RESEARCH

Freezing of Surplus Donated Whole Eyes in the Central Eye Bank of Iran

Use of Defrosted Corneas for Deep Anterior Lamellar Keratoplasty and Report of Postoperative Eye Bank Data

Mozhgan Rezaei Kanavi, MD; Mohammad Ali Javadi, MD; Fatemeh Javadi; Tahereh Chamani, MS

ABSTRACT

PURPOSE: To describe the method of freezing and thawing of donated whole eyes (DWEs), which were surplus to requirements in the Central Eye Bank of Iran (CEBI), and to report the 3-year results of using defrosted corneas in deep anterior lamellar keratoplasty (DALK) in keratoconic eyes.

METHODS: The method of freezing and thawing of surplus DWEs in the CEBI is described. Surplus DWEs at the CEBI were disinfected, processed, and transferred to the freezer (-70°C) for long-term preservation. In case of a shortage of fresh and refrigerated corneas for DALK, a frozen DWE was defrosted and distributed for transplantation either as a whole eye in moist chamber or as an excised corneoscleral disc in Eusol C at 2˚C to 8˚C. Furthermore, eye bank data of the frozen DWEs as well as postoperative eye bank reports of implementation of defrosted corneas for DALK in patients with keratoconus between March 2010 and March 2013 were retrospectively reviewed.

RESULTS: In a 3-year period, 593 surplus DWEs were frozen. Mean duration of freezing was 86.94 ± 38.7 days (range, 5 days to 231 days). All the frozen corneas were used for DALK in patients with keratoconus. Mean follow-up period after transplantation was 20.3 ± 10.4 months (range, 1 month to 34 months). With the exception of 21 grafted corneas (3.5%) that needed regraft due to persistent epithelial defects, other grafted corneas were reported clear and well-epithelialized postoperatively by the surgeons.

CONCLUSIONS: Freezing surplus DWEs is a feasible and practical method for long-term preservation of corneas in the eye banks that harvest whole eyes. Implementation of this technique provides an enlarged and reliable source of donor corneas to meet the requirements for DALK, especially when a shortage exists for fresh donor corneas for transplantation.

KEYWORDS: cornea, freezing, whole eyes, DALK, eye banks, thawing

The Central Eye Bank of Iran (CEBI) is the only eye bank in Iran and is located in Tehran. Tissue requirements for corneal and scleral transplantation in Iran are supplied and preserved by the CEBI.[1] There has been an increasing trend in corneal transplantation in Iran, from 200 grafts in 1988 to 6,053 in 2012 (unpublished data). Keratoconus is the most common indication for penetrating keratoplasty in Iran, accounting for 34.5% of cases.[1] Due to the safety and effectiveness of deep anterior lamellar keratoplasty (DALK) as an extraocular procedure,[2-9] there has been an increasing interest in the implementation of DALK in keratoconic eyes in Iran (unpublished data). DALK is a full-thickness graft without endothelium and Descemet membrane, and the corneal endothelium does not matter for this keratoplasty technique; therefore, donated corneas that have a fair endothelial rating (endothelial cell density less than 2,000 cells/mm^2), whether fresh, lyophilised, or frozen, can be used for this purpose.[10-14] Since fresh corneal tissue suitable for DALK may only be reliably stored in cold storage media for up to 10 days,[15,16] freezing of surplus donated whole eyes (DWEs) was implemented by the CEBI in recent years to provide sufficient tissues to meet the national requirements

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for DALK. Surplus DWEs are those that are not required for transplantation during certain times of the year; these occasions are national holidays or national and international ophthalmology meetings when fewer elective corneal transplantations are performed. In this study, in addition to describing the CEBI protocol for freezing and thawing of surplus DWEs, we report the results of implementation of defrosted corneas for DALK in eyes affected by keratoconus during a 3-year period.

METHODS

From Freezing of DWEs to Transplantation of Defrosted Corneas

Surplus DWEs were considered for freezing if they met the CEBI criteria for harvesting, lacked stromal opacities, and demonstrated at least a good endothelial rating with a minimum endothelial cell density of 2,000 cells/mm^2, which can be estimated through the high magnification of the slit-lamp biomicroscope (Haag-Streit, Bern 900, Switzerland). The CEBI criteria followed the medical standards of the Eye Bank Association of America and the European Eye Bank Association in terms of reviewing donor medical and social history, physical inspection of the donor, evaluation of donor tissue, and donor serologic screening tests for hepatitis B virus, hepatitis C virus, human immune deficiency virus, human T-cell leukemia virus, and syphilis.[17-19] Corneas with at least good endothelial rating were used because the degree of stromal edema and cloudiness in such corneas was less than ones with fair endothelial rating, factors that helped us to easily detect subepithelial and stromal opacities that matter for DALK. After disinfection of whole eyes by immersion in 3% povidone iodine for 3 minutes and irrigation with 0.9% normal saline, gross inspection and slit-lamp biomicroscopic examination of the corneas were performed. Then, the DWEs were transferred to the freezer (-70°C) for long-term preservation. When there was a demand for corneal tissue for DALK, the frozen whole eyes were defrosted by first transferring to a refrigerator and then to room temperature, each for a period of 1 hour to decrease the thermal shock induced by rapid temperature change. Then, under microbiologic hood and sterile conditions, the corneoscleral discs were excised (Fig. 1) and preserved in Eusol C (Al.Chi.Mi.A., Padova, Italy) at 2˚C to 8˚C before transplantation. If both the surgeon and the patient were available, a frozen eye was distributed whilst still frozen and without performing a corneoscleral excision. On receipt, the surgeon would transfer the whole eye to a refrigerator and then to room temperature (as described above) before trephining the donor cornea for surgery. Under such circumstances, no cornea maintenance media were necessary.

Retrospective Eye Bank Data Review

Between March 2010 and March 2013, all the eye bank records pertaining to the frozen corneas used for DALK in keratoconic patients were reviewed, and total numbers of frozen DWEs, enucleation to freezing time, donors' age, and duration of freezing were calculated. Moreover, all the related postoperative reports were reviewed for the presence or absence of (1) post-transplantation corneal clarity reported by the surgeon and (2) adverse reactions such as persistent epithelial defects, corneal melting, and keratitis that led to a regraft.

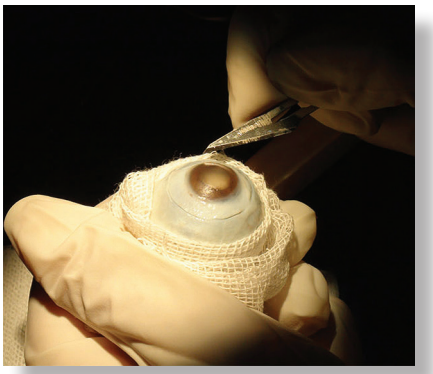
RESULTS

Five hundred and ninety three surplus DWEs were frozen in a 3-year period. Before freezing, all the DWEs were preserved in moist chamber in 4°C for approximately 6.5 hours. The mean donors' age was 39 ± 14 years (range, 4 years to 80 years). The mean duration of freezing of DWEs before defrosting was 86.94 ± 38.7 days (range, 5 days to 231 days). All the frozen DWEs were distributed for DALK in keratoconic patients either as a whole eye or as an excised corneosclera. Mean follow-up period after transplantation was 20.3 months ± 10.4 months (range, 1 month to 34 months). All frozen tissues were used for DALK, and none of them were discarded. Based on postoperative records, all the grafted defrosted corneas were reported clear and well-epithelialized by the cornea surgeons, except for 21 grafted corneas (3.5%) that needed regrafting because of persistent epithelial defects. No corneal melting or keratitis was reported.

DISCUSSION

Our study demonstrated that freezing of surplus DWEs is an easy and efficient method for long-term preservation of the corneas and provides suitable tissue for anterior lamellar keratoplasty techniques. One of the concerns of the eye banks that do not use organ culture media as the long-term preservation method for corneas is receiving a donated eye that is surplus to requirement. This situation can occur at various times throughout the year, especially during events such as New Year, religious holidays, or national and international ophthalmology congresses when the demand is

1a



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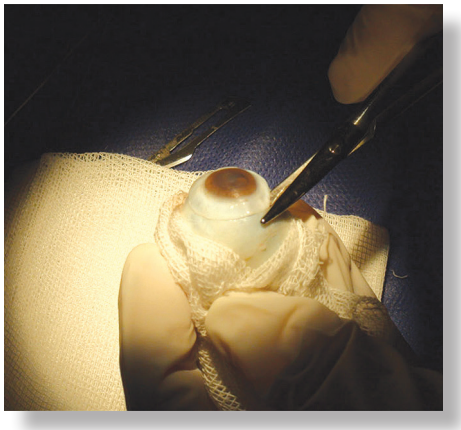

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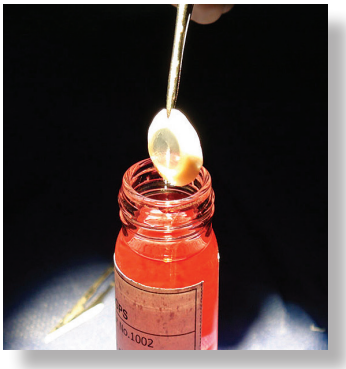

1d

Fig. 1. The excision process is illustrated in Figures 1a to 1d. Fig. 1a depicts a frozen whole eye. Fig. 1b and Fig. 1c illustrate corneoscleral excision of a defrosted frozen whole eye. Fig. 1d shows the transfer of the excised corneal tissue to a cold storage media.

lower. Under such circumstances, freezing of the donated eye may be an efficient method for long-term preservation of the cornea.

Frozen corneas have been reportedly used for penetrating keratoplasty with varying degrees of success and some with a surprisingly long-term survival.[20-23] However, since freezing at -70°C inflicts remarkable endothelial damage,[24,25] such corneas are only suitable for the now popular anterior lamellar keratoplasty techniques. In our study, the vast majority of the grafted defrosted corneas were reported clear and well-epithelialized after surgery. The study took into account the surgeons' postoperative reports in regards to corneal clarity, full epithelialization of the graft, and occurrence of complications leading to regraft. The failure rate of DALK using frozen corneas in keratoconic eyes in the current study was only 3.5%, a figure that is less than the reported failure rate in the literature for DALK in keratoconic eyes that used fresh corneal tissues (eg, failure rate of 6.5% in a study by Jones et al[26]).

Significant damage has been observed in the endothelial cells and keratocytes of the cryopreserved corneas after freezing and thawing.[27] A similar finding is expected to be observed when a whole eye is frozen and defrosted. Loss of keratocytes may be compensated by repopulation of keratocytes from recipient stroma into the donor tissue after DALK. Although the cornea may be slightly stretched by increased internal volume of a frozen whole eye, such a physical change seems to be reversible after thawing. We have already conducted a prospective randomized study to compare the postoperative clinical and paraclinical results of DALK using fresh versus frozen corneal tissues in keratoconic patients. These results will be reported in near future.

Lyophilized corneas are prelathed lenticules that are dehydrated under high vacuum pressure and preserved at room temperature. Lyophilized corneas require rehydration with balanced salt solution just before transplantation.[13,14,28] Although lyophilized corneas are reportedly thinner than the fresh corneal tissues,[13] these corneas are successfully used for DALK in keratoconic eyes with as good as, or even better, clinical and surgical results than the corneas kept in Optisol.[12] In comparison with lyophilized corneal tissues, the frozen DWEs and corneas processed in the CEBI only required a freezer (-70˚C). Additionally, they were not dehydrated and therefore did not need the high vacuum pressure instrument.

Glycerol-cryopreservation provides another form of cryopreserved corneal tissue. Such corneas are preserved at -70˚C in pure sterile glycerol.[10,13] Glycerol-cryopreserved corneas were transplanted safely and effectively in DALK.[10] However, the frozen DWEs and corneas processed in the CEBI are preserved by

simple freezing without using glycerol. Furthermore, a defrosted eye can be either distributed as a whole eye without using maintenance media for immediate graft or be excised and preserved in cold storage media before transplantation. The former, certainly, will be cost-effective for the eye bank because cornea maintenance media are not used.

In conclusion, the current study shows that freezing of surplus DWEs is a feasible and adequate method for long-term preservation of corneas for DALK and can be a reliable source of donor tissue when there is a shortage of donor corneas for transplantation.

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