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To cite this article: Gabriela Wojcik , Stefano Ferrari , Vito Romano , Diego Ponzin , Sajjad Ahmad & Mohit Parekh (2020): Corneal storage methods: considerations and impact on surgical outcomes, Expert Review of Ophthalmology, DOI: [10.1080/17469899.2021.1829476](https://doi.org/10.1080/17469899.2021.1829476)

To link to this article: <https://doi.org/10.1080/17469899.2021.1829476>



Published online: 12 Oct 2020.



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REVIEW



Corneal storage methods: considerations and impact on surgical outcomes

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ABSTRACT

Introduction: With recent developments in the field of eye banking, human corneas are not only procured and preserved, but also processed and prepared for transplantation. However, one of the challenges that still persists is the long-term storage of tissues without damaging the corneal endothelial cells. Thus, the review aims at reporting the influence of tissue storage conditions on the clinical outcomes.

Areas covered: Endothelial cell loss (ECL), graft survival, and contamination from the tissues stored in hypothermic storage and organ culture and; other storage options such as cryopreservation and lyophilization.

Expert opinion: Hypothermic storage and organ culture have shown similar ECL. However, due to the relatively new techniques and limited long-term clinical studies, further evaluation is essential to assess the effect of storage time and conditions on the grafts deemed for endothelial keratoplasty.

ARTICLE HISTORY

Received 25 March 2020

Accepted 24 September 2020

KEYWORDS

Cornea; eye bank; storage; hypothermic; organ culture; cryopreservation; lyophilization; clinical outcomes

1. Introduction

1.1. Importance of corneal endothelium

Maintaining corneal transparency is essential for optimal performance of the visual system and it partly relies on the performance of corneal endothelial cells [1–3]. Corneal endothelium is the posterior monolayer of the tissue that facilitates maintaining hydration levels that is required to keep the tissue transparent. This is usually achieved by the pump-and-leak barrier function that allows the flow of solutes and ions to and from the cornea [3]. Maintenance of the density and function of the corneal endothelium are crucial parameters in eye banking and transplantation outcomes. Therefore, its storage remains one of the critical steps in the eye-banking field [4].

1.2. Corneal donation

It has been noted that corneal blindness is the third reason of blindness in the world. In a recent global survey, it was reported that over twelve million patients are waiting for a corneal transplant [5]. Limited supply of corneal tissues globally has been partially responsible for lower numbers of corneal transplantation. Other obstacles such as access to modern and relatively expensive treatment options are challenging the preventive treatment measures [5–8].

1.3. Eye banking

Eye banks are institutions responsible for harvesting, processing and distributing the ocular tissues [9]. Most eye banks focus on corneal tissues, but other tissues like sclera, retina,

choroid or intraocular lens are also excised either for transplantation or for research purposes [10–12]. Earlier, the eye banks only provided full thickness corneas that were harvested from the cadaveric donors deemed for penetrating keratoplasty (PK). However, with recent developments in the endothelial keratoplasty (EK) procedures, eye banks have not only been at the forefront in preparing tissues for challenging techniques like Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) and Descemet Membrane Endothelial Keratoplasty (DMEK), but also advanced tissue preparation procedures like pre-cut or pre-loaded grafts that are ready for transplantation [13–18]. This does not limit the eye-banking field as they continuously grow in research and development that is focused on the most efficient storage conditions and transportation methods. Tissues prepared by the eye bank technicians are characterized as validated grafts with limited tissue wastage [16,19–23]. Due to the availability of the tissues for research, eye banks have emerged as institutions for cutting-edge research lowering the dependency of the donor material and finding new and effective surgical techniques/tools to reduce surgical trauma [24,25].

1.4. Tissue characteristics

Once the human corneas are retrieved from the cadaveric donors, they are stored to maintain the viability of the endothelial cells, which is one of the important parameters required for a successful corneal transplant. Usually the corneal tissues are retrieved from the cadavers within 24 hours' postmortem however, in certain situations it is extended up to 48 hours. A short interval between death and the tissue retrieval is usually recommended to ensure lower damage to the

Article highlights

- Although the corneal tissues can be stored and have been considered safe up to 14 days in HS and up to 28 days in OC, it could be worth limiting the storage time as short as possible to avoid LEF especially when selective transplantation options are considered.
- Various type of media formulations has been introduced; however, Optisol-GS is still the gold standard for HS.
- Introduction of effective antibiotics/antifungal agents would enhance the use of HS.
- OC has several advantages such as the maintenance of endothelial cells and its metabolism at physiologic temperatures.
- Confounding factors have been reported in terms of donor characteristics and graft survival. Although transplantation of old aged donor tissues has been reported as one of the factors affecting the long-term clinical outcomes, further evidence is required before a systematic conclusion can be drawn.
- As storage condition supports the growth of bacteria and fungus in OC it becomes fairly easy to obtain true positive microbiological results.
- Those eye banks with surplus tissues can store the tissues for long-term thus reducing wastage of corneal grafts for transplantation purposes and it gives enough time for the surgeons to plan the surgeries or the eye banks to prepare tissues for selective transplants.
- Contrary to the above storage methods, cryopreservation of corneas is only limited to emergency cases for PK or DALK and cannot be used for EK as the storage temperature induces huge mortality on endothelial cells.

viable cells [9]. Blood samples are tested for transmissible diseases like HIV or HBV/C. Although the threshold of endothelial cell density (ECD) required for transplantation usually varies between different eye banks, a consensus of tissues with over 2000 cells/mm² without epithelial or stromal scarring and corneal opacity are considered for transplantation by most of the eye banks in Europe and America [26–28]. However, it is also observed that countries with low number of corneal tissues have a threshold as low as 1600 cells/mm². Apart from ECD, a combination of pleomorphism and polymegathism termed as polymorphism and mortality of the cells are other factors considered during tissue evaluation [20].

1.5. Storage solutions

Typically, once the tissues are harvested from the donor they are preserved in storage solutions until they are analyzed and transplanted. The two most popular types of corneal tissue storage methods [26,27,29] include a) hypothermic storage (HS) and b) organ culture (OC) [26,28,29]. HS is popular in the United States and Asia whereas OC is the most preferred option in Europe [27,29]. In HS, the tissues are maintained between 2 and 6°C up to 2 weeks (intermediate storage) [26,29], reducing the metabolic activity of the cells. Most commonly, the tissues are evaluated by specular microscopy [30]. The OC method, however, allows storage of the tissue at the physiological temperature i.e. between 31 and 37°C keeping the cells viable and healthy for up to 4 weeks (long-term storage). During this process, the tissues are tested for microbiological infections to avoid any transmission of pathogens to the recipients [26,29] as the storage conditions support the culture of microbes. Besides the microbiological and

serological checks, tissue quality in terms of endothelial cell morphology, numbers, and viability is evaluated before and after storage using an inverted microscope. OC is relatively more complicated and expensive compared to other methods [30]. In terms of economic difference, HS requires a single vial of storage solution for the entire storage period whereas, OC is divided in three phases of storage, thus requiring different solutions for each phase. These include 1) collection of the tissue from the hospital, 2) storage solution that contains serum to keep the cells viable and 3) transport/de-swelling solution containing dextran to regain the physiological thickness of the cornea which swells during the OC phase. Although OC has several advantages such as, it allows microbiological checks, quality assurance and provides longer time for both, the eye banks and the theater to plan and execute the surgery; it could be limited in many centers due to financial constraints [28]. Cryopreservation has also been considered for long-term storage of donor corneas, which could be an alternative for the countries with surplus of donor tissues that can be preserved and shipped. Frozen corneal tissues have been found to be useful in corneal structure restoration or issues with stromal defects [31,32]. However, cryopreservation can have significant damaging effects on keratocytes, corneal endothelial cells, and graft quality [31]. Cryopreserved tissues without viable cells could be used as an alternative scaffold for other methods such as Boston Keratoprosthesis (Boston KPro) [33].

1.6. Aim of the review

As the primary aim of an eye bank is to harvest, preserve, and distribute the corneal tissue for transplantation purposes, storage of tissue remains one of the critical steps in order to provide a viable graft for surgery. This paper, therefore, reviews different types of storage methods and the related clinical outcomes.

2. Clinical outcomes of tissues stored in HS

2.1. Variety of storage solutions available for HS

The most popular HS solution is Optisol-GS (Bausch & Lomb Surgical, Inc., Rochester, NY, USA), a medium containing 2.5% chondroitin sulfate, 1% dextran, vitamins, and precursors of adenosine triphosphate. Optisol-GS and its alternatives are widely used, especially in the United States [34]. However, many developing countries still rely on the conventional McCarey–Kaufman media mainly as it is easy to manufacture in-house, has a huge financial advantage and it serves the short-term storage purpose (storage between 0 and 4 days) [35,36]. Life4°C (Numedis Inc; Isanti, MN, USA) solution, which is currently used by many eye banks in the United States, has also been studied and recommended [37]. Another alternative to Optisol-GS, not commercially available, is Chen medium (CM; Chen Laboratories, noncommercial product), a FDA-approved isotonic storage solution for corneal tissue [32]. It contains TC199 culture medium with reduced sodium chloride and without bicarbonates. The main supplements

involved are *beta* hydroxybutyrate, gentamicin sulfate, streptomycin sulfate, phosphates, HEPES buffer and 7% dextran. CM has shown to facilitate reduction in lactate formation, stabilizes pH levels and stops glycolysis in the corneal tissue.

2.2. HS for PK

Gupta et al. compared the outcomes of PK using freshly isolated corneas and tissues shipped from the United States to Jordan [38] in Optisol-GS. Fresh tissues were preserved for less than 24 hours before operation. The shipment time from US to Jordan was no longer than 10 days. Success rate of the surgery was 86% and 88% from the local vs imported group, respectively, which was not found to be significantly different. There was no correlation between the graft success rate, visual acuity (VA) and the tissue storage time. This study suggested that intermediate storage time, long transportation routes and the age of donors did not affect the outcome of PK grafts [38].

Bourne WM et al. compared CM with Optisol-GS solution when the tissues were stored up to 11 days. Corneal thickness at day 1 and 1 year after surgery did not show statistical difference between both the groups. The epithelial coverage at day 1 postoperatively was 64% in CM and 65% in Optisol-GS, respectively. First week after surgery, 83% of CM stored corneas were fully epithelialized, compared with 100% after Optisol-GS storage. Two months after surgery, there were no differences in ECL from both the groups. Corneas from CM showed 8% ECL at 2-month follow-up and 19% ECL at last follow-up. The tissues from Optisol-GS showed ECL of 11% at 2-month follow-up and 17% at last follow-up with significant difference between the groups. This study suggested that CM has a similar influence on tissue after PK as Optisol-GS [32].

2.3. HS for EK

Price et al. stored the tissues randomly in Life4°C or Optisol-GS for a maximum period of 7 days to be used for EK [37]. There was no difference between Life4°C and Optisol-GS groups in terms of endothelial cell loss (ECL) at 6 months after the surgery (18% and 20% ECL from Life 4°C and Optisol-GS group, respectively). Recipients' stroma and grafts remained clear during the 6-month follow-up. Although it was suggested that Life4°C behaves similar to Optisol-GS, it has been shown that the addition of glutathione in Optisol-GS facilitates the performance and prolongs the quality of the graft [37].

Lass et al [39]. showed that after 3 years of follow-up, the ECL was 37.3% and 39.7% when the tissues were stored for 0–7 days and 8–14 days, respectively, which was found to be statistically different. However, a further evaluation showed that tissues stored for 14 days resulted in 49% ECL compared with 30% ECL when stored for 0–3 days. As preparing a DSAEK graft requires tissue manipulation using microkeratome, that may increase cellular trauma; therefore, storage time and conditions must be further evaluated for tissues deemed for EK purposes when stored in hypothermic condition [39].

In another study, Terry et al. analyzed data of 362 patients with Fuchs' dystrophy receiving DSAEK grafts from tissues stored in HS [40]. Death to transplantation time was a maximum of 12 days. ECL at 6, 12 and 24 months were 28,

31, and 32%, respectively. ECL values between the groups stored for less than 4 days (183 tissues) and 4 days or more (179 tissues) showed no significant differences at all the follow-up time points. Additionally, comparison of ECL from tissues stored for the longest (7–12 days, ECL 30%) and the shortest time (1.5 days, ECL 33%) did not differ. These results, contradicting to the Lass et al.'s study, suggested that there is no correlation between the storage time and ECL in a relatively uniform patient group [40].

2.4. Identifying risk factors associated with graft survival

Patel et al. used COX regression model to determine the donor risk factors for graft failure and late endothelial failure (LEF) after PK surgeries [36]. Tissues were stored in McCarey-Kaufman media or K-Sol media (McCarey-Kaufman media contains dextran but no chondroitin sulfate and K-sol media has chondroitin sulfate but no dextran) or in OC at 34°C [36]. The main indication of transplantation was keratoconus (68%). Seven grafts failed between 15 and 20 years. ECL at 20 years was 74%, although ECL between 15 and 20 years (5-year period) was merely 0.06%. Graft thickness did not differ between 15 and 20 years, but was found to be thicker at 20 years' time point compared to 2 months after surgery, especially in corneas preserved in McCarey Kaufman media. ECD and pachymetry were stable between 15 and 20 years. Graft failures were mostly due to LEF, which was found to be 31% [36]. This study suggested that patient diagnosis and donor preoperative ECD or coefficient of variance (COV) combined with the postoperative ECD, early ECL are the main factors responsible for the graft failure and LEF [36].

Rosenwasser et al. investigated the influence of storage time on graft survival after successful DSAEK surgeries [41]. Donor tissues were stored in Optisol-GS or Life4°C. 675 eyes received a tissue from postmortem time (PT) <7 days and 655 received tissues from PT of 8–14 days. The most common indication for DSAEK was FECD (94.4%) [41]. The grafts were successful in 95.3% from <7 days PT group and 92.1% in the 8–14 days PT group. In the first post-operative month, the probability of failure was 2.4% in the <7 days PT group and 4.9% in the 8–14 days PT group. An association between longer PT (>11 days) and a lower graft success rate was identified. The success rate was still high in the group with a PT of 12 to 14 days (89.3%), which supported continued use of both the storage solution up to 11 days [41].

3. Clinical outcomes of tissues stored in OC

OC is the most popular corneal storage solution in Europe [30]. Eye banks either make their own culture media or use commercially available mixtures [42] using basal medium such as minimally modified essential medium (MMEM) [43] etc.

3.1. OC for PK

Gauthier et al. investigated the outcomes of more than 90 consecutive PK surgeries using the corneal tissues stored at 31°C up to 29 days in CorneaMax media (Eurobio, Les Ulis,

France) [42]. No incidents during and after the surgery were recorded. ECL was 28% on day 1 and increased to 42% at the last follow-up [42].

Thuret et al. suggested that corneal tissues stored in OC for an intermediate time period (usually between 5 and 12 days) show lower ECL compared to long-term storage (usually between 21 and 35 days) [44]. Tissues from the same donor were assigned randomly and stored in Inosol OC solution (Bausch & Lomb-Chauvin-Opsia, Labège, France) and transferred to de-swelling Exosol media (Bausch & Lomb-Chauvin-Opsia, Labège, France) 48 h before surgery. ECL at the last follow-up (12 months) was 29.9% when the tissues were preserved for intermediate time period compared with 31.3% when the tissues were preserved for long term before PK surgeries, which was not significantly different. There was no difference in the graft rejection rate, highlighting that long storage has no influence on endothelial cell survival [44].

3.2. OC for EK

Dapena et al. reported a 2-year follow-up study after DMEK surgery and the influence on maintenance of the endothelial cells using the corneas stored in OC [43]. Ten corneas were stored for 14 days in minimally modified essential medium (MMEM) at 31°C. There were three cases of graft detachment within 1-week postoperative time (30%). One-month follow-up data showed 28% ECL which increased to 34% at 1 year follow-up [43].

3.3. Identifying risk factors associated with graft survival

Borderie et al. conducted a study to define the correlations between pre-operative donor characteristics and recipient or surgical changes [45]. Two hundred and thirty-one tissues were stored in the Inosol medium (Opsia, Toulouse, France) at 31°C for a maximum of 24 days followed by de-swelling in Exosol media (Opsia) for 1–3 days [45]. This study reported that donor age has an influence on graft survival rate, i.e., donors with age >80 years could survive longer if they meet other criteria [45].

Whether the tissues disqualified for EK or PK are safe for ALK were evaluated by Borderie et al. in a retrospective study using COX regression model [46]. Analysis was carried out on 166 patients suffering from anterior corneal diseases comparing graft characteristics (donor age, storage time, ECD, de-swelling time) and graft survival, post-operative ECL and VA. All the tissues were stored at 31°C in Inosol media. Five-year graft survival rate was 96.5%. Donor age, graft ECD, graft storage time, graft deswelling time had no influence on graft survival or late ECL results. Annual ECL was 8.2% in the early follow-ups and 4.7% at the late phase, respectively. Postoperative VA was lower in patients receiving the tissues from older donors (>80 years). VA improved from 12 to 36 months in patients with grafts from younger donors, but did not improve in the patients with graft from older donors. Graft ECD, storage or de-swelling time did not influence post-op VA. The analysis suggested that donor age strongly influences the visual recovery. Postoperative ECL was independent

of the donor characteristics (ECD, donor age, organ culture time, de-swelling) and patients with the grafts from older donors had lower visual recovery suggesting to avoid collection of grafts from older donors for ALK [46].

Armitage et al. conducted a retrospective 5-year study after PK on 7107 tissues between 1999 and 2005 to determine the influence of the donor factors on corneas in OC and; donor and recipient factors on graft survival [47]. Average storage time was 18.3 days (26% stored <2 weeks; 46% stored 2–3 weeks; 26% 3–4 weeks; 2% >4 weeks). Donor age was 61 years with ECD of 2636 cells/mm² [47]. The overall contamination rate was 5.7% and it was strongly correlated with the cause of donor death. Graft survival after 5 years from surgery was 73%. The risk factors included, gender i.e. male donor tissues had a higher graft failure rate compared to female donors. Graft survival rate decreased with the preoperative diagnosis (bullous keratopathy-59% compared to keratoconus-93%), allograft rejection, trephine diameter >8 mm and difference between the donor and recipient trephine sizes [47].

Bohringer et al. studied the influence of pre-operative characteristics on ECL after PK surgery in a uniform patient population retrospectively [48]. The tissues were preserved in OC for a minimum of 10 days, and had a postmortem time of less than 72 h [48]. The overall ECL was 16.7%. The lack of correlation was recorded between donor and patient age. However, ECD decrease was correlated to postmortem time and donor age, but in case of the storage time, the result was not statistically significantly different. The study suggested that shortening of the post-mortem time and storage time could potentially reduce the risk of LEF [48].

3.4. Synthetic organ culture media

One of the challenges of using serum-based media (standard media for the corneal storage) is the risk of xeno-transfer into the corneal tissue from animal or animal-derived products [29]. Therefore, a synthetic media (Stem Alpha, Argentière, France) was developed to overcome these challenges. There were no statistical differences between the thickness when the tissues were stored in OC supplemented with fetal bovine serum or synthetic media. However, the difference was significant at the de-swelling stage that showed better thinning rate and improved transparency from serum-based media with no difference in ECL between both the media [29]. Advanced synthetic media that use recombinant human serum albumin to replace the animal serum (CorneaSyn, Eurobio, France) have also been tested although clinical outcomes are still awaited [4,28]. Donor selection is one of the most important steps to ensure the corneal qualities before the surgeries [41,48]. This process of eye-banking needs to be regulated to assure the best clinical outcomes.

4. Comparison between HS and OC

Heindl et al. retrospectively investigated the results of deep anterior lamellar keratoplasty (DALK) and DMEK by checking the influence of storage time of split donor tissue and outcomes after surgeries in 110 tissues [49]. The cornea was split

i.e. depending on the DALK or DMEK procedure, followed by the remaining lenticule which was preserved for a maximum of 7 days before the surgery. The corneas (40%) were stored in Optisol-GS and the remaining 60% were stored in Dulbecco's modified Eagle medium containing streptomycin, penicillin, and fetal calf serum. Average donor age was 62 years, post-mortem time was 15 h, storage time was 317 h, and ECD 2581 cells/mm² [49]. Postoperative ECL was 8 and 41% up to 1 year after DALK and DMEK, respectively. No significant association was observed between storage time of split donor tissue as well as total storage time in storage solution with BSCVA, refractive astigmatism, ECD, ECL, central corneal thickness, and complication rate at 1-year follow-up. The data suggested that anterior and posterior lamellas can be preserved safely up to 7 days after they are split, which gives a wider window for surgical logistics and lowers the tissue wastage [49].

Frueh et al. reported that tissues stored in Optisol-GS could survive up to 11 days therefore; it was compared with OC [34]. Corneas from the same donor were stored separately in Optisol-GS or OC and transplanted after 11-days of storage. No graft failure was observed after PK. However, epithelial defects were found in 33% of OC corneas and in 58% of Optisol-GS group during the first 3 days' post-surgery but were not found during future follow-ups. Corneal thickness was increased by approximately 2.5% in OC group compared with 6.4% in Optisol-GS at 24-month follow-up. ECL of 16% was recorded in OC and 22% in Optisol-GS between 1-month and 24-month post-surgery without any statistical difference. This study suggested that there are no major differences in the outcomes of PK surgeries when the corneas are stored in Optisol-GS or OC up to 11 days [34].

Schaub et al. preserved 73 corneas in OC (Biochrome, Berlin, Germany) and 11 tissues in Optisol-GS or Life4°C for an average of 11–17 days [50]. ECL at 12-month follow-up was recorded at 4% and 17% at 3-month post-suture removal between OC and HS groups, respectively. A few postoperative complications including loosened sutures in 20% of grafts, Descemet detachment in 16% of cases, persistent epithelial defects in 8% of grafts were noted. There were no postoperative graft failures. All the complications were related to the surgical issues and none of them were found to have any correlation with tissue characteristics including the storage solution type or length. It suggests that donor qualities, type of storage solution or storage time have no influence on clinical outcomes in DALK or DALK/DMEK surgery [50].

5. Contamination issues with HS and OC

Considering the rising concerns of fungal infection after corneal transplants, Lau et al. conducted a retrospective study, where they compared the donor tissues stored in HS or OC before surgeries. The analysis was performed within 3 European centers between 2014 and 2017 [51]. The tissues in HS were stored up to 2 weeks in Optisol-GS, supplemented with gentamicin, whereas the OC solution was prepared by the eye bank supplemented with penicillin, streptomycin, voriconazole, amphotericin B, caspofungin, and the tissues were stored up to 4 weeks at 28–37°C [51]. Seven eye banks used HS or both, HS and OC (up to 76% corneas from HS were

shipped from outside of the continent). There were 17 cases of fungal infections after surgeries, all stored in HS and shipped from the USA. Comparing the infection rates, HS was most likely to increase the risk of fungal contamination (0.5%) than OC (0.02%). Although with certain limitations in methodology, the study showed that lack of anti-fungicide especially in HS could increase the risk of fungal contamination [51].

Fontana et al. investigated the influence of tissue retrieval techniques and the type of corneal storage method on the positive microbial scleral rims after the PKs. HS -Eusol C (Alchimia) and OC -Tissue C (Alchimia) were analyzed [52]. The study involved data from routine donor rim cultures from one hospital and eye bank with post-mortem time of 24 h, harvested by enucleation or in situ isolation in sterile environments. Tissues in Eusol C (Alchimia) were stored for 3–5 days and Tissue C (Alchimia) between 3 and 4 weeks at 31°C before surgery. Deswelling media, Carry C (Alchimia), was used 24 h before the surgery [52]. Postoperative analyses were conducted on 443 tissues stored in OC and 185 tissues stored in HS. From OC, 1.3% tissues were contaminated, with mostly bacterial infections (fungal to bacterial ratio 0:12). Contamination from HS was 9.8% of all the tissues, mostly fungal origin (fungal to bacterial ratio 2:0). From the tissues that were isolated from the eyeballs in the eye banks, 226 were stored in OC (1.3% positive tissues) and 101 in HS (8% positive tissues). From the in situ isolated tissues, 217 were preserved in OC (1.4% positive tissues) and 84 in HS (12% positive tissues). The method of tissue harvesting or procurement of the tissue did not influence the frequency of the microbial/fungal contamination. The study suggests that the type of tissue storage, especially the use of OC could help eliminate tissue infection rates [52].

Borderie et al. evaluated if the OC at the time of surgery was sterile and analyzed the contamination rate and postoperative endophthalmitis [53]. Tissues were stored in OC for 2–5 weeks, which was supplemented with penicillin, streptomycin, and amphotericin B (media was changed at day 14 and 28 of the culture) and de-swelled for 1–4 days before transplantations. Analysis included 603 tissues from OC (31°C) of which 409 (67.8%) were grafted and 194 discarded, with contamination observed in 69 corneas. Contamination in 69 pre-operatively discarded tissues were bacterial (65%) or fungal (35%), where bacterial contamination occurred at roughly 5 days and fungal at 7 days after culture [53]. Postoperative analysis showed that donor to OC time influenced the contamination risk with 13–24 hours of peak storage time (19.2% contamination rate and less risk with both shorter and longer time). Cornoscleral rims were sterile in 99.3% of grafted corneas and the de-swelling media was sterile in 100% cases. No post-operative endophthalmitis were recorded. Combination of the povidone iodine decontamination of the donor eyes and OC for at least 2 weeks with microbiological examinations could be used as safety measures. Additionally, there is a higher risk of contamination from corneas isolated in situ [53].

6. Clinical outcomes of the tissues preserved using cryopreservation method or lyophilization

Cryopreservation is not a frequently used method of corneal storage, but could have huge potential especially in

emergency cases [33,54,55]. Cryopreserved tissues have been used for the following types of transplantation.

6.1. As an emergency procedure for the treatment of severe fungal keratitis

Yao et al. operated 45 eyes with severe fungal keratitis using PK grafts [56]. Up to 86.7% were successful with eradication of total fungal infection. 91% of grafts were not rejected up to 31 months. Elevated intraocular pressure was observed in 9% of the cases, which in most cases was lowered by proper medication. Most corneas were transparent up to 7 days after the surgery. Complete epithelialization was observed in 44 of 45 eyes at day 5 post-surgery. Corneal edema and opacity was high in all the patients up to 1 month, but it decreased during the follow-up periods. Up to 82% showed good or mild VA. All the eyes showed signs of anterior chamber collapse, corneal tissue irrigation, or corneal perforation when the eyes were transplanted using the corneal tissues that were preserved for 9.5 months at -20°C in balanced salt solution (Alcon Lab, Inc, TX, USA) supplemented with penicillin, streptomycin, neomycin, and amphotericin B. Those results suggest that cryopreserved corneas could serve as an alternative option for fungal infections as an emergency tool [56].

6.2. Treatment of Terrien's marginal degeneration (TMD)

Huang et al. used cryopreserved corneal tissues for the treatment of Terrien's marginal degeneration (TMD) [54]. TMD is a bilateral marginal keratopathy, which leads to thinning of corneal margins, corneal neovascularization, lipid deposition, and corneal stromal atrophy [57]. DALK surgery was performed using tissues stored at -20°C [54]. All corneal reconstructions were successful with full epithelialization achieved within 3–7 days in all patients' eyes. Mild stromal edema was noted, but faded during the recovery phase (up to 2–3 months). Three months' follow up showed that all the grafts attached properly and all corneas regained their normal thickness. During the entire follow-up period, the grafts were found stable, there was no corneal thinning observed, suggesting that TMD was eliminated. All corneas showed normal curvature after six-month postoperative time. However, most of the grafts were opaque or slightly opaque and vascularization was observed in almost 39% of the cases [54].

6.3. Boston Keratoprosthesis

Robert et al. studied the influence of cryopreservation on the success rate of Boston KPro [33]. Same numbers of cryopreserved and fresh corneas were transplanted by the same surgeon at the same time. Frozen corneas were preserved at -80°C as whole globes [storage in Optimyxin ophthalmic solution (Sandoz Canada, Inc, Quebec, Canada)]. At the final follow-up period, the inflammation was observed in 15% of fresh graft cases and 22% of frozen tissues. Patients (31%) that received fresh tissues developed retroprosthetic membrane compared with 16% from frozen tissue group. There was no

corneal thinning, leaks, and tissue necrosis. Additionally, all devices showed 100% retention. This study suggested that cryopreserved tissue could be used as an alternative to fresh tissue [33].

6.4. Comparison between cryopreserved vs fresh corneas

Javadi et al. performed a retrospective study on clinical outcomes and complications after DALK using cryopreserved corneal grafts and compared the results with those after PK and DALK using fresh grafts [58]. Fresh corneas were stored at 4°C in Optisol-GS and cryopreserved globes were stored from 44 to 148 days at -70°C without the cryoprotectant. On the day of surgery, the frozen whole globes were defrosted by transferring to a refrigerator and then at room temperature (each step for 1 h). There were no differences noted in terms of postoperative complications. Persistent epithelial defect (lasting >14 days) was less common with PK than with DALK using either fresh or cryopreserved grafts. None of the patients showed graft failure. Analysis showed no differences in VA, spherical equivalent refraction, or keratometric astigmatism whether a fresh graft or a cryopreserved graft was transplanted. There was higher rate of persistent epithelial defects in the groups using cryopreserved tissues, but none of those were statistically significant. This study suggested that cryopreservation provides similar clinical results to fresh corneal tissues used for PK and DALK. Additionally, long-term cryopreservation does not require lyophilization, or chemical agents [58].

6.5. Comparison between cryopreservation and lyophilization

Cryopreservation can be combined with other storage methods. Farias et al. compared the quality of lyophilized corneas with corneas stored in Optisol-GS [55]. Complete re-epithelialization was found in all the patients, but slightly faster in Optisol-GS preserved corneas during the initial months. No significant statistical differences were found between groups at the final post-op evaluation. Lyophilized corneas were slightly less clear compared to other groups, but the corneal opacity difference was not significant. Corneal curvature and ECD did not show any statistical differences. Pachymetry showed improvement after the surgery with thicker corneas in Optisol group and no corneal edema in both groups. A difference between VA after 6 months was similar to other studies [59], suggesting better visual functions with the use of lyophilized corneas.

7. Conclusions

Based on the literature review, we have noticed that Optisol-GS is the gold standard with maximum clinical studies although the recently introduced Life4 $^{\circ}\text{C}$ has shown promising results. The corneal tissues can be safely stored up to 14 days in HS; however, further evidence is required to understand if a shorter storage time (<14 days) could reduce the ECL when considering selective transplant options such as

DSAEK or DMEK. HS can be further enhanced with effective antibiotics/antifungal agents that would reduce the contamination rates. OC has several advantages such as the maintenance of endothelial cells and its metabolism at physiologic temperatures, possibility of performing microbiological tests on growing organisms due to optimal growth conditions, long-term storage capacity for eye banks that have surplus tissues, and long-distance transport facility available at physiologic temperature and; better planning of the surgeries or large clinical studies especially for selective transplants [26,29,47,53]. Comparatively, cryopreservation of corneas has been limited to emergency cases for PK or DALK and cannot be used for EK. The storage method and temperature for cryopreservation and lyophilization are not suitable for maintaining the viability of the endothelial cells [60,61].

Considering the current trends, the type of storage is guided by the legislation of the country, the number of retrieved corneas and economic stability. According to the World Health Organization in its '*Universal Eye Health: a global action plan 2014–2019*' [6] in 2010 there were an estimated 285 million people with visual impairment out of which 39 million were blind. However, up to 80% of visual impairment has been found to be preventable [6,7]. In the 2018 report, the European Network of Competent Authorities of Tissues and Cells (EURO CET) stated that for the year 2017, the number of all cornea recovery was around 56000 [62]. Just in Italy, a total of 17,600 corneal tissues were retrieved [62], with almost 2,000 donations retrieved from the Veneto Eye Bank Foundation [10]. Therefore, optimum storage conditions to reduce the number of tissue discard rates and increase the long-term survival rate becomes the next big challenge. Alternative transplant options and cultured cell transplants have also been identified in order to reduce the overall demand of the corneal tissue.

8. Expert opinion

Most of the studies suggested that corneas stored up to 14 days in Optisol-GS or its alternatives does not significantly affect the ECL or survival after PK or EK surgeries. HS media help in corneal tissue storage, endothelial cell survival and post-operative re-epithelialization, and therefore it is the choice of storage in most of the countries as it is not labor intensive or have huge associated costs [32,34,37]. Although contradictory, shorter storage time could be considered when the tissues are deemed for selective transplant options. However, lack of optimum antibiotics/antifungal agents compared to OC may increase the postoperative infection rate, which is under consideration for further development of HS [40,52].

No significant ECL, VA or graft quality issues using the OC media were observed when the tissues were grafted for PK [34], DALK or DMEK surgeries [43,50]. It was observed that tissues retrieved from old-aged donors (>80 years) may affect the visual recovery and therefore must be re-considered as a factor for tissue retrieval. Shortening the postmortem interval and storage time could potentially reduce the risk of LEF [48]. Although contamination rate with OC stored tissues are

lower compared with HS, it is still a reason of concern with in situ excision of tissues. The use of OC is safe for corneal tissue, preserves cellular integrity and has a positive influence on the patient's visual outcomes. Synthetic OC media will be of significant value further reducing any potential issues with xeno-transplantation in the future.

Cryopreservation of corneas is subject to consensus due to the negative influence on endothelial cells and stromal keratocytes. Studies suggest that cryopreservation destroys the tissue structure [60,61]. Presented clinical studies suggest that cryopreservation does not influence the VA. Comparison of fresh corneas with cryopreserved corneas at -80 [33] and lyophilized/cryopreserved corneas vs corneas stored in Optisol-GS showed almost the same graft quality and visual outcomes [42]. Therefore, such grafts can be used for emergency procedures but it is unlikely that these grafts could be used for routine transplantation purposes.

One of the major global challenges is not in terms of the availability of the eye-banking facilities or surgeons, but the number of corneal tissues required for transplantation. Although new and advanced techniques in corneal banking provide the possibility of obtaining two grafts for different surgeries from one corneal tissue, it still does not fulfill the global need [7]. Therefore, modern techniques and procedures have been developed where one tissue can be used for at least two or more patients. This is usually carried out by selective transplant procedures such as DALK, DSAEK, or DMEK where the required layer is separated from the tissue and transplanted as two different grafts. More recently, Hemi-DMEK has also been introduced where only Descemet membrane-endothelial complexes of a tissue are further divided into two equal halves and transplanted in two different patients [63]. With the modern eye-banking facilities, tissue preparations for challenging surgeries like DSAEK and DMEK have been standardized and prepared in the eye banks as pre-stripped or pre-loaded tissues that are ready-to-use for transplantation purposes. Therefore, formulations to preserve these tissues for a longer period of time would be beneficial for planning the surgery and would open up long-distance transportation options without the need of local storage. Although the clinical findings of already available storage options have shown positive outcomes, it is important to improve these formulations for optimum long-term storage. To overcome the limitations of corneal tissue donations worldwide, alternative options such as culturing the endothelial cells in vitro and transplanting them as sheets or injecting as cell suspension are being taken into consideration. The necessity to design methods or alternative options that would reduce the dependence on donor tissues by culturing cells in vitro is one of the highly acclaimed research topics [24]. Recently, Kinoshita et al. reported the successful injection of cultured corneal endothelial cells into the patient's eyes. However, a longer patient follow-up would determine the outcomes of this technique, which has a potential to revolutionize the corneal transplantation field [64]. Therapies involving Rho kinase (ROCK) [65] and p38 Mitogen-Activated Protein Kinase (MAPK) [66] have been reported. Tissue-engineered scaffolds could also be an interesting alternative, which still needs to undergo a successful human clinical trial

[67,68]. Once the cells are cultured in the laboratory, these cells should be maintained viable and preserved until transplantation. This will be the next big challenge when long-distance transportation of the cultured cells will be considered or a formulation that will be used as an off-the-counter drug for the treatment of endothelial dysfunction. Thus, future challenges are supposed to focus completely on enhancing HS and OS outcomes, find alternative solutions to culture, and preserve endothelial cells to further reduce the global demand of donor tissues.

9. Method of literature search

The papers were searched on PubMed using the following keywords – Cornea; eye bank; storage; preservation; hypothermic; organ culture; cryopreservation; lyophilization; clinical outcomes or cross combination between them. Those studies that indicated the clinical outcomes were selected and further reviewed.

Acknowledgments

The study was funded through a grant of the Italian Ministry of Health (Bando Ricerca Sanitaria Finalizzata 2016) to the project “Pre-loaded tissues for Descemet Membrane Endothelial Keratoplasty: a new approach to overcome the challenges in posterior corneal surgery” (PI: Diego Ponzin; CUP: E46C18000340001).

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

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