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Preparation of donor corneas: A study of the endothelial population

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PURPOSE. *To quantify the endothelial damage in corneal rim excisions prepared in different ways.*
METHODS. *From the Imola branch of Eye Bank of Emilia Romagna, 24 corneal rim excisions which belonged to 12 pairs of enucleated eye globes were selected. The endothelial mortality was quantified by Trypan blue staining and counting the endothelial cells in a central area of about 5 mm² of each cornea. The mate corneas of each pair were prepared in two different ways: one with an epithelium-endothelium cut using a Hanna trephine with an artificial chamber and the other with an endothelium-epithelium cut using a Hanna trephine with punch. After the cut the endothelium was studied again in the same way as described above.*
RESULTS. *The cell mortality in the corneas before and after the epithelium-endothelium cut using the artificial chamber was increased by 0.9% and the percentage of endothelial loss was increased by 3.9±6.8%. In the corneas prepared with endothelium-epithelium cut using a punch the mortality before and after the cut increased by 8.8% and the percentage of endothelial loss was 20.7±10.9%.*
CONCLUSIONS. *The authors found that in terms of endothelial mortality and endothelial cell density there is less damage to the endothelial population using the epithelium-endothelium cut as compared to the endothelium-epithelium cut. (Eur J Ophthalmol 2008; 18: 341-4)*

KEY WORDS. *Donor cornea, Rim excision, Endothelium, Trephine*

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INTRODUCTION

The advent of the Hanna trephine (Moria, Antony, France) with artificial chamber improved the mechanism of the corneal transplant procedure, allowing for improvement in terms of regularity of the trephinated cornea.
There are two possible methods to produce a corneal explant: an epithelium-endothelium cut or an endothelium-epithelium cut (1, 5).
In using the artificial chamber and the epithelium-endothelium cut, the corneal edge is more regular and the cut is repeatable, and it is possible to assure a complementary match between the donor cornea and the receiving bed.
This complementary match allows a faster and more complete physiologic recovery, and results in lower residual astigmatism (1-9).
Radner et al found that the epithelium-endothelium cut is less damaging for the endothelial population than the punch (2). On the other hand, there are experimental studies on corneas of pigs that do not demonstrate any significant difference between the two methods (3).
In addition, some studies have found that the endothelium-epithelium cut is more damaging to the endothelium than is the epithelium-endothelium cut, especially on the edge of the corneal button (4).
The aim of our study is to quantify the damage caused on the donor cornea in relation to the kind of cut used for its preparation.

METHODS

From the Eye Bank of Imola 24 corneal explants which belonged to 12 pairs of explanted bulbs were selected. Six donors were male and six were female. The average

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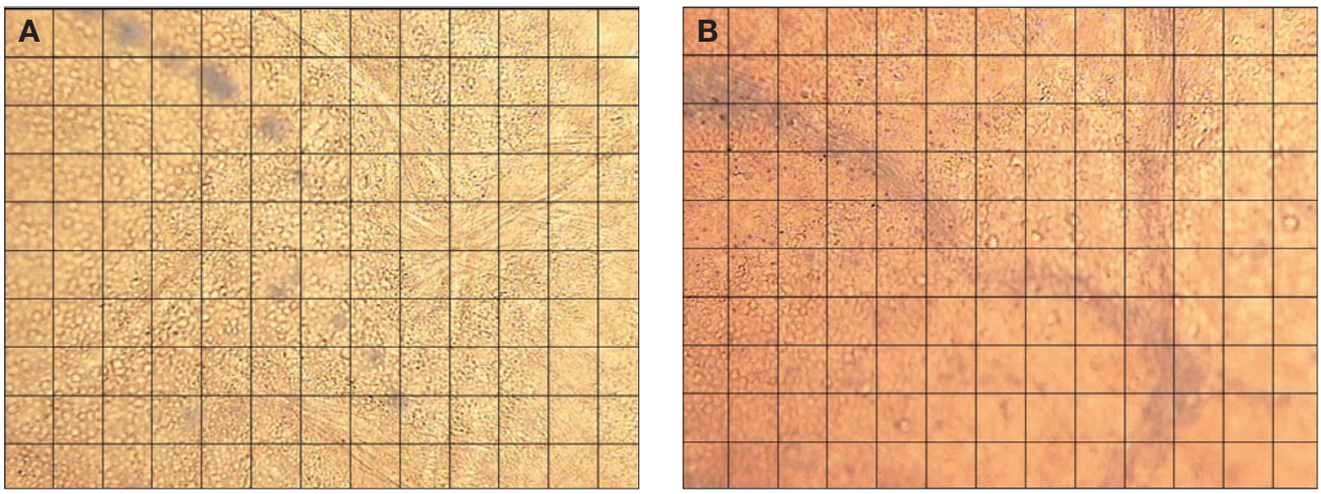

Fig. 1 - A) *Cornea 1. Endothelial population before cutting.* **B)** *Cornea 1. Endothelial population after cutting made by punch (endothelium-epithelium cutting): note the increasing endothelial mortality inside the Descemet folds which were visible before cutting.*

age was 73±16 years.

All the corneas were placed into an organ culture called Tissue-C (Alchimia S.r.l., Padova, Italy) at 31 °C for 14±3 days. Then, 24 hours before trephination, they were put into deturgescence medium called Carry-C (Alchimia S.r.l.). A central area of about 5 mm² was studied as follows (6, 7):

- Trypan blue (Alchimia S.r.l.) colored endothelium was used to evaluate viability and mortality of the cells.
- The endothelial population was then counted manually and automatically by computed software (Navis, Nidek Inc.).

The corneas from the same donor were then studied again and trephined using a different method for each cornea:

1. For the first cornea the Hanna trephine with the artificial anterior chamber was used allowing an epithelium-endothelium cut.
2. For the mate cornea the Hanna trephine with punch was used allowing an endothelium-epithelium cut.

New blades were used by the same operator who obtained regular and complete cuts.

Every cornea was restored in the deturgescence medium for 24 hours and then studied again as before the trephination. This modality allowed the examination of deturgessed corneas with a good thickness.

TABLE I - ENDOTHELIAL MORTALITY BEFORE AND AFTER THE CUT WITH THE TWO DIFFERENT METHODS

	Number of samples	Mortality before cut	Mortality after cut	Difference
Epithelium-endothelium cut	12	4.5%±1.5	5.4% ±2.7	0.9%
Endothelium-epithelium cut	12	4.4%±1.7	13.2%±3.6	8.8%

TABLE II - ENDOTHELIAL POPULATION COUNT BEFORE AND AFTER THE CUT WITH THE TWO DIFFERENT METHODS

	Number of samples	Population before cut (cell/mm²)	Population after cut (cell/mm²)	Percentage of endothelial loss
Epithelium-endothelium cut	12	2223±201	2134 ±193	3.9±6.8%
Endothelium-epithelium cut	12	2231±189	1763 ±193	20.7±10.9%

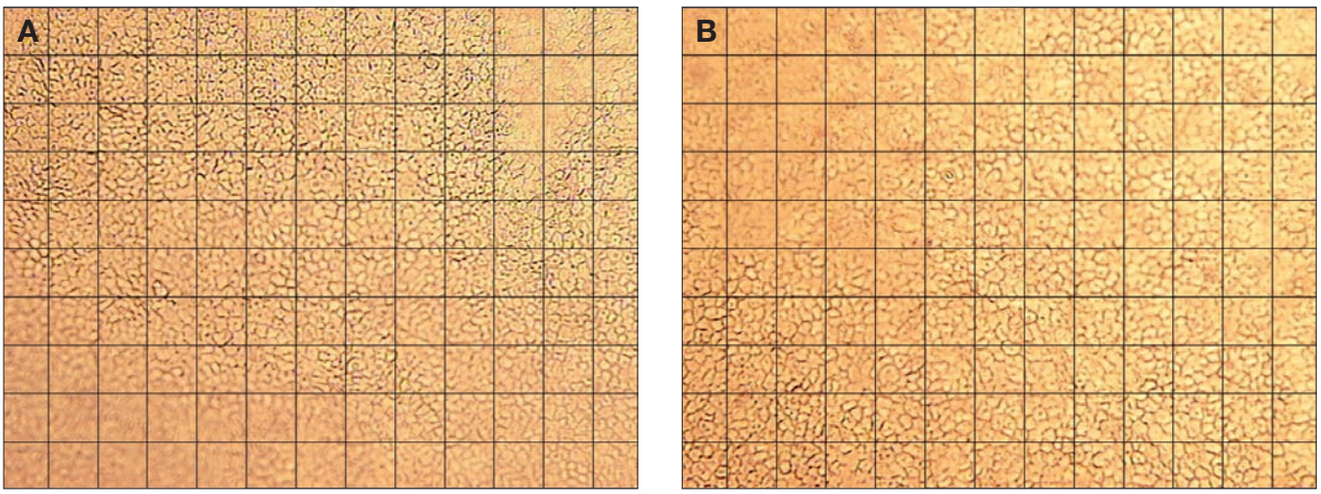

Fig. 2 - A) *Cornea 2. Endothelial population before cutting.* **B)** *Cornea 2. Endothelial population after cutting made by Hanna trephine with artificial chamber (epithelium-endothelium cutting): note that the look of these cells and their mortality are not modified.*

Statistical analysis

Statistical analysis was performed with SPSS/PC 8.0 for Windows (SPSS Inc.). Statistically significant differences between data sample means were determined by a paired-sample Student *t* test. A p value less than 0.05 was considered significant for between-group differences.

RESULTS

Evaluation of the corneas prepared with endothelium-epithelium cut before cutting proved an average population of 2231±189 cells/mm² and an average mortality of 4.4±1.7%.

Evaluation of the corneas prepared with epithelium-endothelium cut before cutting proved an average population of 2223±201 cells/mm² and an average mortality of 4.5±1.5%.

As illustrated in Table I, the corneas prepared with punch (endothelium-epithelium cut: Fig. 1, A and B) had a average mortality of the endothelial population of 13.2±3.6%, with an increased mortality of 8.8% ($p<0.05$); the corneas trephined with the Hanna artificial chamber (Fig. 2, A and B) showed a lower increased mortality of the endothelial population: 5.4±2.7%, with a difference of 0.9% ($p<0.05$).

As illustrated in Table II, the corneas prepared with the endothelium-epithelium cut (made by punch) had an average endothelial cell count of 1763±193cells/mm², therefore decreased by 20.7±10.9% ($p<0.05$) (Fig. 1, A and B); the corneas trephined with the epithelium-endothelium cut (made by Hanna artificial chamber) showed an average endothelial population after the cut of 2134±167cells/mm²: the endothelial population decreased by 3.9% ($p<0.05$) (Fig. 2, A and B).

DISCUSSION

The epithelium-endothelium cut produced with the help of an artificial chamber is less traumatic for the central endothelium (diameter 5 mm²), compared to the cutting obtained with punch, in terms of discomfort and detachment of endothelial cells during the trephination.

In an effort to understand this result, we analyzed the forces involved in the two methods:

- Using punch, there is a pushing action of the blade, so that every fiber of tissue meets only one point of the blade's edge. This action guides the blade's edge inside the deeper layers rapidly and violently, causing crushing of tissue, and, consequently, a strong stretching and sometimes radial tears of the Descemet membrane, with consequent mortality of the endothelial population (5). Moreover, the endothelium is the first layer to meet the blade, and it is involved during the whole cut.
- On the contrary, during the epithelium-endothelium cut, the blade is moved in a parallel direction to the blade's edge, so that each fiber is subjected to the action of

multiple cutting points in a gradual manner. The endothelium is the last layer to be cut (5). In addition the continuous injection of balanced saline solution (BSS) through the artificial chamber during the whole procedure maintains the stretching of the cornea and decreases the twisting effect on the Descemet membrane. During the penetration of blade into the inner stromal layers, the tissue is dragged by the rotation of the blade. This causes a deforming effect also on Descemet and endothelium (8), but these forces are neutralized by the artificial chamber, which maintains the inner pressure constant, through the continuous injection of BSS.

- The precision of the cut and the evaluation of endothelial cell density and mortality before and after cutting were accomplished through the use of corneas cultivated at 31 °C and then passed in a deswelling medium called Carry-C. This procedure provides an uniform thickness of each cornea, giving the possibility of obtaining complete and precise cutting without the necessity of completing the cut manually.

In conclusion, in our study the epithelium-endothelium cut of the donor cornea proved to be the best method to preserve endothelium. Our findings are contrary to the studies carried out by Damiano et al (4), in which they used fresh corneas. The Hanna trephine did not exist at that time (1978) but our results agree with those obtained in 1999 by Radner et al (2).

The authors have no proprietary or financial interest in any aspect of this article.

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