Review Article

A review of long-term corneal preservation techniques: Relevance and renewed interests in the COVID-19 era

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The growth of eye banking in India was showing positive trends until the nation was hit by unprecedented times as a result of the COVID-19 pandemic. The impact of this has led to a downward spiraling in eye banking activities globally. Several measures had to be implemented to tide over the crisis and strategies planned for future to prepare for the needs of corneal transplantation. While eye banks in India have been practicing short- term and intermediate storage preservation media, there is a definite need to evolve other methods of very long-term preservation. This review discusses various methods of long term corneal preservation, their relevance and applications in the present times. We reviewed relevant medical literature in English from PUBMED with the key words "Corneal preservation", "Cryopreservation", "Glycerol preservation", Gamma Irradiation", "Eye Banking" ,"COVID-19".

Key words: Corneal preservation medium, COVID-19, cryopreservation, eye banking, gamma irradiated cornea, glycerol preservation

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Eye banking in India made a significant transformation with improved collection, processing and distribution from the time of establishment of first eye bank in Regional Institute of Ophthalmology, Chennai in 1947.[1-3] In a recent global survey on eye banking and transplantation,[4] India was depicted in the "almost sufficient" category, with the second highest number of corneal tissue procurement and transplantation after USA. In spite of the positive trends in eye banking in India, there existed a disparity in tissue retrieval and utilization, in several geographical regions of India. Approximately 70% of the collection and utilization are from states of Andhra Pradesh, Telangana, Gujarat, Karnataka, Maharashtra, and Tamil Nadu.[5] Most eye banks in India rely on short term (3-4 days) and intermediate term (14 days) storage of corneas. Hence, donor corneas beyond 14 days cannot be utilized for transplantation. As per the EBAI statistics, the overall utilization rates have been close to 50% over the past several years.[6] A national overall discard rate of 50% is huge economic burden and overlooks the cost and human resources involved in eye banking activities.[7]

Microbial keratitis is one of the leading indications for corneal transplantation in India.[8] Therapeutic penetrating keratoplasty remains the second most common type of corneal transplant and may be performed with non-optical grade tissues as the primary goal of surgery is eradication of infection. Utilization trends in India show that nearly 40% of corneas retrieved were of non-optical grade and hence, were discarded after reaching near expiry of preservation time.[8]

The unprecedented COVID-19 pandemic witnessed the suspension of donor cornea retrieval activities. As an aftermath of this crisis, the gap of demand versus supply of cornea is likely to widen and become unpredictable globally. To prepare for the world post COVID-19, eye banks are expected to modify earlier practices, prepare and adopt strategies to tide over the crisis of shortage of corneas.[9] While elective transplantation may be postponed, therapeutic keratoplasty, patch grafts and tectonic keratoplasty cannot be deferred.[8,9] In context of the present scenario, it is crucial for the eye banks to adopt methods of very long-term corneal preservation that would minimize the wastage related to non-utilization of the corneas and also prepare the eye banks for periods of uncertain availability of corneas for any reasons in future.

Hence, the purpose of this study is to review the existing literature on long-term corneal preservation techniques. All relevant literature cited in PubMed in English language was reviewed with the key words "Corneal preservation", "Cryopreservation", "Glycerol preservation", Gamma Irradiation", "Eye Banking", "COVID-19".

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Long-Term Corneal Preservation Techniques

A. Cryopreservation and vitrification techniques

Cryopreservation techniques came into vogue following experiments by O Neill *et al*.[10] and Capella *et al*.[11] on storage of corneas at -196°C. These techniques help to preserve the cornea by freezing for extended periods of time.

1. Principle of cryopreservation

Cryopreservation crystallizes extracellular water into ice and increases extracellular electrolyte concentration. Increased osmotic concentration of extracellular solutes and loss of water from the cells leads to cell shrinkage. As the freezing temperatures drops, the water molecules in the cell cytoplasm also turn to ice crystals causing lethal injury to the endothelial cells. In order to overcome this, addition of cryoprotectants was recommended. Cryoprotectants are believed to act by permeating into cells and limit the rate of freeze-thaw thermal injury. With increasing concentrations, cryoprotectants themselves can have cell toxicity and osmotic stress.[12]

2. Principle of vitrification

During freezing water turns into ice crystals, this leads to structural and functional damage of the endothelium and loss of graft clarity. The alternative method of cryopreservation is by vitrification which involves a liquid to solid transition without formation of crystalline ice. This is achieved by increasing the viscosity of the added solution by using a mixture of cryoprotectants and other solutes. The constituents typically consist of a mixture of dimethyl sulfoxide (DMSO), acetamide, 1,2 propanediol, 2,3 butanediol, formamide, polyethylene glycol etc.[13] When viscosity increases beyond a critical point a liquid to solid transition occurs, known as, the glass transition temperature. During vitrification, ice crystallization does not occur and the molecules remain in a random phase such as in liquid while the assuming the properties of a solid. Experimental studies have reported retention of functionality of corneal endothelial cells following vitrification.[14-17] The rate of cooling of 1°C/min following incorporation of cryoprotectants in the storage medium influences endothelial functionality as a faster or slower rate of cooling adversely affects the endothelial viability.[18]

3. Challenges in cryopreservation and vitrification

Intracellular ice formation and cryoinjury caused due to swelling of cells during freezing and expansion of ice crystals leads to cell rupture. A second cycle of cell destruction occurs due to thermal stress during non-uniform warming of the tissues. This is so because of low thermal conductivity and high specific heat of biologic material. Vitrification that has been proposed as an alternative to cryopreservation requires rapid cooling $>10^6$ ^{0}C/min or very high osmolar levels of cryoprotective agents (4-8M) that is toxic to cells and tissues. Vitrified organs are brittle and fractured by thermal stress if not heated uniformly.[19] The techniques of cryopreservation are presently too complex to be incorporated into routine eye banking protocols though there is a feasibility of preserving a functional endothelium. If a simpler procedure is made available, then in the future, cryopreservation techniques may have a wider application in eye banking.

B. Glycerol preserved corneas

Glycerol preserved cornea (GPC) were popularized following experiments by JH King and associates on feline cornea long term storage, with anhydrous glycerol and vacuum, the technique was further extended for preserving human corneas.[20,21] Corneas may be stored for extended periods of time from 5-23 years at room temperature.[22,23] Glycerol is a colorless, odorless viscous liquid with hygroscopic properties. Glycerol is also a known cryoprotectant, as it prevents ice crystal formation. It has antimicrobial properties and dehydrates corneal tissue. The endothelium is not viable by glycerol preservation. The technique involves placing cornea in 95% glycerol in a glass tube and vacuum sealing the tubes. Following this the corneas were preserved in room temperature. During transplantation the glass tubes were cut and the glycerol was decanted off. The tissue was warmed in BSS for 10-15 minutes before surgery. Tissues prepared by this technique was stored for 2 years at room temperature and transplanted to 50 patients. The graft clarity was reported to be comparable to fresh tissues. The vacuum preparation for GPC involved a technology intensive process of 6-8 hours. Due to this limitation, the technique was modified in later publications from King *et al*.[21] by adding molecular sieves (sodium and calcium alumina silicates) which dehydrated the commercial glycerol during storage to very low levels of vapor pressure. In this medium, corneal tissue could be stored for 10 years and had comparable graft clarity to fresh tissues.

1. Modifications in glycerol preservation techniques

The technique has undergone modifications with several groups experimenting with concentration of glycerol and temperature of tissue storage. Li *et al*.[24] stored GPC with and without alumina silicates at -78°C, -20°C, and at room temperature. Tissues were stored for 3 months following which they examined the tissues for light transmissibility, tensile strength, thickness, immunohistochemistry, and tissue collagen arrangement. They concluded that GPC at -78°C were most pliable, least edematous, and clearer. Storage at all temperatures had low levels of HLA antigens such as HLA-ABC, HLA-DR and CD45 and the stromal collagen architecture was most preserved for GPC stored at -78°C. Tripathi *et al*.[25] in a similar experiment concluded that comparable tissue clarity and tensile strength with acellular organized stromal architecture of the collagen lamellae was seen on GPC stored at 4°C. These studies suggest that cryopreservation in addition to dehydration with glycerol maintained long term acellular corneal anatomy with organized stromal architecture. The potential benefit of acellular corneal stroma is in terms of reduced expression of HLA antigens that may lead to reduced risk of allogenic graft rejection.

2. Clinical experience with cornea transplantation using GPC tissues

Clinical studies on GPC, Gupta *et al*.[26] in a series of 34 cases reported anatomic integrity in 91.2% eyes (n = 31/34) using GPC with a median follow up of 195 days and visual acuity of light perception with accurate projection of rays in 88.2% patients (n = 30/34). The most common postoperative complication was glaucoma in 35.1% (n = 12/34). There were no episodes of graft rejection.

Lin *et al*.[23] performed tectonic keratoplasty using GPC in eyes with no visual potential. They reported anatomic integrity in 57.1% (n = 8/14) the remaining grafts had a delayed epithelial healing with graft melting requiring conjunctival flaps. No episodes of graft rejections were reported in this series. GPC tend to remain opaque post-transplant and cosmetically

unsatisfactory. However anecdotal reports of spontaneous graft clearing has also been described in cases where the surrounding host endothelium is healthy and migrates into the graft.[27] The advantages of GPC tissue includes simple preparation, long shelf life, no rejection episodes, and less expensive to prepare. In anterior lamellar keratoplasty Li *et al.*[28] reported a BCVA of 20/40 or better after DALK using glycerol cryopreserved corneal tissue (GCCT) 57.6% (n = 19/33) compared to fresh corneal tissue (FCT) 54.8% (n = 17/31). There were no episodes of graft rejection in the GCCT arm and the overall rejection free graft survival rate of 2 years was higher compared to FCT arm (100% versus 78.8%, P = 0.006).

Lin *et al.*[23] used GPC for perforated corneal ulcers with poor visual potential by combining patch grafting and anterior vitrectomy. The preoperative visual acuity was light perception to hand motions. Anatomic integrity was maintained in 57.1% (n = 8/14) delayed epithelialization was noted in 48.8% (n = 6/14), which required conjunctival flaps. The study recommended GPC as a viable alternative to evisceration. GPC tissues have also been reported to cover glaucoma drainage devices. In a study by Wigton *et al.*[29] the risk of erosion of glaucoma valves with overlying glycerol preserved cornea was 83% less compared to covering with pericardium. The type of glaucoma was not associated with the risk of erosion and the mean time to exposure was 252 days for pericardium and 440 days for the corneal grafts (P = 0.0017).

3. Utility of glycerol preservation techniques in countries where eye banking infrastructure is less than adequate

Feilmeir *et al.*[30] propose that in an emergency situation and in the context of developing nations fresh corneal tissue is not readily available. In these countries the availability, low shelf life and distribution networks for corneas are challenges to eye banking. Patients have difficulty in follow up, poor compliance to medications and in general corneal transplants have a poor outcome. In these scenarios tectonic procedures performed in an emergency setting will be helpful to salvage the eye and in favorable cases a follow up optical PKP can restore sight.

In summary, glycerol preservation is a simple to adopt and easy to use technique. The tissues may be stored at room temperature or refrigerated for prolonged periods of time up to years. The acellular cornea has low rates of rejection. Therefore, GPC is recommended when a regular supply of cornea is not available either due to lack of suitable donors, limited functioning of eye bank, prohibitive cost of tissue or in the setting of lamellar surgery where a high-quality tissue may not be required.

C. Lyophilised Cornea (LC)/freeze dried method of cornea preservation

Transplantation of lyophilised cornea was attempted in early 1940s. Subsequently Katzin, and Leopold and Adler carried out similar experiments with frozen rabbit corneas.[31] McNair and King described a method of effective lyophilisation in an animal experiment.[32] Grafts dehydrated in glycerine and stored in vacuum without refrigeration can be used for lamellar keratoplasty. Similar experiments were repeated in humans and animals, and later as homograft and heterograft.[33-35]

Initially, lyophilisation was mostly used for preservation of grafts. However, subsequently this was used for reduction of antigenicity of heterogeneous graft[35] and long-term preservation of corneal tissue.[36]

1. Techniques for corneal lyophilisation

Lyophilisation or freeze drying, is a low temperature dehydration process that involves freezing the product, lowering vapour pressure and then removing the ice by sublimation or a change of phase from solid to vapour without passing through liquid phase. This is used for preservation of heat-sensitive drugs and biological agents.

Lyophilisation of cornea can be performed by different techniques.[37] It can be done with or without cryopreservation. Whole cornea or flap of cornea can be lyophilised. Farias *et al.* have described four different techniques of lyophilisation.[37] In cases of without cryopreservation, the bottles with corneal flap and balanced salt solution were kept in dry ice with isopropyl ethanol for one hour, after which flaps were rinsed with balanced salt solution (BSS). The bottles were kept in a lyophilisation machine (Modulyon D) for 23-hours at -40°C and the vacuum was set at 1-2 mbar. The bottles were sealed after subjecting to one hour of vacuum pressure. Some corneal flaps were cryoprotected using 2.3 mol of saccharose for 40 minutes. Corneas were rehydrated for 30 minutes in three washouts of balanced saline solution.

Decellularization of tissue can also be done by various physical and chemical methods. Cell depletion and lyophilisation makes the cornea opaque. Glycerol was used to transparise stroma. Rovere *et al.* used cell depletion and transparised stroma for lyophilisation (LTDC) for long-term preservation and to decrease immunogenicity.[36]

2. Characteristics of lyophilised cornea

Quantock *et al.* studied organisation of collagen lamellae in lyophilised porcine corneas by synchrotron x-ray diffraction and transmission electron microscopy (TEM).[38] They concluded that collagen fibres are compact in lyophilised cornea and become wide-spaced when rehydrated to prior interfibrillar spacing. Farias *et al.* had examined lyophilised cornea after rehydration with BSS, phosphate-buffered saline or distilled water, by light microscopy and electron microscopy.[37] Rehydration with distilled water and phosphate buffer saline did not maintain corneal structure. However, rehydration with BSS for 30 minutes, preserved corneal structures, such as, parallel arrangement of stromal lamellae, the epithelial basement membrane, and Bowman's layer are maintained.

Rovere *et al.* had examined LTDC corneas for histocompatibility antigens.[36] Histocompatibility antigens were not detectable by antibody staining. TEM of LTDC cornea revealed spatial arrangement of collagen fibres and presence of cellular debris in an ultrastructural arrangement of collagen fibres close to native cornea. Pepose *et al.* compared distribution of HLA-ABC (Class I) and HLA-DR, DQ and DP antigen in fresh non-lyophilised cornea with rehydrated lyophilised epikeratoplasty lenticules.[39] While Class I antigens were present in both control and epikeratophakia lenticules, Class II antigens were present only at the limbus of control cornea.

3. Clinical application and outcomes with lyophilised corneas

Lyophilised cornea (LC) has been used for emergency or tectonic purposes.[40] Most common indications are for anterior

lamellar keratoplasty in corneal scars and keratoconus.[37,41-44] Frozen lyophilised donor lenticule has also been used for epikeratophakia.[45,46]

Tayyib *et al.* used LC in lamellar corneal transplants for corneal scarring in 6 patients.[42] Visual acuity improved in all eyes post operatively. Five grafts epithelialized rapidly, whereas one graft had delayed epithelialisation. Coombes *et al.* used LC for deep lamellar keratoplasty in 44 eyes with keratoconus.[43] The mean time to complete epithelialisation was 3.8 days. The median post-operative visual acuity was 6/9. Farias *et al.* compared efficacy of LC with corneas stored in Optisol for deep lamellar keratoplasty patients with keratoconus.[37] No statistical difference was noted between both groups with regard to topography, pachymetry, endothelial count and best spectacle corrected visual acuity. Optisol stored corneas had greater keratocyte density while the keratocyte density improved in LC during follow-up.

Buratto and Ferari compared outcomes of lyophilized and freshly prepared myopic epikeratophakia lenticles for refractive errors ranging from -14 to -28 diopters.[45] No difference in postoperative refractive outcomes were noted between two groups. Though the rate of postoperative epithelialisation was slower in freeze-lyophilised group, all eyes were re-epithelialized by 3-weeks.

D. Gamma irradiated sterile cornea (GISC)

Gamma Irradiation is a strategy to sterilize the tissue grafts against microbial contaminants. It is effective in the eradication of diverse microorganisms that includes bacteria, fungi, viruses and prion agents.[47,48] The technique has been commercially used in irradiating donor corneas by Tissue Banks International (Baltimore, MD) to produce Vision Graft Sterile Cornea.[49] Gamma irradiated cornea is a decellularized, stromal collagen matrix.[47,50] The Vision Graft gamma irradiated corneas is categorised as HCT/P (human cells, tissues, and cellular and tissue based products); hence its regulatory and distribution guidelines are the same as standard corneal tissue.[47,51]

1. Preparation technique of gamma irradiated sterile corneas

The donor corneas deemed unsuitable for transplantation for keratoplasty due to poor endothelial health, but with clear stroma, are selected for gamma irradiation and frozen for long term preservation and storage.[49] The frozen corneal tissue is first submerged in a storage medium containing human serum albumin in a sealed container, which is sterilised with a validated gamma irradiation process to a sterility assurance standard levels as per the recommendations of American National Standard Sterilization of health care products. The corneas are subjected to gamma radiation of 17-23kGy from a cobalt -60 source. The irradiated corneas are approved for storage at room temperature for 2 years.

2. Characteristics of gamma irradiated donor corneas

The effect of gamma irradiation has been studied in various tissues. Most studies have indicated a substantial change in physical and/or biological properties in the collagen.[52,53] Normal corneal architecture and viable corneal cells are needed to maintain corneal transparency.[54] Chae *et al.* studied the biophysical characteristics of gamma irradiated corneas and compared it with fresh donor corneas. The physical properties such as light transmittance, hydration and elastic modulus of gamma irradiated corneas were found to be comparable with fresh donor corneas.[50,55,56] However, the density of collagen fibrils and DNA content was found to be lower than fresh donor tissue.[55] Stevenson *et al.* studied the cellular viability, graft clarity, graft survival and T-cell alloreactivity (using mixed lymphocyte reaction assays and delayed type hypersensitivity assays) after transplantation of gamma irradiated corneas in murine eyes.[47] The allogenicity of gamma irradiated corneas was found to be reduced. This is likely related to the devitalization of potentially antigenic corneal cells, including resident antigen-presenting cells in the process of irradiation.[47] Additionally, gamma irradiation may offer increased resistance to keratolysis by collagenases.[57]

3. Clinical applications and outcomes

The gamma irradiated corneas have been used for a variety of clinical procedures that includes therapeutic/tectonic penetrating keratoplasty, patch grafts, anterior lamellar keratoplasty, glaucoma patch grafting, and keratoprosthesis implantation.[58-66] In a multicentric case series[58] consisting of 23 patients, 6 patients underwent deep anterior lamellar keratoplasty (DALK) (3-keratoconus, 3-stromal dystrophy), 15 patients underwent anterior lamellar keratoplasty (ALK) or lamellar patch grafts (9-infective keratitis, 5-autoimmune keratitis, 1-limbal dermoid), 2 patients underwent keratoprosthesis (both for multiple failed grafts). In 7 cases (6-DALK, 1-ALK), the grafts epithelised well on an average of 6.6+/-1.3 days and maintained clarity over a median of 12 months follow up. Two patients who had keratoprosthesis did well in the initial post-operative period, but developed endophthalmitis between 4-7 months after surgery, presumed to be unrelated to the donor cornea. In another clinical series[59] of 10 patients, lamellar keratoplasty using gamma irradiated corneas was performed for corneal melts with microperforation (n = 6), keratoprosthesis associated melt (n = 2), and non-inflammatory corneal lesions (n = 2). All grafts except 1, with a progressive systemic disease, epithelised in 1-13 days and remained clear over a period of 7-15 months. In a large retrospective series of 319 aqueous drainage device implantation, the outcomes of GISC as coverage patch in primary and secondary glaucoma was analysed.[62] Ten out of 319 eyes experience patch graft failure (defined as tube erosion through patch graft) with a mean follow-up of 15.4+/-9.8 (SD) months. Nolan *et al.* reported successful use of GISC in a majority of pediatric Ahmed drainage valve surgery.[63] In the laboratory experiments, femtosecond laser was shown to provide smoother interface in lamellar cuts in gamma irradiated corneas compared to fresh corneas.[67]

Discussion

Very long-term corneal preservation techniques are limited by lack of viable cells in the tissue leaving behind an acellular corneal stromal scaffold. However there are several ways in which it may be advantageous.[68] 1) Lack of expression of MHC antigens, hence these grafts are less liable for immune rejection. 2) Epithelium, keratocytes and endothelial cells can re populate the acellular cornea that helps regain corneal structure and function. 3) Very long-term preservation times provides a ready source of cornea for emergency and lamellar procedures. Disadvantages of very long corneal preservation techniques

are: 1) Cannot be used for optical purposes, 2) Prolonged time to epithelialization of the grafts, 3) Edema, swelling and opacification of grafts post transplantation.

Current techniques in cryopreservation alone are complex and expensive to be adopted universally and are inadequate to maintain endothelial viability. On the other hand, there are exciting developments in methods of viable preservation of endothelium for potential use in cell based therapies.[69] Hence, strategies for direct endothelial cell culture therapy using the acellular corneas as scaffolds can be potential areas of research interest.

Glycerol preservation techniques are relatively easy to adopt and under the current COVID-19 crisis in India and elsewhere this technique of preserving corneas for an indefinite period of time is feasible and replicable across eye banks. The storage conditions for GPC are also broad as the original descriptions from King *et al*.[20,21] recommended storage of corneas at room temperature, whereas the newer experiments recommended better tissue quality, pliability, transmissibility and collagen architecture when stored from -4°C to -80°C.[24,25] Glycerol is inexpensive and easily available from most pharmacies. Sterile vials of glycerol may be prepared and stored under adequate refrigeration in most eye banks that are equipped to process tissues. The technique of glycerol preservation has been proposed as viable alternative for developing countries where expensive and technology intensive processes for improving shelf life of corneas are limited. Keratoplasties are performed for therapeutic or tectonic purposes and patients have challenges in compliance to post transplant treatment regimens and follow up.[30] The obvious disadvantage of a lack of viable endothelium can be easily mitigated by subsequent vision restoring full thickness or endothelial keratoplasty.

Lyophilized corneas require specific equipment, the process is cumbersome and are relatively expensive. In addition it will need trained technicians to process the tissue. The corneal tissues are decellularized during processing which reduces its antigenic load with reduced levels of HLA antigens.[36,39] However, the cornea becomes opaque during processing.[37] Lyophilised corneas can be used for emergency, tectonic purposes, and lamellar corneal surgeries. There are concerns regarding delayed epithelialization post-transplant though most of re-epithelialization is complete by 3 weeks.[42,45] The disadvantages of lyophilized corneas are difficulties in tissue handling during surgery and lack of tissue transparency while adding to the cost of the procedure.

Gamma irradiated sterile corneas use a patented technology and are commercially available from Tissue Banks International, USA. These tissues have a good shelf life of 2 years that can be stored at room temperature. The transmissibility, elastic modulus and hydration of gamma irradiated corneas are comparable to fresh tissue. The antigenicity of gamma irradiated tissues are reduced as the process of irradiation decellularizes tissue leaving behind an acelluar stromal shell therefore decreasing the risk of rejection.[47,58] Thus irradiation increases shelf life and decreases chance of microbial contamination. The disadvantages of gamma irradiation is the need to procure the technology and/or the cost of bearing the tissue processing charges. These in addition to limited indications such as in therapeutic, tectonic and anterior lamellar surgeries grossly limits the usage of gamma irradiated corneas for clinical use.

Cryopreservation techniques attempted to preserve corneal collagen architecture while maintaining the viability of the endothelium. The major drawback of this technique was its complexity which hindered a wider adaptability. From a physiologic standpoint the thermal injury induced during cryopreservation by intracellular ice crystal formation and increased osmotic gradient in the extracellular space due

Table 1: Summary of long-term cornea preservation techniques

Techniques of long-term corneal preservation	Benefits	Drawbacks
Glycerol Preservation	Extended preservation time of 5-23 years Relatively Inexpensive Minimum use of technology Easy to adapt by eye banks in developing nations Tissues may be stored at room temperature or cryopreserved at -4°C to -80°C Reduced risk of rejection due to lack of viable cells	Acellular corneas lead to loss of graft clarity post-transplant Tissues may be used for tectonic, therapeutic and anterior lamellar keratoplasty
Lyophilised (freeze dried) Corneal preservation	Cornea may be stored for extended period of time Tissues may be used for patch grafts or as epikeratophakia lenticles	Technology intensive Expensive to process Not easily replicable Lack of viable cells leads to corneal opacity post transplantation
Gamma Irradiation	Cornea can be stored for 2 years Tissues may be used for patch grafts or covering glaucoma valves as an alternative to sclera	Requires patented technology for processing tissues Additional cost of preparing and procuring tissues Lack of viable cells leads to corneal opacity post transplantation
Cryopreservation	Cornea can be stored indefinitely Viability of cells can be maintained	Technology intensive process Requires expensive equipment and specialized expertise for tissue processing Not easily replicable Thermal stress during tissue rewarming leads to loss of tissue function

cryoprotectants themselves led to loss of cell viability.[13,14,17] The benefits and drawbacks of each technique of long term corneal preservation are summarized in Table 1.

Conclusion

To conclude, adoption of some form of very long-term preservation methods should be considered at every eye bank to manage the uncertainties in availability of donor corneas. Further, if there is some semblance to 'normalcy' restored post COVID-19 phase, India is expected to reach a state of sufficiency/surplus corneas. Exploring the avenues of very long-term preservation will help in maintaining surplus corneas for emergency use at all times. Possibly, surplus donor corneas in long term preservation methods, may be considered for international use in those countries where eye banking is primitive/embryonic with respect to changing times and existing laws for the same in future.

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Conflicts of interest

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