Comparison of in situ Corneoscleral Disc Excision versus Whole Globe Enucleation in Cornea Donors Regarding Microbial Contamination in Organ Culture Medium – a Prospective Monocentric Study over 9 Years

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Keywords

Cornea donation · Whole globe enucleation ·
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Summary

Background: Corneas needed for keratoplasty can be harvested using two techniques: whole globe enucleation and in situ excision of the corneoscleral disc. This study evaluates the rate of microbial contamination of the donor cornea organ culture medium according to the method of retrieval. **Methods:** All donor corneas of our cornea bank received between January 1, 2001 and December 31, 2009 put into organ culture and microbiologically tested were prospectively analyzed for microbial contamination of the organ culture medium. **Results:** 2,805 donor corneas could be included in this study in total. 975 of them were retrieved by whole globe enucleation (group 1) and 1,830 by in situ corneoscleral disc excision (group 2). 15 corneas of group 1 (1.5%) and 46 corneas of group 2 (2.5%) showed a contamination of the organ culture medium. The difference was shown not to be statistically significant ($p = 0.082$). **Conclusion:** The rate of microbial contamination in organ-cultured donor corneas does not seem to be dependent on the method of their retrieval.

Schlüsselwörter

Hornhautspende · Bulbusentnahme ·
Korneoskleralkomplex-Entnahme · Organkultur ·
Mikrobiologie

Zusammenfassung

Hintergrund: Spenderaugenhornhäute für Keratoplastiken können mit Hilfe zweier Entnahmetechniken gewonnen werden: der Enukleation des gesamten Spenderauges und der In-situ-Korneoskleralkomplex-Entnahme. Diese Studie untersucht die Rate mikrobieller Kontaminationen des Organkulturmediums in Abhängigkeit von der Entnahmemethode. **Methoden:** Alle Spenderaugenhornhäute unserer Hornhautbank, die zwischen dem 1. Januar 2001 und dem 31. Dezember 2009 entnommen, organkultiviert und mikrobiologisch getestet wurden, fanden Einschluss in diese Untersuchung hinsichtlich der Rate mikrobieller Kontaminationen des Organkulturmediums in Abhängigkeit von der Entnahmemethode. **Ergebnisse:** Bei insgesamt 2805 Spenderaugenhornhäute ist der Anteil unsteriler Hornhäute bei Bulbusentnahme 1,5% (15 von 968) und bei der isolierten In-situ-Korneoskleralkomplex-Entnahme 2,5% (46 von 1830). Der Unterschied ist nicht statistisch signifikant ($p = 0,082$). **Schlussfolgerung:** Die Rate an mikrobiellen Kontaminationen des Organkulturmediums scheint nicht abhängig von der Entnahmemethode der Spenderhornhaut zu sein.

Introduction

Cornea transplantation has become a standard procedure to cure certain eye diseases. The donor corneas needed for keratoplasty can be harvested using two techniques: whole globe enucleation and in situ excision of the corneoscleral disc.

Both methods have different strength and weaknesses. According to the 2010 directory of the European Eye Bank Association, 34 of the member banks perform whole globe enucleation only, 12 banks in situ corneoscleral disc excision only, and 16 both.

Yet a matter of debate is the risk of microbial contamination of the donor cornea with both methods. The whole globe can be additionally treated with disinfecting agents after enucleation, whereas no disinfection of the corneoscleral disc is possible after in situ excision. Therefore, we studied the rate of microbial contamination of organ culture cornea medium according to the retrieval method in our cornea bank.

Material and Methods

All donor corneas of our cornea bank received between January 1, 2001 and December 31, 2009 put into organ culture and microbiologically tested were included in this study. Cornea donor selection and recovery followed the guidelines of the German Medical Association and the German good tissue practice for cornea banks [1, 2].

Whole Globe Enucleation

The closed eyelids and the periocular region were treated with 10% iodine solution for at least 10 min (Braunol®, Braun, Melsungen, Germany). After that the conjunctiva with its fornices and the corneal surface were rinsed with sterile isotonic 2% PVP iodine solution twice with a total incubation time of 5–7 min, followed by rinsing with 20–40 ml sterile and isotonic NaCl solution. The person performing the procedure was wearing sterile gloves as well as a surgical cap and mask. After performing a 360 degree peritomy and disinserting the outer ocular muscles and connective tissue using forceps and scissors, the optic nerve was localized and cut to allow complete enucleation. The two globes were stored separately in sterile plastic containers (100 ml Polypropylene container, Sarstedt, Nümbrecht, Germany), stabilized with sterile gauze and moistured with steril 0.9% sodium chloride solution and transported on a cool pack in a Styrofoam box to our cornea bank. Here the donor globes were stored in a refrigerator at 4–6 °C to a maximum of 24 h until excision of the corneoscleral disc. This excision was performed under sterile conditions in a laminar air flow bench. The donor globes were immersed separately in 40 ml sterile, isotonic 2% PVP iodine solution for 5 min, followed by 5 min in 40 ml of sterile, isotonic sodium thiosulfate solution and 5 min in 40 ml of sterile, isotonic saline solution. After that the corneoscleral disc was removed and placed on a cornea holder (Böhnke Donor Cornea Holder, Bausch and Lomb, Heidelberg, Germany) and put into tissue culture flasks (Becton Dickinson Labware, Franklin Lakes, NJ, USA) filled with 40 ml of culture medium.

At our cornea bank the whole globe enucleation was used exclusively for the retrieval of corneas from organ donors and mainly performed by the residents of the department of ophthalmology of the Charité or the organ donation coordinators of the German Organ Transplantation Foundation (DSO). The tissue retrieval was performed after organ explantation in the operating room.

In situ Corneoscleral Disc Excision

Skin and ocular surface disinfection were performed the same way as described above. The face and head of the donor were covered with surgical drapes leaving the region of the eyes open. The person performing the procedure (only well trained personnel of the cornea bank) was wearing a surgical cap and mask as well as a sterile gown and sterile gloves. The procedure was performed in the autopsy room of the donor hospitals which was not used for other purposes at the same time.

After performing a 360 degree peritomy and partial removal of the conjunctiva, a trephine with a diameter of 15 mm was used to cut the sclera around the cornea. This cut was then completed using a scalpel or scissors. The corneoscleral disc was carefully separated from the iris and uveal tissue using two forceps and transferred to a sterile plastic container filled with 20 ml of cornea organ culture medium. The donor corneas were directly brought to the cornea bank and stored at 32 ± 2 °C in an incubator until transfer into tissue culture flasks with culture medium on cornea holders.

For organ culture an antibiotic-containing minimal essential medium (MEM) with the following composition was used: 2,000 ml contain 200 ml MEM with Earl's salts (10×) ; 40 ml fetal calf serum (concentration of 2%), 20 ml penicillin/streptomycin 10,000 U / 10,000 µg/ml (final concentration of 62.5 µg/ml penicillin und 100 µg/ml streptomycin), 20 ml amphotericin B 250 µg/ml (final concentration 2.5 µg/ml), 20 ml L-glutamin (200 mmol/l), 25 ml HEPES buffer (1 mol/l), 58.6 ml NaHCO₃ (7.5%), and 1,616.4 ml distilled water.

At day 7 ± 1 of organ culture, 10 ml of the medium of each cornea were given to aerobic and anaerobic blood culture bottles containing resins for inactivation of the antibiotics (BD BACTEC™ Plus Aerobic / F, Anaerobic / F, Becton Dickinson and Company, Sparks, NV, USA) and were analyzed at the Institute of Microbiology and Hygiene, Charité – Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany. Culture medium with obvious microbial contamination (clouding, decoloration) was sent directly to the Institute of Microbiology and Hygiene, Charité – Universitätsmedizin Berlin, where microbial growth was verified using thioglycollate medium and blood agar plates.

The sample proportion test was used for statistical analysis of the results [3].

Results

2,805 donor corneas could be included in this study in total. 975 of them were retrieved by whole globe enucleation (group 1) and 1,830 by in situ corneoscleral disc excision (group 2). 15 corneas of group 1 (1.5%) and 46 corneas (2.5%) of group 2 showed a contamination of the organ culture medium. The difference was shown not to be statistically significant ($p = 0.082$; $Z = -1.74$). The number and rate of microbial contaminations per year are shown in table 1.

All medium contaminations were detected already in the cornea bank by its clouding and decoloration and confirmed by microbiological examination. All other medium samples tested with blood culture bottles showed negative results.

In 34 of the 64 microbial contaminations of the organ culture medium, a germ differentiation was performed. 12 cases of *Staphylococcus haemolyticus*, 7 of *Enterococcus faecium*, 4 of *Escherichia coli*, 2 of *Pseudomonas aeruginosa*, 2 of *Klebsiella pneumoniae*, 1 of *Pseudomonas fluorescens*, 1 of *Enterobacter cloacae*, 1 of *Chryseomonas indologenes*, 1 of *Kocuria*

Table 1. Number and rate of microbial contaminations per year

Year	Number of whole globe enucleations	Number (rate) of microbiological contaminations	Number of in situ corneoscleral disc excisions	Number (rate) of microbiological contaminations
2001	93	1 (1.1%)	144	1 (0.7%)
2002	113	0	217	1 (0.5%)
2003	114	2 (1.8%)	157	4 (2.5%)
2004	102	3 (2.9%)	188	7 (3.7%)
2005	162	2 (1.2%)	184	3 (1.6%)
2006	117	3 (2.6%)	236	2 (0.8%)
2007	103	1 (1.0%)	183	6 (3.3%)
2008	86	1 (1.2%)	205	9 (4.4%)
2009	85	2 (2.4%)	316	13 (4.1%)
Total	975	15 (1.5%)	1830	46 (2.5%)

rosea, 1 of *Enterococcus faecalis*, and 1 of *Staphylococcus warneri* were found. No fungi were found among the analyzed microorganisms.

Discussion

For many decades, cornea transplantation is a well-established method to treat several eye diseases. Since the population in the developed countries is getting older and age is a risk factor for cornea dysfunction, the need for cornea grafts might be rising. Therefore, the harvesting procedure and preservation methods of donor corneas should be optimized to increase the number of available grafts.

There are two retrieval techniques for donor corneas in use today. Both have different advantages and disadvantages. Whole globe enucleation – the older method – is easily to learn and to perform. The whole globe needs to be transported cooled what might be difficult at warm ambient temperatures. Furthermore, an additional preparation of the corneoscleral disc at the cornea bank is necessary requiring additional time, materials, and skilled personnel also during weekends and holidays; however, a second disinfection under sterile conditions can be performed.

The in situ corneoscleral disc retrieval requires strict asepsis because no further antiseptic cleaning is possible after excision. Also handling of the donor cornea has to be done carefully at the site of harvesting, what is not always unproblematic since the conditions at the donor site are much more variable compared to the setting in the cornea bank. Therefore, in situ corneoscleral disc excision requires experience for successful results and is more prone to mistakes. On the other hand, this technique needs no further preparation steps at the cornea bank, thus saving time and resources. The transport media – in case of later organ culture – allows a safe transport at temperatures between 10 and 40 °C. Furthermore, the in situ excision shortens the death-to-preservation interval because the donor cornea is given directly into culture medium after removal before transportation.

It was reported that the permission for cornea donation from the next of kin is granted in a higher number of cases with the in situ excision technique [4, 5], probably because harvesting of the cornea is much less invasive and defacing compared to whole globe enucleation. This corresponds to our own experience at the Cornea Bank Berlin, Charité.

Since the in situ corneoscleral disc excision takes place in a non-sterile environment, it has been supposed that corneas retrieved by in situ excision have a higher microbial contamination risk. It would be a significant disadvantage when more donor corneas would be lost to microbial contaminations when using in situ corneoscleral disc excision. Therefore, we studied the rate of microbial contamination of organ-cultured donor corneas according to the retrieval method in our cornea bank between 2001 and 2009.

With 1.5% contamination rate in the group of whole globe enucleation and 2.5% in the group of in situ corneoscleral disc excision we found low contamination rates for both retrieval techniques, showing no statistically significant difference.

Linke et al. [6] reported a much higher microbial contamination rate (10.3–19.4%) in organ-cultured corneas with in situ excision since 2008, while this rate was lower (3.3–12.5%) when whole globe enucleation was performed in earlier years. The authors assumed that the different disinfection protocols for the two methods might cause this difference.

To our knowledge, no other study was published comparing the contamination rate in organ-cultured corneas for different retrieval techniques. Some studies have investigated this topic for donor corneas preserved with cold storage [6, 7]. The microbiological testing in these studies differs from ours making it difficult to compare the results.

Lane et al. [7] found more positive limbal swabs in their in situ group compared to the whole globe group, but after cold storage for 7 days there was no difference in the number of positive cultures of the corneoscleral rim and of the culture medium between the two groups. Jhanji et al. [8] also found similar results with a higher number of positive limbal swabs in the in situ group and comparable positive corneoscleral rim cultures of 20 versus 24% after cold storage [8].

Hudde et al. [5] reported a microbial contamination rate for in situ corneoscleral disc excision of 5.5% being slightly higher than our rate [5].

The small difference between our two groups is especially interesting because there were only organ donors without any clinical infections in the whole globe enucleation group and many donors with septicemia in the in situ excision group. Spelsberg et al. [9] compared the rate of microbial contamination in organ culture medium for in situ excised donor corneas from septic or non-septic donors and was not able to find any significant difference between both (contamination rate 8 or 11%).

Since 2010, we harvest all donor corneas with in situ corneoscleral disc excision to have minimal tissue removal from the donor, less processing time in the cornea bank, and shorter death to preservation intervals.

The whole globe enucleation method does not seem to have advantages with regard to the rate of microbial contamination in organ culture. Our data allow the conclusion that in situ excision is an effective and safe method of donor cornea retrieval.

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Disclosure Statement

The authors declared no conflict of interest.

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