

Effects of Povidone-iodine Dissolved in the Cornea Storage Media on Endothelial Cell Health

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ABSTRACT

Introduction: Fungal keratitis and endophthalmitis are uncommon but devastating complications of corneal transplants, however no current FDA-approved cornea storage media contain antifungal coverage.

Objectives: To examine the effects of various concentrations of povidone-iodine (PI) dissolved in cornea storage media on endothelial cell health.

Methods: Donor corneas deemed fit for research but unfit for transplant were examined with light microscopy and 14 corneas of high quality were chosen for inclusion. Optisol GS was used as the cornea storage media. Two corneas were randomly selected as controls, and the remaining 12 were evenly distributed among four different concentrations of PI obtained through serial dilutions of an original 10% PI solution. Backlight microscopy¹² and specular microscopy were obtained and compared with similar studies performed at 24 hours, 48 hours, and 7 days following placement of the corneas in the PI solutions.

Results: Specular microscopy of all four PI concentrations tested was unable to detect individual endothelial cells as a result of PI toxicity. Significant corneal haze developed in all four concentrations as early as the 24 hour mark. Betadine crystals were noted on both backlight microscopy and specular microscopy in the 1% PI and 0.75% PI specimens.

Conclusions: Although povidone-iodine may be safe and effective at decreasing fungal contamination, the toxicity of this medication to the corneal endothelium as noted in this study indicates that it is not a safe additive to cornea storage media.

Key words: Betadine, Cornea, Endothelium, Povidone Iodine

Fungal keratitis and fungal endophthalmitis¹ are rare but potentially devastating complications of corneal transplants. Given the frequency with which these procedures are performed, it is essential to minimize the risk of these complications. There are currently four FDA-approved corneal storage media available for use: Optisol-GS, Eusol-C, Life 4C, and Cornea Cold Storage.

None of these provide antifungal coverage. Amphotericin B can be added as a supplement but this is not the standard protocol, and there is limited data investigating the efficacy and safety of other medications when dissolved in Optisol-GS. The addition of various concentrations of voriconazole and amphotericin B to corneal storage media have shown promise in some studies,^{2,3} but they have not been proven to be a perfect solution to date.

Studies have shown that corneoscleral rims used in penetrating keratoplasty (PKP) that test positive for fungal elements are significantly more likely to result in fungal infection in the recipient eye,⁴ and that there is an overall 0.12-0.16% incidence of fungal infections following PKP.^{4,5} It has also been shown that donor rim culture results for endothelial keratoplasty-processed eyes are three times more likely to be positive for fungi than corneas used for other purposes.⁶ Given the increasing frequency of endothelial keratoplasty procedures relative to penetrating keratoplasties, this places increased importance on decreasing the risk of fungal infections through contaminated donor tissue.

Increasing the povidone-iodine (PI) exposure time during preparation of the donor cornea has been described to decrease the incidence of rim cultures positive for fungus. Furthermore, doubling the PI exposure time additionally decreased the percentage of positive rim cultures and trended towards significantly decreasing the percentage of postoperative fungal infections without increasing epithelial toxicity.⁷ Despite this, we did not identify any studies published to date looking at the effects of adding PI to the corneal storage media itself. Our study aims to examine the safety of various concentrations of PI dissolved in cornea storage media on endothelial cell health.

METHODS

Human donor corneas with healthy endothelium at the Lions Eye Institute for Transplant and Research in Tampa,

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FL were examined upon arrival. A selection of those that had been deemed fit for research purposes but not for transplant for a variety of reasons were examined with backlight microscopy. 14 of these donor corneal buttons were then selected for inclusion in the experiment. The control corneal storage media used in the experiment was Optisol-GS, the most commonly used corneal storage medium in the United States. Two of the selected corneas were used as controls and placed in the standard Optisol-GS solution. The remaining 12 corneas were then evenly distributed among the four experimental groups consisting of the following concentrations of PI: 1%, 0.75%, 0.5%, and 0.25%. These various concentrations of PI were obtained via serial dilution of an original 10% PI solution with Optisol-GS until the desired concentration of PI was reached. 20mL of each desired concentration was used to store the corneas.

Baseline testing consisted of backlight microscopy¹² and specular microscopy. Backlight microscopy was performed after staining with trypan blue and photos were obtained of all specimens. Backlight microscopy of all specimens then took place at 24 hours, 48 hours, and 7 days following initial placement in the betadine solutions and additional photographs were obtained for every specimen. Specular microscopy (Cell Check 10, Konan, Japan) was also performed. Endothelial cell density was recorded and representative images were captured. These same tests were then performed at 24 hours and 48 hours following initial placement in the PI solutions with additional photographs were obtained for every specimen. All testing was performed at the Lions Eye Institute for Transplant and Research in Tampa, FL. No statistical analysis was able to be performed.

RESULTS

The control corneas exhibited no appreciable increase in corneal haze on backlight microscopy (Figure 1). Images of the various experimental corneas are shown in Figure 2. These images demonstrate significant corneal haze that developed in all specimens beginning as early as 24 hours after placement in the Optisol-GS-povidone-iodine solutions and clearly worsening over the course of the 7-day experiment. This is evidenced in part by the fact that the endothelial defect (likely a result of tissue processing) clearly present at baseline in image (a) is barely visible after 24 hours in image (b) as a result of increasing corneal haze. The corneal haze was increasingly prominent with increasing concentrations of PI in the corneal storage media. There were no instances of new corneal endothelial defects occurring in any specimen. Crystalline structures (Figure 3) were noted to develop in specimens stored in the 1% and 0.75% PI media.



Figure 1. Control cornea stained with trypan blue at baseline (a) and after 24 hours in Optisol GS cornea storage media (b)

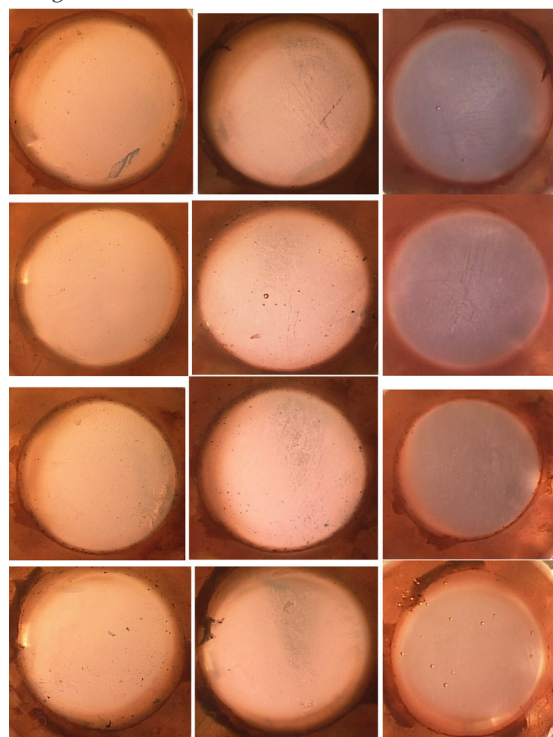


Figure 2. Representative backlight microscopy photos of experimental corneas stained with trypan blue. Top row: 1% PI cornea at baseline (a), 24 hours (b), and 7 days (c). Second row: 0.75% PI cornea at baseline (d), 24 hours (e), and 7 days (f). Third row: 0.5% PI cornea at baseline (g), 24 hours (h) and 7 days (i). Bottom row: 0.25% PI cornea at baseline (j), 24 hours (k), and 7 days (l). Notice the significant haze that develops in all concentrations as early as 24 hours after exposure to the various PI solutions

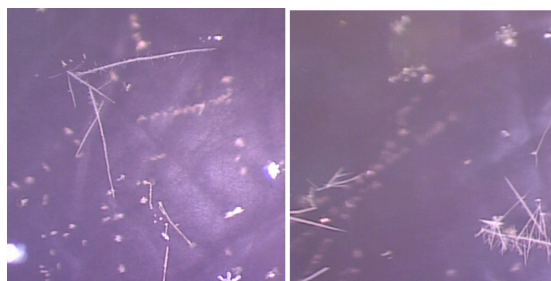


Figure 3. High-magnification backlight microscopy photos of PI crystals. Although etiology of crystals is unknown, the crystals only occurred in the 1% PI and 0.75% PI samples.

Specular microscopy demonstrated the mean endothelial cell densities shown in Table 1. There was no significant decrease in endothelial cell density after 24 hours in the control corneas (Figure 4). Endothelial cells were unable to be detected after 24 hours in any experimental specimens. Crystalline structures were identified (Figure 5) in specular microscopy in the 1% povidone-iodine and the 0.75% PI specimens.

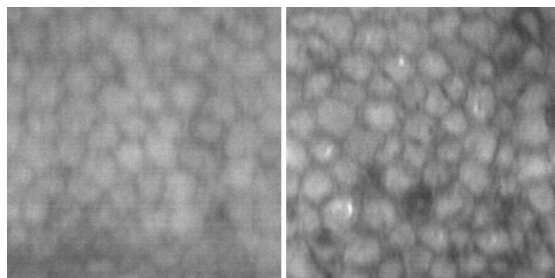


Figure 4. Specular microscopy of sample control corneas. No clinically significant difference between endothelial cell density at baseline (a) and at 24 hours (b).

Table 1

	Baseline (cells/mm ²)*	Day 1 (cells/mm ²)*	Day 2 (cells/mm ²)*
Control	2868	2771	Not tested
1% povidone-iodine	2367	Undetectable	Undetectable
0.75% povidone-iodine	2743	Undetectable	Undetectable
0.5% povidone-iodine	2620	Undetectable	Undetectable
0.25% povidone-iodine	2477	Undetectable	Undetectable

* Numbers represent the mean endothelial cell densities of all samples in a given group and listed as cells per square millimeter.

DISCUSSION

Povidone-iodine is an indispensable anti-septic which has shown to be an efficient antimicrobial against bacteria, virus, and fungus. Its excellent safety profile, broad spectrum coverage, and cost effectiveness underline its inclusion by the World Health Organization as an essential medication.⁸ It is routinely used preoperatively on the skin and eyes to provide a sterile field. Its mechanism of action works through iodination and oxidation of nucleic acids and amino acids thus destabilizing cellular membranes and resulting in permanent death of multiple organisms.⁹ With recent increases in corneal transplant being performed, as well as a parallel increase in fungal contamination of donor

tissues, there is interest in potentially using PI in corneal storage media to reduce fungal contamination. Our goal was to investigate the effect of betadine-infused corneal storage media on the health of corneal endothelium.

Several studies have looked at PI and its effect in bovine and porcine corneas both in vivo and in vitro studies. Current in vitro studies of bovine endothelium and its toxicity to diluted PI show that a 0.1% concentration or less does not induce any corneal endothelium dysfunction or toxicity as shown with specular microscopy.¹⁰ Additionally, PI concentrations of 5% and 10% confirmed severe corneal toxicity with immediate corneal haze when injected into rabbit anterior chambers.¹¹

Our study confirms that the same mechanism that makes PI an effective antimicrobial agent also makes it extremely toxic to corneal endothelial cells even at lower concentrations. In all four concentrations of PI we see severe corneal endothelial dysfunction within 24 hours from instilling it into the corneal storage media. We hypothesize that disequilibrium of the endothelial cell membrane likely resulted in an increase in cell permeability with a subsequent dysfunction of the endothelial cell pump function. With time, we noticed a violet hue to the corneal endothelium by 7 days. Interestingly, when we stained the corneas with trypan blue, the trypan blue did not detect a significant number of dead cells as evidenced by the lack of focal staining. The cell membranes appear to have remained intact. Further studies may be needed to investigate whether endothelial apoptosis or dysfunction results in cell loss. Higher concentrations of betadine resulted in betadine crystals that were evident on specular and backlight microscopy.

There are several limitations to our study design. We were limited by availability of corneal tissue, and thus could only test a limited range of concentrations of PI. We did not have the means to test the chemical reactions leading to the PI crystal content. It may have been useful to look at the specular microscopy of corneal tissue immediately or sooner than 24 hours to closely monitor the reaction soon after exposure to PI. The limited sample size and single center study were additional limitations to the study. Future direction for studies include investigating short PI rinse rather than storage, following up on PI exposure time, or investigating applications of amphotericin B and voriconazole with corneal storage media.

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