium consists of tissue culture medium TC-199, dextran (5% 40.000 molecular weight), bicarbonate and antibiotics. The colloidal osmotic agent dextran prevents excessive swelling of the excised corneas in liquid medium. The pH of the original medium was unstable as the bicarbonate in the medium has to be balanced with carbon dioxide gas which is present in too low concentrations in the air. A synthetic buffer 0.025 M HEPES (N-2-Hydroethylpiperazine-N'-ethane-sulphonic acid) has been added. This new formula is referred to as modified MK.²⁷¹ The antibiotics added are penicillin/streptomycin for both MK and modified MK. The storage solutions are not supplemented with antimycotics.

b2. Optisol (GS)

The nowadays most commonly used medium Optisol (GS) consists of a mixture of the tissue culture media TC-199 and Minimum Essential medium (MEM), HEPES buffer and antibiotics. The macromolecules chondroitin sulphate (2.5%) and dextran (1%) prevent corneal swelling in vitro. Other additions are vitamins, pyruvate, hydroxyproline, ATP precursors and other nutrients. The difference between Optisol and Optisol GS is that the antibiotic added in Optisol is gentamycin while in Optisol GS streptomycin is also added as a second antibiotic.²⁷² Contrary to dextran that has no preservative function, chondroitin sulphate protects the donor cornea. Probably it acts as an antioxidant and free radical scavenger. It might also regulate cat ion fluxes across cell membranes.²⁷³ However, the exact mechanism is not known. Optisol corneas are thinner than the corneas preserved in MK.

Cooling reduces metabolic demand and increases tolerance to anoxia but there are cell and tissue specific limitations. The epithelium and keratocytes appear to be less tolerant to hypothermic storage than the corneal endothelium.²⁷⁴ A relation exists between storage time and percentage dead cells in the endothelial monolayer, with nearly 100% cell death observed after 8 days in MK.²⁷⁵

In the Optisol (GS) solution, this process is much slower: 9.5% endothelial damage in one week, 11% after 2 weeks and 95% to 100% of endothelial cell viability loss after 67 days.²⁷⁶

Storage time

As the extent of epithelial defects increases with storage time,²⁷⁷ the generally used storage time for MK is 2-3 days and for Optisol 6-10 days, which is shorter than the maximum time advocated by the suppliers.

Microbiological testing

Microbiological tests with samples of the storage solution are generally not performed, as the storage time is (too) short to have the results available before surgery. In addition, with proper decontamination before storage the number of contaminating microbes will be low and will not grow at this temperature (see chapter 2.3.2).

Tissue evaluation method

For tissue evaluation slitlamp biomicroscopy and specular microscopy are available. Special fixation devices for the corneoscleral disc facilitate inspection of the cornea in a non-invasive way. Because the appearance of the endothelial cells varies with temperature, type and time of preservation and media, evaluation at room temperature is recommended. Determination of endothelial cell density is required according to the Medical Standards of the Eye Bank Association of America effective since 2001. In general this will be done by a pre-storage inspection of a small area of the endothelial surface (see chapter 2.4). Evaluation of the endothelium by light microscopy is not recommended as it is an invasive technique and the possibility for microbiological tests before surgery is limited. At the time of inspection two aspects have to be taken into account: at the hypothermic temperature, wound healing of endothelial cells is not expected as this process is dependent of a high metabolic activity. Cell damage may progress. Apoptosis and cell necrosis have been observed in particular if factors known to affect endothelial viability such as post mortem delay are involved. Even though these processes will hardly proceed during the hypothermic storage, they will continue when the temperature rises after grafting.²⁷⁴

Pros and cons of hypothermic storage

The technique is simple. It requires no complex and expensive equipment. Corneal tissue is available for direct use. The storage solutions are commercially available. The storage time for MK might be considered as too short, but the more modern Optisol offers the possibility to schedule surgery.

Because the hydration is artificially maintained, in Optisol reaching normal levels, the tissue is easy to handle for the surgeon. It also offers good storage conditions for corneal lamellae or pre-cut tissue, required today in modern keratoplasty practice.

A disadvantage might be the not well defined microbiological status of the cornea as shown by the variation in rate of positive scleral rim cultures after surgery. Another one is the limited endothelial wound healing. For these reasons, one is generally more selective concerning donor criteria. This might reduce the possible pool of donors, but on the other hand the yield is generally higher than with organ culture storage.¹²¹

Organ culture procedure



Figure 14. Tissue culture flasks: right for preservation left for transport.

Technical aspects

a. Storage condition

In organ culture the corneoscleral discs are suspended in a tissue culture medium (modified MEM) supplemented with foetal or newborn calf serum (2-10%), antibiotics and antimycotics and stored in an incubator at 30° - 37°C in tissue culture flasks or vials (glass or plastic). Macromolecules as dehydrating agents necessary to maintain a normal corneal hydration in vitro are ingested by the corneal cells at a physiological temperature.²⁷⁸ Rather than preventing corneal swelling to about twice the normal thickness due to corneal edema during organ culture, the swelling is reversed shortly before transplantation. The cornea is transferred to the transport solution (figure 14), storage medium supplemented with dextran because addition of dextran to the first phase storage solution is technically not possible. The cornea is transported at ambient temperature. Sperling has demonstrated that the cornea is able to tolerate these circumstances.¹¹³ Banks use various compositions of storage solutions, different base tissue culture media, different concentrations of dextran and different antibiotics resulting in corneas differing in hydration status and thickness (see table).¹¹³ Also the moment the corneas are transferred to the transport solution before surgery may vary.¹²¹

b. Survival of the corneal cells

During storage, the epithelium reduces from 6-7 layers to 3-4 layers. Epithelial defects that may be present due to post-mortem delay and/or trauma before storage are repaired within a week by growth of epithelial cells from the limbal area. Most of the keratocytes survive.²⁷⁹ Although a direct relation between storage time and cell loss has been observed, it takes in general about 4-5 weeks before the cell loss becomes significant. During OC, endothelial wound healing takes place.^{109,280} On the one hand reversible damage will be restored, irreversible damage e.g. due to post-mortem delay will be repaired. On the other hand affected endothelium will be reflected by significant and extreme cell loss and this way organ culture serves as a stress test.²⁸¹ The transport solution containing the dextran reduces the epithelial cell layer within 3-4 days to 1-2 layers. Because the dextran T500 is ingested by the keratocytes and the endothelial cells, the storage time is limited. Conflicting results have been published about the degree of the toxic effects.

c. Storage time

Considering a minimum quarantine period, 4-5 weeks storage is generally accepted provided the endothelium is inspected at the end of the storage period shortly before surgery. Up to 7 weeks has also been described if the medium has been renewed during storage. The time in transport medium is limited from 1 to 7 days.

Microbiological testing

Microbiological testing of medium samples before surgery is mandatory as well as a quarantine period before releasing the corneas. Microbiological safety of the tissue stored by OC is obtained by discarding contaminated tissue before grafting. Contamination detected during culture varies between eye banks.²⁸² This may be dependent on the antibiotic cocktail present in the medium, collection procedures, in situ excision or enucleation and the post-mortem time of collection and storage. Thanks to these possibilities, donors with an increased risk of contaminated tissue, e.g. sepsis, might safely be accepted for storage by OC.²⁸³

Tissue evaluation method

Tissue evaluation is an important aspect of this technique since its introduction by Sperling in Europe in 1978. The endothelium is observed by light microscopy (bright field or phase contrast), allowing inspection of the whole endothelial surface, irrespective of corneal swelling (see chapter 2.4). In addition to the evaluation of the endothelium, optional before storage and mandatory at the end of the storage, slitlamp biomicroscopy and specular microscopy is applied. Assessment of the regenerative capacity of the corneal endothelium is only possible during OC. The endothelial morphology before and after storage, the cell loss during storage, the presence of reformation figures in the mosaic,⁴⁷ are all signs

of endothelial wound healing. In this way donor corneas with reversible or irreversible damage by post-mortem delay or disease will be detected and might be identified and even discarded.

Pros and cons of organ culture

With organ culture storage for up to four weeks or even longer is possible.²⁸⁴ It offers sufficient time for the scheduling of grafts and for managing variations in donor supply and surgery capacity. Tissue with a well defined endothelial quality and microbiological state is released for grafting.¹⁸⁴ By the available tissue and cell selective tools one can be less selective concerning the donor on beforehand by increasing the general supply. Specific selection takes place afterwards at the price of a higher percentage of discarded corneas. These advantages cannot be obtained with a simple technique. Disadvantages: OC is relatively complicated, requires well-equipped facilities and intensive and long training of the staff. A minimum storage period required as quarantine period because of the microbiological testing. Tissue is not available for direct use (see chapter 4.1 and 4.2), due to the fact that thinning of the cornea needs to take place before surgery. The medium contains bovine serum, chemically not well defined. Although storage solutions are nowadays commercially available, many eye banks still prefer to continue with the local production as long as they still have the expertise, equipment and facilities.

