Scientific Section

Corneoscleral discs excised from enucleated and non-enucleated eyes are equally suitable for transplantation

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ABSTRACT.

Purpose: **To assess whether the biological quality of corneoscleral tissue dissected in situ is, after organ culture, comparable to that harvested after enucleation.**

Methods: **Corneoscleral discs were prepared from 23 donor eyes, either after enucleation, under laminar flow conditions (right eyes; group 1) or by direct excision in situ (left eyes; group 2). Endothelial cell counts were made and the degree of tissue contamination assessed both prior to and upon termination of organ culture.**

Results: **Microbial growth was found in 12/22 conjunctival swabs collected from group 1 eyes and in 14/22 of those obtained from group 2 globes ($p = 0.76$). Bacterial growth was detected in four primary culture media, two from each group, at low colony densities. No significant difference in endothelial cell counts were encountered between the two groups, either immediately after dissection [group 1: 2940 ± 308 (2100-3500) c/mm^2; group 2: 2947 ± 345 (2200-3700) c/mm^2; $p = 0.945$] or upon termination of organ culture [group 1: 2646 ± 321 (1895-3200); group 2: 2723 ± 312 (2100-3650); $p = 0.413$].**

Conclusion: **Dissection of corneoscleral discs in situ may serve as an alternative to the conventional technique if consent is obtained to remove only the cornea. The risk of contamination is no higher and endothelial cell viability no lower than in tissue derived from enucleated globes, provided that the excision is performed by a skilled surgeon and a rigorous disinfection protocol is instigated.**

Key words: corneal transplantation - dissection in situ - contamination - organ culture - corneal endothelium.

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The removal of organs from potential donors is governed by national and/or local laws and regulations. Hence, under certain circumstances, permission to enucleate the globe as a whole will not be granted, although consent may be given to excise a corneoscleral disc. Efforts to perfect the technique of dissecting such tissue in situ so as not to compromise its usefulness for transplantation are therefore worthwhile. This alternative has been proposed by several investigators (Vannas 1975; Polack 1977; Barraquer et al. 1980), and the main drawback associated with its execution appears to be an increased risk of microbial contamination by conjunctival flora (Vannas 1975; Gallagher 1986). Indeed, Lane et al. (1994) reported the incidence of both contamination and endothelial trauma to be significantly higher in corneoscleral tissue dissected in situ and stored for short periods at 4°C in McCarey-Kaufman medium. In the current study, the influence of dissection technique on these two parameters is investigated in organ cultured corneae.

Material and Methods

Data presented in this study derive from 27 consecutive donors, the right eyes having been enucleated and the left ones utilized for dissection in situ. The following standard protocol was instigated for each eye: skin decontamination with 10% polividon iodine (PVP; Mundipharma, Hamilton, Bermuda) for two minutes; collection of a conjunctival swab from the inferior fornix; global rinsing with 2 ml of 0.6% PVP; washing of the ocular surface with 1% chloramphenicole; removal of superfluous fluid from the conjunctival sac. The eye region was then covered with sterile gauze. After insertion of a lid specula, a smooth circular peritomy of the conjunctiva was effected in both eyes, particular attention being paid to avoid remaining projecting flaps as a possible source of tissue contamination.

The right globe was removed after excision of extraocular muscles, orbital adnexae and optic nerve, and transferred to a sterile moist chamber for transportation purposes, the cornea being covered by a gauze soaked in sterile phosphate buffered saline solution (pH 7.4). A 20 mm-diameter orbital implant was inserted into the eye socket and covered by

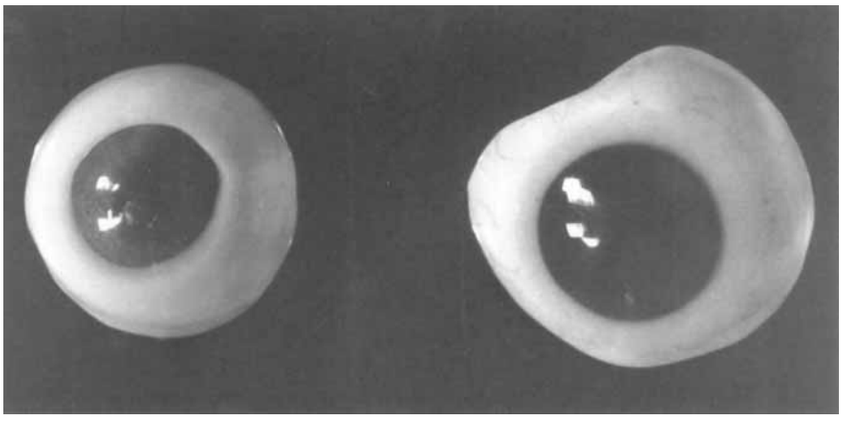

Fig. 1. Left: Conventional epibulbar prosthesis. Right: Newly-developed epibular prosthesis, adapted to orbital anatomy, for use after dissection of corneoscleral discs in situ.

a conventional glass eye prosthesis. Upon receipt in the laboratory, the globe was disinfected for three minutes in 2% PVP, and then rinsed copiously with 100 ml of sterile phosphate buffered saline prior to tissue dissection under laminar flow conditions. The eyeball was fixed in a vacuum globe holder (Storz Instruments, Heidelberg, Germany), and after manual trephination of the corneoscleral disc with a 15 mm-diameter hand trephine, the dissection was completed using Castroviejo scissors. The corneoscleral tissue was carefully separated from the ciliary body with a hockey knife and transferred to 3 ml of primary culture medium (group 1).

The left eye was injected with air via the pars plana to obtain an intraocular pressure of approximately 25 mmHg (measured with a Schiötz tonometer) in order to avoid torsional damage during trephination (Draeger et al. 1985); this latter procedure was performed precisely as described above, and the excised corneoscleral disc similarly transferred to 3 ml of culture medium (group 2). A specially designed epibulbar prosthesis (Fig. 1) was inserted and attached using 10% methylcellulose (Dispersa Inc.), and the lids closed, if requested, with a single Vicryl 6-0 suture.

The complete endothelium of corneoscleral discs (groups 1 and 2) was examined by inverted phase-contrast microscopy in order to assess the extent of preexisting and dissection-induced damage, and changes remarked on a standardized tissue evaluation form. A Polaroid® photograph of a representative central area (magnification × 200) was taken immediately after dissection of corneoscleral tissue in right eyes, and after their excision and transfer to the laboratory in the case of left ones. Endothelial cell density was estimated from the photographs by counting in a 0.01 mm^2 area; this was undertaken by a single person in a masked fashion, using the fixed frame technique, in duplicate from each photograph and the average of both counts used. The primary corneal storage medium was collected for microbiological examination using standard laboratory procedures.

Corneoscleral discs were cultured in 40 ml of medium [Eagle's Minimum Essential Medium (Gibco, Basel, Switzerland) supplemented with 10% foetal calf serum (Seromed, Berlin, Germany), Penicillin (100 µg/ml), Streptomycin (100 µg/ml) and Amphothericin B (0.3 µg/ml; all antimicrobials from Gibco, Basel, Switzerland)] at 32°C in a humidified atmosphere containing 5% CO_2 for three to fifteen days. One day prior to the termination of incubation, the culture medium was replaced and supplemented with 6% Dextran 500000 (Pharmacia, Upsala, Sweden). Final examination of the endothelium was performed in parallel on corneoscleral discs derived from fellow eyes. Estimation of endothelial cell density at this stage was made after induction of intercellular swelling using Balanced Salt Solution (BSS®, Pharmacy, Univerity Hospital Bern, Switzerland).

Paired data of both groups were compared using Student's two-armed t-test and ANOVA test for repeated measures (quantitative findings), Fisher's Exact test (qualitative findings), assuming a statistical error of $\alpha = 0.05$ and ES (t) = 0.4. The comparison of Student's two-armed t-test and ANOVA did not reveal differences; therefore, only the t-test results are detailed. As the ANOVA for repeated measures is expected to be more sensitive in detecting a difference, the power between groups was calculated for ANOVA and found to be 0.76.

Results

From the 27 donors included in this study, four pairs had to be excluded from the statistical analysis owing to the absence of data pertaining to endothelial cell density after organ culture. Of the 23 evaluated, the age was 16-79, on average 55 years; the time from death to dissection of corneoscleral discs did not differ between the two groups [group 1: 8-63, on average 28 hours; group 2: 6-60, on average 25 hours; $p = 0.476$]; and the average tissue culture time was 8.0 ± 2.8 (3-15) days.

Microbial growth was detected in 12/22 (55%) of the conjunctival swabs taken from group 1 eyes and in 14/22 (64%) of those from group 2 ($p = 0.76$; Table 1). In both groups, microbial growth was found in 2/23 of the primary organ culture fluids ($p = 1.0$), the number of colonies being less than twenty in each case. Amongst the four pairs excluded from this study, bacterial growth was detected in one culture from group 1 and in two from group 2; in each instance, the isolated organism was Pseudomonas aeruginosa (Table 2).

Endothelial cell density did not differ significantly between the two groups, either at the time of dissection [group 1: 2940 ± 308 (2100-3500) c/mm^2; group 2: 2947 ± 345 (2200-3700) c/mm^2; $p = 0.945$] or after tissue culture [group 1: 2646 ± 321 (1895-3200) c/mm^2; group 2: 2723 ± 312 (2100-3650) c/mm^2; $p = 0.413$; ANOVA: $p = 0.314$]. Endothelial cell loss incurred during storage was significant for each group [group 1: $p = 0.003$; group 2: $p = 0.026$], but there was no difference between the two [group 1: 294 ± 258 (150-750); group 2: 223 ± 209 (50-750); $p = 0.314$].

Discussion

The results presented in this study demonstrate that the biological quality of corneoscleral discs harvested by dissection in situ is comparable to that of tissue obtained from enucleated eyes. The former technique may have a legal relevance, and its adoption may lead to permission for tissue collection being granted in a greater number of cases by the next of kin. The time required for dissection in situ is also less than that needed for excision from enucleated globes, since this procedure is performed under semi-rather than rigorously sterile conditions. Albeit so, when stringent disinfection of the conjunctival sac is instigated, tissue culture

Table 1. Microbial contamination of donor conjunctivae.

	Enucleated globes (Group 1)	Non-enucleated globes (Group 2)
n_{swabs} (contaminated/total)	12/22 (55%)	14/22 (64%; p = 0.76)
Bacterium	n_{abs} (%)	n_{abs} (%)
Staph. epidermidis	3 (17)	4 (26.6)
Staph. aureus	2 (11)	2 (13.3)
Micrococcus spp.	1 (5.5)	1 (6.7)
Bacillus spp.	3 (16.5)	2 (13.3)
Coryne	4 (22)	2 (13.3)
Streptococcus spp	3 (17)	3 (20)
E. coli	1 (5.5)	0 (0)
Enterococcus spp.	1 (5.5)	0 (0)
Proteus vulgaris	0 (0)	1 (6.7)
$n_{bacteria}$ total	18 (100)	15 (100)

Table 2. Microbial contamination of corneoscleral discs in primary culture media.

	Enucleated globes (Group 1)	Non-enucleated globes (Group 2)
$n_{cultures}$ (contaminated/total)*	2/23 (9%)	2/23 (9%; p = 1.0)
Bacterium	n_{abs}	n_{abs}
Staph. epidermidis	0	1 (a,b)
Micrococcus spp.	1 (a,b)	0
Coryne	0	1 (a,b)
Pseudomonas spp.	1 (a,*)	0 (*)
$n_{bacteria}$ total	2	2

(a) Maximally 10-20 colonies detected.
(b) Secondary contamination of the bacterial cultures could not be excluded.
(*) Pseudomonas aeruginosa was detected in one culture from group 1 and in two of those from group 2 of the four pairs excluded from stastical analysis.

contamination is not markedly higher than when the conventional method is adopted. An increased risk of contamination associated with dissection in situ has been alluded to in the past (Vannas 1976; Gallagher 1986), and Liesegang et al. (1984) suggested that this problem may be minimized by skillful excision of corneoscleral discs with smooth contours. For this reason, peritomy of the conjunctiva was effected with particular care in order to avoid the protrusion of tissue flaps, serving as potential harbingers of pathogens, onto the surface of the corneoscleral discs.

Testing for bacterial contamination revealed the existence only of single colony growth in only four of our primary cultures (two in each group), and in three of these, a secondary contamination of plates could not be ruled out, since the bacteria detected were all highly sensitive to the antibiotics included in culture media. These results fall within the range expected from previously published data (Garweg et al. 1989; Hagenah et al. 1995; Frueh et al. 1995). Due to a low overall incidence of contaminations we would have needed a 6-fold higher number of donor pairs to detect an anticipated difference in the contamination rate of 10%. Nevertheless, in three of the eight corneae excluded from statistical analysis in this study and in one of those referred to above (group 1), Pseudomonas was detected. This pathogen is not sensitive to the antibiotics conventionally employed in culture media, and it therefore plays an important role in tissue contamination (Payne 1986). If transferred with a graft, the ensuing infection may become a difficult one to treat (Khodadoust & Francklin 1979; Leveille et al. 1983), and accounts, indeed, for 25% of cases of postoperative endophthalmitis after penetrating keratoplasty (Payne 1986). Pseudomonas bacteria may not have been detected in conjunctival swabs because contaminating spores have to develop first before their growth can be detected. Thereby these bacteria are readily overwhelmed by other organisms. Contamination of organ cultures by Pseudomonas, therefore, can be ruled out only after a minimal period of three to four days. As 4° culture is run maximally over an interval of 48 hours, this is a considerable advantage of organ culture regarding transplantation safety. That Pseudomonas was detected in the excluded cases may not be entirely coincidental; our inability to distinguish endothelial cell borders in these instances after induction of intercellular swelling may be linked to the presence of this pathogen.

Dissection in situ is naturally carried out using sterile instruments and solutions, but the excision itself cannot, of course, be effected under sterile conditions, and rigorous local decontamination is therefore crucial. Employment of a fast-acting and effective disinfection with 2% PVP, as is used for the decontamination of whole globes (followed by extensive rinsing in sterile buffered saline), is not appropriate in our situation, owing to the highly toxic effect of this reagent on endothelial cells, which will be endangered if inadvertant contact with it is incurred during excision (Hasany & Basu 1989). However, even when utilized at lower concentrations, i.e. 0.6%, as advocated for conjunctival surface decontamination, there is a potential threat, and we circumvented this difficulty by removing all fluid from the conjunctival sac and by additional rinsing prior to trephination.

The dissection technique itself is also important. Hypotony of the globe increases torsional forces during trephination, and we avoided this problem by raising the intraocular pressure to physiological levels, either by use of a suction globe holder (group 1) or by inflation with air (group 2). Probably the most relevant factor contributing to the remarkably low cell loss incurred during dissection in situ lies in its having been performed by a skilled surgeon using microsurgical techniques. Although a competent technician may be present in the cornea laboratory, such personnel are not usually in attendance to remove tissue in

Table 3. Endothelial cell viability.

Endothelial cell density	Enucleated globes (Group 1)			Non-enucleated globes (Group 2)			Student's t-test
	Mean	±SD	Range	Mean	±SD	Range	
Before culturing	2940	±308	2100-3500	2947	±345	2200-3700	p = 0.945
After culturing	2646	±321	1895-3200	2723	±312	2100-3650	p = 0.413
Loss	294	±258	0-750	223	±209	0-750	p = 0.314

the pathology department. This consideration may account for the discrepancy between our data and those reported by Lane et al. (1994). However, in this previous study, corneoscleral discs were cultured at 4°C rather than at 32°C, and this may also have a bearing on the extent of endothelial cell loss.

The determination of endothelial cell density with the fixed frame technique performed by us has the disadvantage to miss peripheral cell damage. However, 32°C organ culture allows repair of endothelial defects by migration from healthy areas to damaged ones. Consequently, we might have detected a possible peripheral damage by a higher regression of endothelial cell density during culture in either group. As paired corneoscleral discs from one donor were always examined in parallel, a time dependant difference in the endothelial wound healing is precluded. In the statistical strength analysis we might have detected a difference between groups of less than 10% which was not the case.

A short death-to-dissection time has also been shown to be an important determinant influencing contamination (Harbour & Stern 1983), but this factor was not relevant in our study owing to the relatively long post-mortem times (Matsuda et al. 1986). Indeed, most European eye banks harvest tissue after post-mortem times which are long compared with those prevalent in the United States (European Eye Bank Association 1996). Prolonged delay after donor death will increase the risk of folds developing in Descemet's membrane and of other degenerative changes being incurred. Such phenomena will decrease adhesion of the endothelial monolayer, and this may generally influence the degree of cell loss incurred during dissection (Böhnke 1991), but did not show an influence on the results in our series. The damage of a given donor tissue with a high post mortem time by the dissection procedure may be detected after organ culture, so that a primary exclusion of donor tissue from transplantation should not be based on this factor alone, independently of the dissection method used. Long post-mortem times, on the other hand, preclude the undertaking of reliable serologic HLA typing. And for this reason, eye banks relying on the receipt of tissue after protracted periods have developed methods for assessing histocompatibilty data using cultured retinal pigment epithelium (Baumgartner & Grabner 1992); testing for HIV and Hepatitis B/C can be successfully performed using uveal tissue (Böhnke et al. 1988). Such material is obviously not available when corneoscleral discs are excised in situ.

For a cosmetically satisfying coverage of the donor eye, we have developed a new epibulbar prosthesis, which is constructed strictly according to the anatomy of the conjunctival sac and centralizes spontaneously with almost no risk of dislocation. Such a prosthesis optimally replaces the corneoscleral disc. The transparent area, 11-12 mm in diameter, is eccentric, according to the localization of the globe within the orbit; its temporal margin is larger than the nasal one and the upper one broader than the lower (Fig. 1). Using this prosthesis, a closure of the lids by suture was requested on behalf of only one donor.

In our opinion, dissection of corneoscleral discs in situ may serve as an alternative to the conventional technique, if relatives consent only to the removal of the cornea, and if legal interests preclude enucleation of the globe as a whole. The biological quality of the tissue thereby gleaned depends upon the instigation of a rigorous decontamination protocol and a skilled surgical technique.

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