Antimicrobial Effect of Cryopreservation on Donor Ocular Tissue

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Purpose: The aim of this study was to assess the antimicrobial effect of cryopreservation on donor globes with a previously positive culture. More specifically, our study aims at determining whether microbial organisms can still be cultured after cryopreservation in previously culture positive donor whole globes.

Methods: This is a prospective quality assurance study of microbiological cultures using donor ocular tissues obtained by the Lions Eye Bank of Manitoba and Northwestern Ontario from January 2009 to January 2010. Enucleated globes were soaked in 2.5% povidone iodide for 5 minutes, rinsed with sterile normal saline, and cultured in chocolate and Sabouraud agar and thioglycolate broth. The whole globes were then preserved in Optimyxin Plus and an antibiotic solution before being cryopreserved for 1 month. Culture-positive whole globes were thawed to room temperature and recultured on the same media to determine the effect of the cryopreservation protocol of our eye bank on bacterial counts.

Results: Twenty-seven donor whole globes were included in our study. Upon primary culture, all specimens had positive bacterial growth. The most common isolate on primary culture was coagulase-negative Staphylococcus (62.8%). Upon secondary culture of the thawed cryopreserved whole globes, no bacterial growth was detected on any of the culture media.

Conclusions: Our study demonstrates that harvested donor whole globes with positive microbial cultures became culture negative after secondary culture by the Lions Eye Bank of Manitoba and Northwestern Ontario's cryopreservation protocol. This suggests that ocular tissues treated in this manner may be microbiologically safe and therefore able to be used for transplantation in patients.

Key Words: cryopreservation, donor ocular tissue

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One of the most feared complications of transplantation of donor ocular tissues is postoperative infection. Contamination of these tissues may occur anytime during the harvesting process from enucleation to preservation, during storage, and at the time of transplantation.[1] Sources of contamination are contaminated storage solutions, the patient's endogenous microbial flora, and intraoperative surgical contamination.[2–4]

Cryopreservation is the only available method permitting the storage of ocular tissues for an indefinite period.[5–7] Transplantation of human corneas that have been cryopreserved has been successful in both the short term[8–10] and long term.[11] Cryopreserved corneal tissue affords long-term graft durability[12] and has been used in deep anterior lamellar keratoplasty[13] and as a tectonic graft for perforations caused by trauma[14] and foreign bodies.[15,16] In light of the variable and unpredictable rates of endothelial cell loss seen after cryopreservation,[17] these corneas are most useful in emergency situations when tissue availability is a problem. These include therapeutic keratoplasty secondary to infectious keratitis,[18–20] autoimmune disease causing corneoscleral melt[21] such as in rheumatoid arthritis[22,23] and Mooren ulcer,[24] and corneoscleral melt after pterygium surgery.[25] Human donor sclera is routinely used in glaucoma surgery to cover drainage implants.[26]

The purpose of this study is to assess the antimicrobial effect of cryopreservation on donor globes with a previously positive culture. More specifically, our study aims at determining if microbial organisms can still be cultured after cryopreservation in previously culture-positive donor whole globes. It is critical to determine whether donor ocular tissues remain microbiologically active after the storage process before implanting the tissue into a patient's eye.

MATERIALS AND METHODS

All donor ocular tissues obtained by the Lions Eye Bank of Manitoba and Northwestern Ontario from January 2009 to January 2010 were examined for inclusion in our study. Whole globes from humans were procured per the standards and procedures of the Eye Bank Association of America and the Lions Eye Bank of Manitoba and Northwestern Ontario. There were no age or gender eligibility restrictions and no limitations on the health of the donor other than preexisting ocular infections. The standard practice at our eye bank was to perform microbial testing on all globes before preservation. In this study, any whole globes with positive microbial cultures were included. This included all donated tissues intended for educational purposes only and those that were otherwise deemed unusable for scleral or

corneal transplantation. An exclusion criterion was any active ocular infection at the time of donor death regardless of whether treatment was received before or at the time of demise. Any ocular tissues that may present any risk of communicable disease were likewise excluded. The Institutional Review Board of the University of Manitoba approved this study.

Cryopreservation Protocol

Enucleation was carried out according to the standard procedures of the Eye Bank Association of America. After enucleation, all globes were brought to the eye bank and prepared for storage and plating by the same technician under aseptic culture techniques, including the use of a sterile gown and gloves and a microbiology hood. The whole globes were placed in a sterile container and soaked for 5 minutes in a solution of 2.5% povidone–iodide. Next, the globes were transferred to a second sterile container filled with normal saline and rinsed. They were then removed using sterile forceps, and swabs for microbial cultures were taken at the limbus and plated on chocolate and Sabouraud agar plates and in thioglycolate broth and were allowed 3 days of aerobic incubation at 37°C. The chocolate agar plate was stored in a carbon dioxide foil pouch to allow for the growth of fastidious organisms. The whole globes were then placed into a sterile bottle containing a preservation solution of Optimyxin Plus (Sandoz, Quebec City, Quebec, Canada) and Polymyxin B 10,000 U, neomycin 2.5 mg, gramicidin 0.025 mg, and benzalkonium chloride 0.005%. The tissue was then stored for a minimum of 1 month in a temperature-controlled freezer between −16°F and −18°F. After 30 days, each globe was allowed to thaw to room temperature on its own, without any intervention and cultured using the same 3 media as indicated above.

TABLE 1. Donor Causes of Death

Cause of Death	No. Patients (%)
Cancer	9 (33.3)
Lung	4
Pancreatic	2
Breast	1
Prostate	1
Gastric	1
Heart disease	8 (29.6)
Congestive heart failure	2
Cardiomyopathy	2
Myocardial infarction	2
Ischemic heart disease	1
Cardiogenic shock	1
Other	
Cirrhosis	3 (11.1)
Chronic renal failure	2 (7.4)
Renal cyst	2 (7.4)
Alzheimer disease	1 (3.7)
Cerebrovascular accident	1 (3.7)
Motor vehicle accident	1 (3.7)
Total	27

TABLE 2. Specimen Collection Details

Factor	Mean, h	Median, h	Range, h
Death to enucleation	6.11	5.5	2–13.5
Enucleation to preservation	8.18	6.25	1.25–23
Death to preservation	14.29	11.75	4.25–35.7
Refrigeration time (after enucleation, before culture)	2.65	0	0–11.5

RESULTS

Over a 1-year period, 27 donor globes were analyzed for culture: 7 bilateral donors and 14 unilateral donors. The most common cause of donor death was cancer (including lung, pancreatic, breast, prostate, and gastric) in 33.3% and heart disease (including congestive heart failure, cardiomyopathy, and myocardial infarction) in 29.6%. Other causes of death are specified in Table 1. The average age of the donors was 69 years (SD, 12.2). The majority of the globes (89%) were harvested within 12 hours from donor death; the remainder were harvested within 24 hours. Details regarding specimen collection are given in Table 2. Globes were cryopreserved for a mean time of 1.44 months (SD, 0.89).

Upon primary culture, all samples were found to have positive growth. The most common type of bacteria isolated from the donor whole globes was coagulase-negative Staphylococcus (47.1%), which is consistent with the findings in the literature.[1,27–29] Other bacterial isolates are shown in Table 3. Table 4 shows the sensitivities of the initial isolates to the various antibiotics tested. Microbial isolates were sensitive to all the antibiotics tested, except for coagulase-negative Staphylococcus and *Staphylococcus aureus*, which exhibited resistant strains. Upon secondary culture of the donor whole globes, all culture media were observed to be negative; no microbial organisms were isolated in all the samples after cryopreservation.

TABLE 3. Spectrum of Bacterial Isolates From Initial Donor Culture

Type of Isolate—Primary Culture Report	No. Isolates (%)
Gram-positive cocci	
Unspecified	3 (8.8)
Staphylococcus species (unspecified)	1 (2.9)
Coagulase-negative Staphylococcus	16 (47.1)
Staphylococcus aureus	5 (14.7)
Staphylococcus epidermidis	2 (5.9)
Micrococcus	1 (2.9)
Enterococcus	1 (2.9)
Gram-positive cocci in clusters	
Unspecified	2 (5.9)
Gram-positive rods	
Corynebacterium species	1 (2.9)
Bacillus species	1 (2.9)
Gram-negative rods	
Escherichia coli	1 (2.9)

TABLE 4. In Vitro Antibiotic Sensitivity Pattern of Bacteria Isolated on Primary Culture

Type of Isolate	Total N (%)	Gentamicin (%)	Moxifloxacin (%)	Tobramycin (%)	Vancomycin (%)	Cefazolin (%)	Ciprofloxacin (%)
Gram-positive cocci (unspecified)	3	1/3 (33.3)	NT	1/3 (33.3)	1/3 (33.3)	1/3 (33.3)	NT
Staphylococcus species (unspecified)	1	1/1 (100)	NT	1/1 (100)	1/1 (100)	NT	NT
Coagulase-negative Staphylococcus	15	10/15 (66.7)	5/15 (33.3)	8/15 (53.3)	10/15 (66.7)	10/15 (66.7)	NT
Staphylococcus aureus	5	4/5 (80)	4/5 (80)	4/5 (80)	4/5 (80)	4/5 (80)	NT
Staphylococcus epidermidis	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	NT
Micrococcus	1	NT	NT	NT	1/1 (100)	1/1 (100)	NT
Enterococcus	1	1/1 (100)	NT	NT	NT	NT	1/1 (100)
Gram-positive cocci in clusters (unspecified)	2	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	NT
Corynebacterium species	1	1/1 (100)	NT	NT	NT	NT	1/1 (100)
Bacillus species	1	NT	NT	NT	NT	NT	NT
Escherichia coli	1	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	NT
	33 (100)						

Type of Isolate	Ceftazidime (%)	Clindamycin (%)	Ceftriaxone (%)	Erythromycin (%)	Penicillin (%)	Unable to Determine Sensitivities (%)
Gram-positive cocci (unspecified)	NT	NT	NT	NT	NT	2/3 (66.7)
Staphylococcus species (unspecified)	NT	NT	NT	NT	NT	1/1 ((100)
Coagulase-negative Staphylococcus	NT	NT	NT	NT	NT	4/15 ((26.7)
Staphylococcus aureus	NT	NT	NT	NT	NT	1/5 (20)
Staphylococcus epidermidis	NT	NT	NT	NT	NT	
Micrococcus	NT	NT	NT	NT	NT	
Enterococcus	NT	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
Gram-positive cocci in clusters (unspecified)	NT	NT	NT	NT	NT	
Corynebacterium species	NT	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	
Bacillus species	NT	NT	NT	NT	NT	1/1 (100)
Escherichia coli	1 (100)	NT	1/1 (100)	NT	NT	

Note: Unable to determine sensitivities because of a small number of organisms present in culture media.
%, Percentage of bacterial isolates; n, total number of bacterial isolates; N, number of bacterial isolates; NT, not tested.

DISCUSSION

Cryopreservation has allowed for the virtually indefinite storage of ocular tissues. This is of paramount importance given the paucity of donors faced by most eye banks. Given the obvious deleterious implications of transplanting infected ocular tissues into a patient who will subsequently undergo local immunosuppression in the form of topical steroids, it is important for eye banks to try to achieve sterility in the ocular tissues they distribute.

We have shown that a significant number of whole globes are culture positive after treatment with povidone–iodide, the only agent proven to decrease bacterial counts and the risk of developing endophthalmitis in patients undergoing an intraocular surgery.[30] These whole globes would not be eligible for immediate transplantation without further processing. In this proof-of-concept study, we have demonstrated that all povidone iodide–treated donor whole globes that remained culture positive had negative microbial cultures after undergoing our cryopreservation protocol.

Although culture sampling bias may have been a factor in our results, we tried to minimize interobserver variability by having the same technician perform the cultures before and after cryopreservation. Another weakness of our study was our small sample size of 27 whole globes. In light of our highly encouraging results, we recommend that further studies be undertaken with greater numbers.

In conclusion, we report a cryopreservation protocol that is adept at eliminating microbial counts in ocular tissues, potentially rendering these tissues safe for transplantation. We hope that this will be beneficial in minimizing the amount of ocular tissue waste at eye banks and that these results will reassure clinicians that tissue that is preserved using our method may be used in patients.

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