Cryopreserved Corneal Tissue: A Practical Guide to Its Use

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The use of cryopreserved corneal tissue stored at −179° C in liquid nitrogen for up to six months has become more popular during the past two years. This seems to be a useful technique for the future, not only because it will allow a better utilization of the still sparsely available corneal tissue, but also because it will make the life of the corneal surgeon easier, by allowing him to schedule his transplants during routine operating time rather than in the evenings or during holidays.

This paper will not deal with the actual technique of cryopreservation and the studies done to insure us of the viability of the corneal endothelium if the technique of cryopreservation has been scrupulously followed. Neither will we discuss the actual results obtained when using cryopreserved tissue. Our personal experience with this type of tissue is limited to only a handful of cases, meaningless for statistical purposes, and we would have to rely solely on published results or personal communications from other corneal surgeons.

What we will explain in detail is the technique we have developed to actually use cryopreserved tissue in the operating room. Anyone who has followed the development of this type of transplant material has heard and read how important it is to follow exactly certain steps, but nowhere in the literature is there a guide as to how, in practice, the different steps are to be carried out. We know of no other procedure in ophthalmology where absolute adherence to the strictest set of guidelines is absolutely necessary.

Earlier attempts to use frozen corneal tissue frequently failed because the absolute need for a very exacting thawing process had not been recognized. The time element for proper thawing is counted in seconds, which makes meticulous preparation and a carefully thought out technique absolutely necessary if we are to achieve clear grafts.

The cornea has been preserved in a solution of sucrose and dimethylsulfoxide (DMSO) and albumin, and it is well known that DMSO is toxic to the warm endothelium; therefore, DMSO is added during the freezing process in an ice bath and does not injure the endothelial cells when it is frozen and kept frozen in the storage cannister. When thawing, the process has to be done rapidly, but not so rapidly that the DMSO can injure the endothelium. Once the cornea has been thawed it is placed in 25% salt-poor albumin at which time the metabolism of the cornea starts again. It can be kept in that solution for a maximum of 10 minutes, but should be placed in the recipient eye as soon as possible so that the normal metabolites can start repairing some of the damage done to the endothelial cells, even with the best freezing technique, and a fatal process of anoxia will not further damage the endothelial cells.

Surgical Technique.

1. The cornea arrives in the operating room from the eye bank in a widemouthed thermos bottle. On the top tiny holes have been made to allow some venting of the liquid nitrogen which fills half of the thermos bottle. Taped to the side of the thermos is a long forceps which permits pick up of the plastic vial floating freely in the nitrogen and which contains the

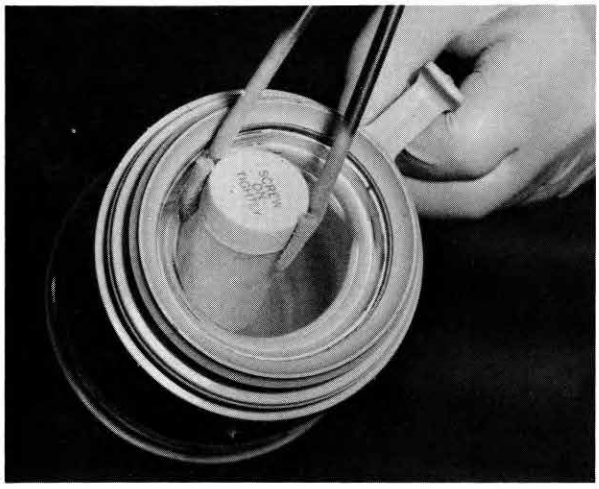

Fig. 1—Removal of plastic vial from widemouthed thermos filled with liquid nitrogen.

small glass tube with the frozen cornea and preservative (fig. 1).

2. The patient is prepared in the usual fashion with the surgeon wearing two pairs of gloves. The surgeon then removes one pair of gloves, drapes the patient, and gives the usual O'Brien and retrobulbar blocks for the routine ocular anesthesia. The operating microscope has been positioned and focused either before the preparation of the patient or at the end of this step #2.
3. The surgeon will have to do the critical thawing himself unless he can rely on an assistant who is totally familiar with the procedure and will not deviate one iota from the course to be outlined. For our purposes here, we will assume that the surgeon himself will want to be in charge of this most important step. While still wearing the gloves, the surgeon steps out of the operating room either into the utility room or into the wash up area, opens the thermos, and picks up the plastic bottle floating in the nitrogen. While a circulating nurse closes the thermos again, the surgeon wraps the plastic bottle in a towel and opens it. As there may be liquid nitrogen inside the plastic bottle, the precaution of using a towel in opening it is necessary to prevent accidental spilling of the nitrogen onto the surgeon's face, which could happen with a rapid warming up (fig. 2).
4. The small glass tube is removed from the outer plastic vial and is placed either in a water bath or under the faucet (if previously it has been established that the temperature is 60°) for exactly 50 seconds. The glass vial is rotated slowly during this 50-second warming process so that the water reaches all areas of the glass vial. It is important not to move the glass vial too rapidly as the thawing ice crystals could damage the endothelium. At the end of 50 seconds, a small ice ball should remain attached to the center of the cornea, and this is allowed to thaw at room temperature, which usually takes about 20 to 30 seconds. If, after removal from the hot water, there is no ice ball, the thawing has been too fast, the so-called thermal runaway has occurred, and the cornea is not suitable for a penetrating keratoplasty (fig. 3).
5. The preserving fluid bathing the cornea is now poured off and 1 to 1.5 cc of 25% salt-poor albumin is added to the cornea. The glass tube is brought back to the operating room and placed on a sterile towel in an ice bath (fig. 4).
6. The surgeon will now remove his gloves, put on gown and fresh gloves, place the bridle suture, and trephine the recipient eye with a Castroviejo trephine of whatever

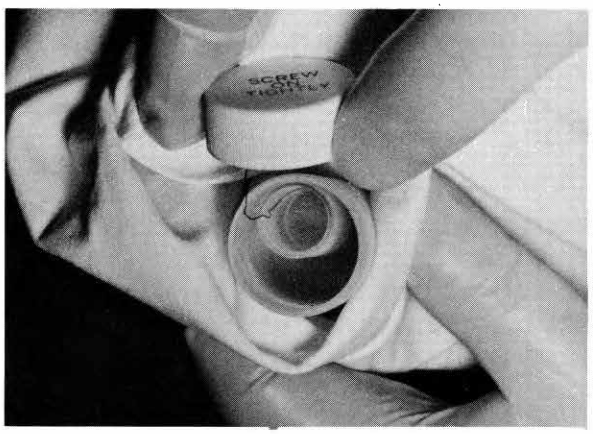

Fig. 2—Plastic vial open, showing the inner vial with frozen cornea and suture.

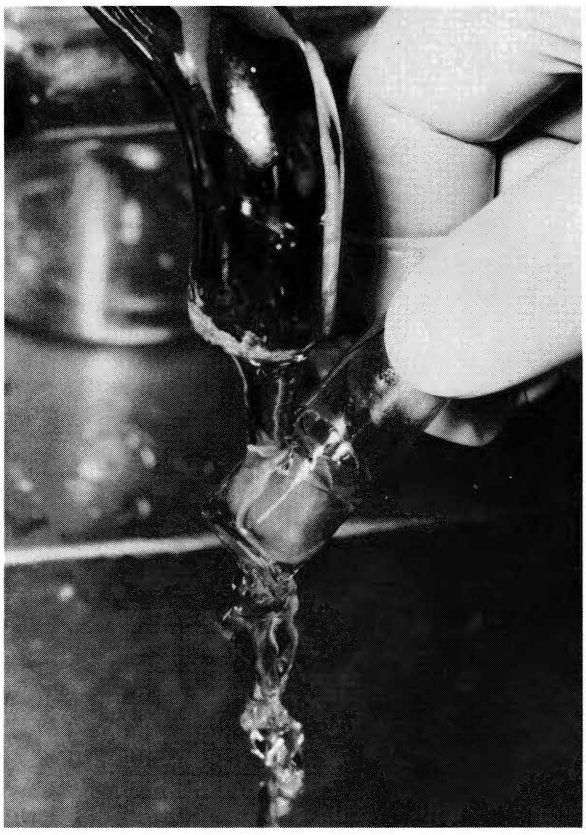

Fig. 3—Cornea being thawed for 50 seconds under 60°C water.

Fig. 4—Thawed preserving fluid being poured off and albumin being added.

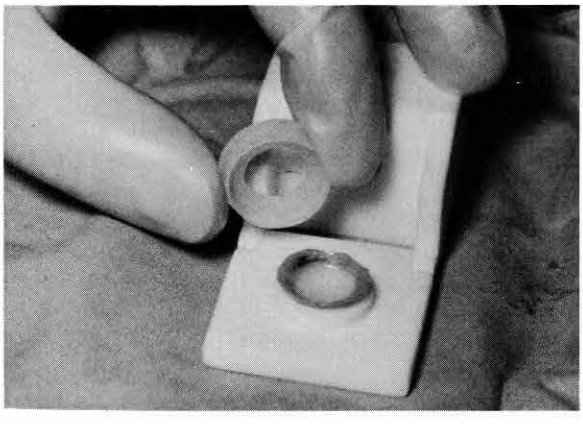

Fig. 5—Cornea, endothelial side up, being placed on the punch.

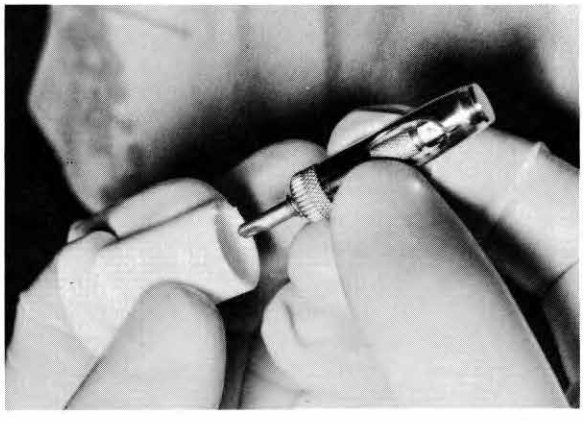

Fig. 6—Castroviejo trephine is being fastened to the punch head.

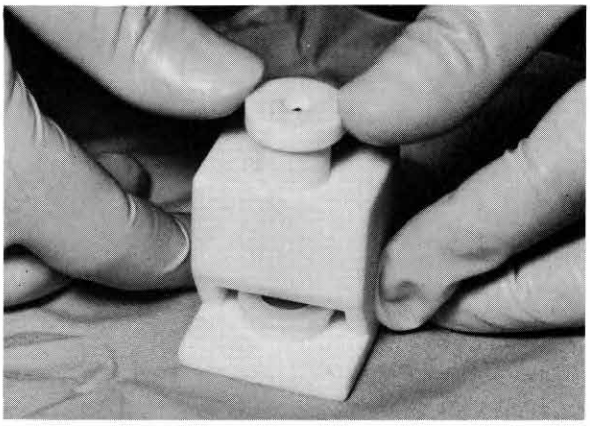

Fig. 7—Cutting of the graft by pushing down the plunger of the punch.

size the surgeon has decided beforehand. It is important to note that the cut has to be made with a Castroviejo trephine and no other. The corneal button, after having been cut completely, is left in the recipient eye.

7. The thawed out cornea with its small suture in the scleral rim still attached is now removed from the glass tube with albumin and placed, endothelial side up, in the corneal punch. The suture is cut, and the plastic ring is placed over the cornea for better fixation (fig. 5).
8. The corneal punch is built in such a fashion that any size Castroviejo trephine can be screwed into its top. The same trephine used on the recipient eye should now be fastened to the top of the corneal punch (fig. 6).
9. The punch is lowered by pressing with both thumbs over the top which should allow a clean cut of the cornea (fig. 7).
10. The trephined button is then placed inside the recipient bed after the previously cut recipient button has been removed. The transfer from the punch to the eye can be accomplished with any spatula or lens loop.
11. The previously described steps 3 through 10, can be easily accomplished in less time than the 10 minutes maximum in which the thawed cornea can remain in albumin.

Special Measure in Aphakia. Obviously in those cases where a Bonacoloto-Flieringa ring has to be used and possibly an anterior vitrectomy needs to be done, the time limit of 10 minutes is too short for comfortable operating, and the technique has to be changed slightly. In those cases, we advise, after draping of the patient, that the surgeon put on gown and gloves, anesthetize the patient, and then suture the ring in place. The proper thawing can then be carried on as outlined, and after the surgeon returns to the O.R. with the cornea in albumin, he should change gown and gloves and proceed as described.

In summary, the thawing of the cryopreserved cornea needs to be done in 50 seconds, and the thawed cornea has to be inside the recipient eye within 10 minutes. These facts require a completely thought out technique with which the surgeon needs to be thoroughly familiar in every minute detail before he ever attempts to use cryopreserved tissue.