Increasing Povidone-Iodine Exposure in Endothelial Keratoplasty Tissue Processing and Fungal Infection Impact

C. Drew Salisbury, MD, Carter N. Kirk, MD,† W. Barry Lee, MD,*‡ Stephen M. Hamilton, MD,* Alan M. Kozarsky, MD,*‡ Eric Meinecke, CEBT,‡ and R. Doyle Stulting, MD, PhD‡§*

Purpose: To evaluate the effect on donor rim cultures and postoperative infections of doubling the povidone-iodine exposure time during corneal tissue recovery before its use in keratoplasty.

Methods: Consecutive donor cornea recoveries were evaluated for positive donor corneal rim cultures and postoperative infections before and after a protocol change of doubling the exposure time of povidone-iodine during donor preparation.

Results: In 631 consecutive cornea donor recoveries, 18 (2.9%) had positive fungal rim cultures and 41 (6.5%) had positive bacterial rim cultures. Three (0.48%) developed postoperative fungal infections, and no bacterial infections occurred. After doubling the povidone-iodine exposure time during the recovery process, 725 consecutive corneas were reviewed. Four (0.6%) had positive fungal rim cultures, and 29 (4.0%) had positive bacterial rim cultures. No postoperative fungal or bacterial infections occurred. No noticeable increase in epithelial toxicity developed between the 2 groups.

Conclusions: Increasing the povidone-iodine exposure time during the donor cornea recovery process decreased the rate of positive donor corneal rim fungal cultures ($P = 0.001$), positive donor corneal rim bacterial cultures ($P = 0.04$), and postoperative fungal infections ($P = 0.06$).

Key Words: povidone-iodine, betadine, endothelial keratoplasty, fungal keratitis, fungal endophthalmitis, cornea tissue recovery, DMEK, DSAEK, corneal transplant, eye banking

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Fungal infection after corneal transplantation is a rare event that occurs at a reported rate between 0.014% and 0.023% for all keratoplasty procedures.[1–3] Despite this low rate, there has been a nonstatistically significant trend of increasing rates of fungal infections with keratoplasty over the past 10 years.[3] This trend correlates with the increasing popularity of endothelial keratoplasty (EK), which has increased from 1429 cases in 2005 to 30,336 cases in 2018.[4] Donor rim cultures for EK-processed eyes are 3 times more likely to be positive for fungi when compared with processed tissue for other uses. The higher rates of positive donor rim cultures during EK are speculated to be secondary to the extended tissue warming time during donor tissue preparation at the eye bank.[5]

The rate of positive donor rim cultures for fungi has been reported to be between 0.42% and 2.1% for all forms of corneal transplants.[5,6] There is a significant correlation of positive fungal donor rim culture results with the development of subsequent clinical keratitis or endophthalmitis, indicating that the source of these clinical infections is contamination of the donor tissue.[1,7] The development of clinical fungal keratitis or endophthalmitis has been shown to occur in 5.6% to 13.5% of the transplanted corneal tissues that have positive donor rim cultures.[5,6]

A similar trend of increasing fungal keratitis and/or endophthalmitis after EK was observed with tissue supplied from a single large eye bank in the Southern United States (Fig. 1). All cases of positive fungal rim cultures and clinical infections from tissue prepared by this eye bank were investigated. There was no correlation between these cases and higher death to preservation times, cause of death, or cooling of body times. In an attempt to decrease the rate of positive donor rim cultures, the medical advisory board at this specific eye bank reevaluated their own tissue recovery process and specifically focused on the use of povidone-iodine solution during the recovery process.

The Eye Bank Association of America Medical Standards for tissue recovery (E1.100)[8] specifies that povidone-iodine solution must come in contact with the surface of any ocular tissue intended for transplantation; however, it does not specify the duration of contact or the percentage of povidone-iodine solution that must be used. The eye bank medical advisory committee implemented a change in the tissue recovery protocol that increased the duration of povidone-iodine usage during tissue recovery with the goal of decreasing positive fungal donor rim cultures and clinical infections.

METHODS

Institutional Review Board approval was obtained at Eye Consultants of Atlanta. Before October of 2016, the tissue recovery process at this single large eye bank consisted of a 5-minute soak of ocular and periocular areas with 5% povidone-

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From the *Cornea Service, Eye Consultants of Atlanta and Piedmont Hospital, Atlanta, GA; †Kirk Eye Center, Chicago, IL; ‡Georgia Eye Bank, Atlanta, GA; and §Woolfson Eye Institute, Atlanta, GA.
W. B. Lee and R. D. Stulting are unpaid co-medical directors for the Georgia Eye Bank. E. Meinecke is the President and CEO of the Georgia Eye Bank.
Correspondence: C. Drew Salisbury, MD, Cornea Service, Eye Consultants of Atlanta, 3225 Cumberland Blvd SE, Suite 900, Atlanta, GA 30339 (e-mail: cdsalis@gmail.com).

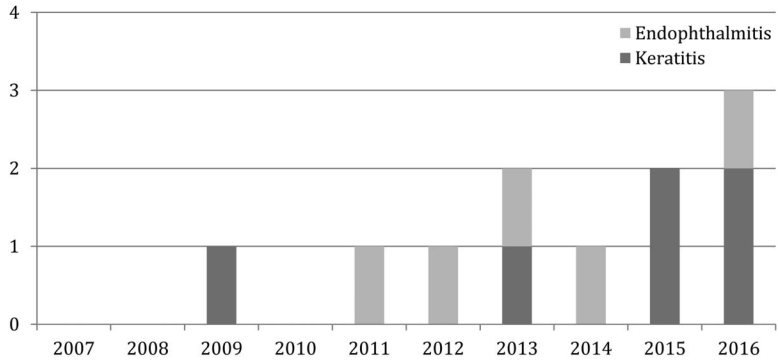

FIGURE 1. Ten-year trend of keratoplasty-associated fungal endophthalmitis and keratitis from tissue supplied by a single large eye bank.

iodine solution. In October of 2016, the tissue recovery process was changed by the eye bank's medical advisory committee to include 2 separate 5-minute soaks with 5% povidone-iodine solution separated by a 5-minute break in between the soaks. The povidone-iodine solution was irrigated from the ocular and periocular surfaces with eyewash after each soak. No other element of the tissue recovery process was changed.

Corneal transplants performed at 3 separate outpatient surgical centers that receive corneal tissue exclusively from this single eye bank were reviewed between the years 2015 and 2018. Corneoscleral rims of all transplanted tissues during this time were sent for bacterial and fungal cultures at Piedmont Atlanta Hospital or Emory Montgomery Laboratory. Consecutive donor cornea recoveries were evaluated for positive donor corneal rim cultures and postoperative clinical infections before and after the tissue recovery protocol change.

The primary outcome measures were the incidence of positive fungal rim cultures and the incidence of fungal endophthalmitis and/or fungal keratitis. The secondary outcome measures were the incidence of positive bacterial rim cultures and the incidence of bacterial endophthalmitis and/or bacterial keratitis. The recovered corneal tissues were evaluated at the eye bank under slit-lamp examination for any evidence of epithelial toxicity. The eye bank technicians were specifically monitoring for any increased signs of epithelial impact from the tissue recovery process and surgeons were monitoring for any delay in epithelial healing times of these transplanted tissues during the postoperative course. All primary graft failures of corneal transplantation before and after the protocol change that occurred between the years 2015 and 2018 at these outpatient surgical centers were recorded.

Statistical Analysis

The rates of positive fungal rim cultures and fungal endophthalmitis and/or fungal keratitis were calculated before and after the protocol change. In addition, the rates of positive bacterial rim cultures and bacterial endophthalmitis and/or bacterial keratitis were calculated before and after the protocol change. A χ^2 test was used to calculate the statistical significance of the change in rates of positive fungal rim cultures, fungal endophthalmitis and/or keratitis, positive bacterial rim cultures, and bacterial endophthalmitis and/or keratitis. Statistical analyses were conducted using Microsoft Excel, version 12.1.9 (Microsoft Inc, Redmond, WA).[9]

RESULTS

The total number of corneal transplants performed before the protocol change was 631. EK transplants represented 78% of these surgeries including 295 Descemet stripping automated endothelial keratoplasty and 198 Descemet membrane endothelial keratoplasty procedures. The remaining 138 surgeries consisted of penetrating keratoplasty (PKP) and deep anterior lamellar keratoplasty (DALK) procedures. The overall positive fungal rim culture rate was 2.9% (n = 18). Of the 18 donors that had positive fungal rim cultures, 17 were from EK-processed tissues (Table 1). The clinical fungal infection rate of endophthalmitis and/or keratitis was 0.48% (n = 3) before the protocol change. All 3 cases that developed clinical fungal infections occurred after EK procedures, and the donor rim cultures from all 3 cases grew *Candida albicans*. Two of these cases resulted in fungal keratitis, whereas 1 resulted in fungal endophthalmitis. All 3 of the cases required additional surgery, and 2 of them had subsequent poor visual outcomes with final best-corrected visual acuity of 20/400 and light perception vision only (Table 2). Corneal transplants with positive donor rim cultures developed clinical fungal infections at a rate of 16.67% since all 3 of the clinical infections originated from tissues that had positive fungal donor rim cultures at the time of surgery. The positive bacterial rim culture rate was 6.5%

TABLE 1. Breakdown of Positive Fungal Rim Cultures and Clinical Fungal Infections Based on the Type of Keratoplasty

Transplants Before Protocol Change	631	Positive Fungal Rim Cultures	Clinical Fungal Infections
DSAEK	295	12	2
DMEK	198	5	1
PKP	117	1	0
DALK	21	0	0
Transplants After Protocol Change	**725**	**Positive Fungal Rim Cultures**	**Clinical Fungal Infections**
DSAEK	284	2	0
DMEK	212	2	0
PKP	205	0	0
DALK	24	0	0

DMEK, Descemet membrane endothelial keratoplasty; DSAEK, Descemet stripping automated endothelial keratoplasty.

(n = 41), and the clinical bacterial infection rate of endophthalmitis and/or keratitis was 0.0% (n = 0) before the protocol change.

The total number of corneal transplants performed after the protocol change was 725. EK transplants represented 68% of these surgeries including 284 Descemet stripping automated endothelial keratoplasty and 212 Descemet membrane endothelial keratoplasty procedures. The remaining 229 surgeries consisted of PKP and DALK procedures. The positive fungal rim culture rate was 0.6% (n = 4). All 4 of the positive donor rim cultures were from EK-processed tissues. The clinical fungal infection rate of endophthalmitis and/or keratitis was 0.0% (n = 0) after the protocol change. The positive bacterial rim culture rate was 4.0% (n = 29), and the clinical bacterial infection rate of endophthalmitis and/or keratitis was 0% (n = 0) after the protocol change.

There was a statistically significant decrease in the rate of positive fungal rim cultures after the protocol change (*P* value = 0.001). The rate of clinical fungal infections decreased; however, it was not statistically significant (*P* value = 0.06). There was a statistically significant decrease in the rate of positive bacterial rim cultures after the protocol change (*P* value = 0.04). There was no change in the rate of clinical bacterial infections because there were no cases of bacterial endophthalmitis and/or keratitis before and after the protocol change (Table 3).

There were no increased signs of epithelial disruption of the recovered corneas after the protocol change based on slit-lamp examination of corneal donor tissue at the eye bank. Also, the participating surgeons did not notice a clinically significant increase in delayed epithelial healing after the protocol change. In addition, there were no reported primary graft failures of any corneal transplant procedure before or after the protocol change.

TABLE 3. All Corneal Transplants Performed at Three Outpatient Surgery Centers Between 2015 and 2018

Total Transplants Before Protocol Change	**631**	**Positive Culture (%)**	
Positive fungal rims	18	2.9%	
Clinical fungal infections	3	0.48%	
Positive bacterial rims	41	6.5%	
Clinical bacterial infections	0	0.0%	
Total Transplants After Protocol Change	**725**	**Positive Culture (%)**	***P***
Positive fungal rims	4	0.6%	0.001
Clinical fungal infections	0	0.0%	0.06
Positive bacterial rims	29	4.0%	0.04
Clinical bacterial infections	0	0.0%	

DISCUSSION

Doubling the povidone-iodine exposure time from 1 to 2 separate 5-minute soaks with 5% povidone-iodine solution during corneal tissue recovery through 725 corneal transplants significantly decreased the positive fungal and bacterial donor rim culture rates. More importantly, the change in protocol resulted in a zero-percent clinical fungal infection rate. The decrease in clinical fungal infection rate was not statistically significant (*P* value = 0.06) because there were very few clinical fungal infections (n = 3) before the protocol change. Although statistical significance was not proven, this study did show that EK-related fungal infections can result in significantly poor visual outcomes as seen in 2 of the 3 patients who developed infections before the protocol change. It is important to note that all of the clinical fungal infections developed from EK-processed tissue and all of the 22 positive fungal donor rim cultures except for 1 case were from EK-processed tissue. Because the number of EK procedures being performed around the world continues to increase, tissue recovery, processing, and storage methods should be modified to minimize the risk of EK-associated positive fungal donor rim cultures and clinical fungal infections.

TABLE 2. Description of 3 Clinical Fungal Infections Before Protocol Change

Clinical Fungal Infections	**Time to Diagnosis**	**Additional Surgery**	**Final Postop VA**
Case 1: Keratitis (C. albicans)	3.5 months	PKP	20/50
Case 2: Endophthalmitis (C. albicans)	6 days	EK removal/ DSAEK/RD repair	20/400
Case 3: Keratitis (C. albicans)	3.5 months	EK removal/PKP/ CPC	Light perception

CPC, cyclophotocoagulation; RD, retinal detachment; VA, visual acuity.

There was a concern from the eye bank medical advisory committee that increasing the duration of povidone-iodine exposure during tissue recovery could lead to epithelial toxicity and subsequent primary graft failures after transplantation. The protocol change included irrigation of the povidone-iodine solution from the ocular surfaces after each soak and a 5-minute break in between each soak to limit the amount of potential epithelial toxicity caused by the increased soaking time. There was no increase in clinically significant epithelial toxicity based on slit-lamp examination of the corneal donor tissue at the eye bank, and clinical observation of postoperative epithelial healing times were normal after the PKP and DALK procedures. Persistent epithelial defects after keratoplasty can lead to primary graft failures, so it is important to note that there were no primary graft failures of any corneal transplants after the protocol change.[10,11] This indicates that the potential epithelial toxicity caused by the increased duration of povidone-iodine solution exposure was not significant enough to affect corneal transplant viability. Despite these observations, the biggest weakness of this study is that epithelial changes were not objectively measured at the eye bank or during the postoperative course. Potential epithelial toxicity concerns that can occur during the tissue recovery process will become less impactful as the trend of EK surgery continues to grow because epithelial defects have not been shown to contribute to graft failure in EK procedures.[11] Given that there were no clinically significant delays of epithelial healing in PKP and DALK transplant tissues and no primary graft failures, the benefit of reducing postoperative fungal infections outweighs the risks of

having subclinical epithelial toxicity from increased povidone-iodine exposure during tissue recovery.

Given the documented trend of increasing rates of keratoplasty-associated fungal infections over the past decade, there have been significant efforts to develop a safe and effective antifungal storage medium. Adding antifungal prophylaxis to storage media could be a viable option in the near future, but the safety and efficacy of these products are still being investigated, and these products will only add to the already increasing cost of performing corneal transplants. This study outlines a cost-effective measure that can be instantaneously implemented by eye banks around the world to decrease the rate of positive fungal and bacterial donor rim cultures of recovered corneal tissue. Eye banks should evaluate their tissue recovery processes and consider increasing the duration of povidone-iodine exposure as outlined in this study. Increasing the povidone-iodine solution exposure time can significantly decrease the positive fungal and bacterial rim culture rate, which can subsequently decrease the rate of clinical fungal and bacterial infections that are associated with corneal transplantation.

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