

Methods of Evaluation Cadaveric Donor Cornea for Keratoplasty (Literature Review)

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Abstract

In the present for a large part of patients with corneal pathology necessarily the keratoplasty therefore there is a quite high demand of constant stock donor corneas. In this article methods of analysis cadaver donor corneal material in sequence that necessary for qualitative assessment before surgery or for the choice of the optimal method of preservation in the Eye Bank are described. As we know, cornea endothelium cells and its viability perform a major role in maintaining of clarity transplanted corneal flap after penetrating keratoplasty and endothelial keratoplasty. We need the highest quality of donor material with the storage of a large number of viable cornea endothelium cells for these types of keratoplasty. Corneal flaps with the worst quality of endothelial layer can be successfully used for different types of anterior lamellar keratoplasty and inter lamellar keratoplasty. Therefore, the adequate evaluation of donor corneal material before surgery reduces the risk of postoperative complications associated with the unfitness of the cornea endothelial layer and increases the viability of the transplant in postoperative period. In addition, the choice of the optimal type of preservation for the maintaining of biologically valuable corneal material in the long run for various types of transplants make a figure in its accessibility.

Keywords: Penetrating Keratoplasty; Endothelial Keratoplasty; The Donor Material; Cornea Endothelium Cells; Cell Viability

Introduction

According to the WHO, corneal diseases, as the cause of blindness and low vision, take 4th place after cataracts, glaucoma and age-related macular degeneration [1]. More than 200 thousand keratoplastics are performed worldwide annually [2], so the need for high-quality donor material and its constant supply is high. Obtaining cadaver donor material suitable for keratoplasty remains one of the most pressing problems in modern transplantation of the cornea of the eye [3-7].

Depending on the layers of the cornea that need to be replaced, or at the level of which an intervention is performed, several types of keratoplasty are distinguished: pass-through (UPC) - full-layer replacement of the cornea of the recipient with donor material; anterior layered (PKP) - replacement of the anterior layers of the cornea up to the descemet membrane; endothelial (ECP), including the replacement of the posterior layers of the cornea with a graft with a thickness of 20 to 250 microns; interlayer (MCP), or interlamellar - implantation of donor tissue of various thicknesses and shapes into the stroma of the cornea of the recipient.

UPC is still the most popular type of radical corneal surgery. Also, in recent years, ECP has become widespread with bullous keratopathy [8]. It is for these types of keratoplasty that the highest quality of the donor material is required, while maintaining a large number of viable endothelial cells.

It should be noted that the preservation of the epithelium of the donor cornea is not an essential criterion for successful keratoplasty, since a few days after the operation it is completely replaced by the recipient's epithelium. At the same time, given its high antigenicity, it is believed that the epithelium should be removed before keratoplasty or preservation [9,10]. In the stroma, the so-called collagen "matrix", which after transplantation is populated by the recipient's own cells, there should be no clouding, roughness of the front surface, signs of lysis. The main criterion for the viability of corneal donor material for SKP and ECP is the state of the endothelial layer, namely the quality and quantity of endothelial cells (PEC) [11,12], which ensure normal hydration and transplant transparency due to pumping and barrier functions [13-15].

Currently, there are many methods for assessing corneal donor material. All these methods are divided into laboratory (invasive), or auxiliary, and clinical (non-invasive), or basic. After invasive methods, the donor cornea becomes unsuitable for clinical purposes, they are intended for scientific research. These methods include: laboratory-morphological (histological, histochemical, electron-microscopic) and laboratory-functional (cultural, radioautographic, biophysical, biochemical). Clinical methods for assessing the donor cornea preserve its structural and functional integrity and include an assessment of its viability and transplant ability before preservation and keratoplasty. Among the latter, clinical-morphological (biomicroscopic, mirror-microscopic, morphometric) and clinical-functional (vital staining and biophysical methods) are distinguished [3].

Enucleation of eyeballs during autopsy of the donor with the simultaneous selection of cadaver venous blood is performed by employees of the Tanatology Department together with employees of the Eye Bank. Transportation of donor material to the corneal bank should be carried out no later than 24 hours from the date of death.

The stages of assessment of corneal donor material can be represented as follows:

1. Serological examination of cadaver venous blood for the presence of viral infections.
2. Morphological assessment of the donor cornea in the composition of the whole eyeball in order to identify biomicroscopic defects.
3. Evaluation of the quality and density of EC of the native cornea using specular and confocal microscopy or the method of vital staining, and canned - using a special device - keratoanalysis.
4. Physiological assessment of an energetically significant criterion for the viability of cadaveric corneal tissue using an adrenaline test.

Below is a detailed algorithm for assessing donor corneas.

Study of cadaver venous blood samples for infection with HIV, hepatitis B and C, syphilis

In the clinical laboratory of an ophthalmic medical institution, an enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) perform serological blood tests for viral infections such as HIV, hepatitis B and C, RW. In addition, contraindications for the use of donor corneas for transplantation purposes include (accepted by the European Conference on Corneal Banks, Leiden, 1990): active viral encephalitis; Creutzfeldt-Jacobs disease; rabies; oncological diseases; subacute sclerosing panencephalitis; congenital rubella; Reye's syndrome; septicemia; leukemia; generalized lymphoma; jaundice of unknown origin [16]. Data on these pathologies are provided by the forensic morgue staff, in which donor material is collected.

Elderly age is not a contraindication to donation, but, nevertheless, 65 years should be considered the optimal upper limit of the norm. The main selection criterion is the quality and quantity of EC. At the same time, cadaveric corneas from older donors with a high amount of EC are the most suitable material for transplantation of the descemet membrane, since it becomes thicker and less elastic with age, which means it will be easier to straighten in the anterior chamber, which, in turn, will lead to a decrease in damage to the endothelial layer [17].

Uninfected cadaver eyes are transferred to the next stage for further research on viability and transplantability [18].

Biomicroscopic assessment, or morphological screening, of the cadaveric cornea is performed as part of a whole eyeball using a slit lamp and is the first mandatory method for selecting donor corneas for preservation and transplantation. This method is carried out on the basis of morphological indicators on a scale of biomicroscopic signs of cadaveric donor corneas [3]:

- 3 points - The stroma is completely transparent, not thickened. There may be single folds of the descemet membrane. The endothelium is absolutely transparent and intact over the entire surface area.
- 2 points - A stroma with initial signs of edema in the deep layers, practically not thickened, transparent. Descemet's membrane with single folds radially directed from the center. The endothelium is almost intact, it is permissible to have a barely noticeable swelling in the form of opaque opalescence on individual sections along the folds of the descemet membrane.
- 1 point - The stroma is completely swollen, matte. Descemet's membrane with pronounced multidirectional folds. The endothelium is opaque, interrupted along the contours of folds that appear transparent.

In addition, biomicroscopic signs of poor quality corneal material include (accepted by the European Conference on Corneal Banks, Leiden, 1990): malignant tumors of the anterior segment of the eye; retinoblastoma; active inflammatory process (scleritis, keratitis, uveitis), dystrophic and cicatricial changes in the cornea, keratorefractive and intraocular surgical interventions. When these signs are identified, corneal donation should be excluded [16].

Analyzing the data of the above scale of biomicroscopic features, we can state the following:

- Corneas of the first group (3 points) can be successfully used for all types of keratoplastics, especially those involving the replacement of the endothelial layer, i.e. for UPC and ECP; the material of this group may be susceptible to various methods of conservation, among which the optimal are hypothermic (conservation in a nutrient medium for up to 4 days for UPC and ECP and up to 7 days for PCP and MCP) [19-22] and normothermic (preservation in organ culture with t + 340C up to 35 days for SKP and EKP) [23,24].
- Corneas of the second group (2 points) should be used for PCP and MCP, for which the usefulness of the endothelial layer of donor material does not matter, and in urgent cases for SKP; the optimal types of preservation for the material of this group will be hypothermic and normothermal for SKP, PKP and MKP, as well as the practically unlimited cryothermal method (preservation at subzero temperature) for PKP and MKP [25-29].
- Corneas of the third group (1 point) are considered non-transplant; this material can be preserved by a virtually indefinite conservation method, namely, silico-desiccation (drying on silica gel) [30] with a view to its further use for epikeratoplastics ("non-waste technology" of Eye banks).

To provide a complete picture of the state of the cadaver donor cornea, the studies listed below are necessary.

The quality and density of EC of the native donor cornea are assessed using specular [31] and confocal microscopy [32] or by vital staining [31,33] and canned staining using a keratoanalysis [3].

High PEC with the presence of homogeneous hexagonal cells and a low coefficient of variation, which is caused by the absence of pleomorphism (changes in the shape of EC) and polymegatism (changes in the size of EC) is the normal state of the endothelial layer of the cornea [34,35]. According to modern requirements, according to the recommendations of the European and American Eye Bank Associations, PEC should be at least 2200 per 1 mm² [36,37].

The method of specular (endothelial) microscopy is non-contact and is carried out using a specular microscope (for example, Eye Bank Kerato Analyzer EKA-98, Konan Medical Inc.). The device allows you to automatically or semi-automatically reproduce the image

of the endothelial layer after manual installation of the donor cornea and perform its pachymetry and morphometric analysis of EC. The disadvantage of this method is that the estimated region of the endothelium is limited to one zone, usually the center of the cornea, which is associated with the device [31]. At the same time, the main criterion in assessing the suitability of the donor cornea for keratoplasty or preservation is PEC of at least 2500 per 1 mm² and for SCP after preservation - at least 2000 unchanged cells per 1 mm² (according to the keratoanalyser) [11].

Confocal (transfocal) microscopy is a non-invasive highly informative method that allows you to study the morphological features of all layers of the cornea, including EC. In this case, the normal morphological picture of the endothelial layer is represented by hexagonal cells connected to each other by tight intercellular contacts, with a light uniform surface and with clear dark intercellular borders, while the cell nuclei are not visualized. The device allows you to manually or automatically calculate the PEC, EC area and coefficient of variation [32]. For the selection of cadaveric corneal material of widespread use, confocal microscopy did not find in view of the high cost of the device itself.

The morphometric method is carried out using an automated computer keratoanalyzer. This method allows non-invasive (without removing the cornea from a bottle with a preserving medium) pachymetry of the donor cornea and the density, area and shape of EC without the use of dyes. This advantage absolutely prevents the possibility of infectious contamination of donor material at the time of analysis. The most common keratoanalyser for Eye Banks is Konan 98 (Japan) [3].

It should be noted that for a reliable assessment of the quality of the cadaver donor cornea, it is recommended to use the average value of at least 3-fold PEC measurements using any of the above methods for assessing PEC. These methods are comparable and equivalent [38].

The method of vital staining of the endothelium (for example, 0.25% trypan blue solution) does not cause structural damage to EC in a non-toxic dye concentration. According to the European Association of Eye Banks, to eliminate toxicity, the concentration of trypan blue should be in the range of 0.2 - 0.5% and the exposure time should not exceed 30-90 seconds (European Eye Bank Association Directory). The viability of donor corneas is judged by the number of stained and unpainted cells. Viable cells with an intact cell membrane remain unpainted. On the contrary, EC staining in full indicates damage to the latter. However, due to the high risk of contamination of donor corneas under light microscopy [31], the method of vital staining in the activities of the Eye Bank is practically not used for clinical purposes.

Physiological screening for the determination of an energetically significant viability criterion in the cadaveric corneal tissue.

The determination of an energetically significant criterion for the viability of cadaveric corneas using a non-invasive adrenaline test was proposed by the MNTK Eye Microsurgery named after Fedorov and is currently used only in some eye banks.

The essence of the method is to irrigate donor eyes with a 0.1% solution of adrenaline hydrochloride. The conclusion is made by the appearance of the first signs of pupil elongation in one of the iris meridians (the "cat's eye" phenomenon).

The examination of the pupil is carried out using biomicroscopy. When this phenomenon occurs, after 5 minutes from the moment of instillation (Grade A), the sample is considered to be sharply positive (post-mortem ATP loss in EC according to 31P-NMR spectrometry is 0 - 55% of the initial value), after 10 minutes (Grade B) - positive (post-mortem ATP loss in EC according to 31P-NMR spectrometry is 56 - 69% of the initial value), and after 15 minutes (Grade C) is doubtful (post-mortem ATP loss in EC according to 31P-NMR spectrometry is 70 - 100% of the initial values). In the absence of a pupil reaction after 15 minutes (Grade "0"), the sample is considered negative (the residual ATP fraction in EC is practically 0%).

For SKP and EKP, donor material corresponding to degrees A and B is considered physiologically suitable; for PKP and MCP - degrees A, B, C and "0" [3,18].

Conclusion

Given the shortage of high-quality donor material for keratoplasty, it is advisable to rationally use cadaveric corneal tissue, including a full assessment of its condition before surgery and the choice of the optimal type of preservation in the conditions of the Eye Bank. Adequate assessment of donor corneal material before surgery can reduce the risk of postoperative complications associated with corneal insufficiency, and, consequently, increase the transplant viability in the operated patients. The choice of the optimal type of conservation for maintaining the corneal material biologically complete for a long time for various types of transplantations plays an important role in its availability.

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