Assessing Environmental Contamination During Corneal Processing

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ABSTRACT

Purpose: This study was performed to assess the potential introduction of biological contamination during eye bank processing of corneal tissue, including femtosecond laser-assisted keratoplasty (FLAK) and endothelial keratoplasty (EK) tissue. The study was designed to determine if FLAK or EK processing could be performed without introducing bioburden or environmental contamination of the corneal tissue.

Methods: We evaluated the processing of FLAK tissue in a laser processing suite and EK processing in a laminar flow hood, following established Standard Operating Procedures (SOPs) for FLAK and EK. In-process cultures were taken from corneoscleral rims before and after tissue preparation using a femtosecond laser. Environmental and processing cultures were also taken from sterile soft contact lenses substituted for the corneoscleral disc during EK processing before and after processing. Environmental settling plates were taken before and after FLAK and EK processing of the corneal tissue.

Results: The processing cultures taken from the corneoscleral rims pre-and post-femtosecond laser processing and those cultures taken of the sterile soft contact lenses pre-and post-simulated femtosecond laser processing all resulted in no growth. The sets of cultures taken of the sterile soft contact lenses pre-and post-simulated EK processing resulted in no growth.

Conclusions: Results demonstrate that eye banks may reliably provide contaminant free FLAK and EK corneal tissue processed in an open-room or laminar flow hood. Aseptic technique as well as industry accepted corneal decontamination with povidone iodine and environmental monitoring are required. Sterile contact lenses may be a valid substitute in simulated processing of the corneoscleral disc during environmental and process validation studies.

Key Words: endothelial keratoplasty; femtosecond laser assisted keratoplasty; bacterial contamination; settle plates; processing; eye bank; laser suite; laminar flow hood.

New keratoplasty techniques for selectively replacing dysfunctional portions of the cornea have been mainstream for over a decade. Procedures such as Descemet's stripping automated endothelial keratoplasty (DSAEK), Descemet's membrane endothelial keratoplasty (DMEK) and deep anterior lamellar keratoplasty (DALK) have become the standard methods of keratoplasty. Additionally, femtosecond lasers and femtosecond laser-assisted keratoplasty (FLAK) have altered how penetrating keratoplasty is performed.

Traditionally, corneal surgeons received whole corneas or corneoscleral rims from eye banks and prepared that tissue for their PK and EK procedures using manually trephined or mechanically prepared corneas using a microkeratome in the operating suite. The new norm is for the eye banks to process this tissue using manual techniques, mechanical microkeratomes and femtosecond lasers to pre-cut tissue for the surgeon. This allows the eye bank to provide surgeons with custom, quality tissue saving the surgeons' valuable operating room time and a consistently processed tissue.

The additional processing of corneoscleral tissue, after recovery and placement into an appropriate storage solution, is defined as "processing" by the Eye Bank Association of America (EBAA).[1] Processing of corneal tissue is currently performed in a variety of environments including clean

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rooms, laser suites and laminar flow hoods. The EBAA Medical Standards are used as a guidance by eye banks and include standards to decrease the likelihood of contamination of processed donor tissue. The donor cornea, the recipient's endogenous flora, and airborne bacterial contamination during preparation of corneal tissue are all potential sources of microbial contamination not only at the eye bank but at the transplant surgery site that could potentially lead to corneal infection. The EBAA Medical Advisory Board has previously recommended that the environment where corneal tissue is processed be able to meet airborne contaminant standards of fewer than 25 colony-forming units (CFUs) per 90-mm settling plate per 1-hour exposure.[2]

Fortunately, the incidence of post-keratoplasty infection remain quite low.[3-5] Although endophthalmitis after penetrating keratoplasty is more likely with a culture-positive donor rim,[6] the rate of infection is much lower than the prevalence of positive cultures of donor tissue.[5,7,8]

As keratoplasty procedures continue to evolve, concerns have been expressed whether processing, which involves warming the tissue, additional handling, and contact with equipment, could result in an increased risk of microbial contamination and result in clinical infection. Rauen and colleagues[9] reported that the additional steps required in processing of "pre-cut" tissue by one eye bank did not increase the risk of microbial donor rim contamination compared to "no-cut" tissue provided by the same eye bank.

Several studies have shown that tissue processed by eye banks does not increase the risk of graft failure[10-12] or infection[4, 5] compared to surgeon-prepared tissue. Adverse events or decreased survival rates in endothelial keratoplasty (EK) have more commonly been associated with the recipient's clinical history, surgeon volume or experience, or other surgical factors, rather than with tissue processing or donor factors.[12-14] The purpose of this study was to determine whether mechanical and laser processing of corneal tissue for endothelial keratoplasty can be conducted within environments such as clean rooms, laser suites and laminar flow hoods without introducing bio-contaminants.

METHODS

Thirteen corneoscleral rims and twenty contact lenses were processed either mechanically (EK Study) or with a femtosecond laser (FLAK Study) at two different eye banks. For each of the 33 sampling events, the cornea or contact lens was swabbed pre-and post-processing (2 swabs), and settling plates were used to assess potential airborne bacterial contamination during processing of the tissue. All swabs and settling plates were evaluated for microbial growth.

FLAK study

In the femtosecond laser-assisted keratoplasty (FLAK) study, thirteen corneoscleral rims and 10 contact lenses were processed as partial-thickness grafts for anterior lamellar keratoplasty (ALK). The thirteen corneoscleral rims were recovered using aseptic technique and were preserved and stored in Optisol GS storage media (Bausch + Lomb) prior to the study initiation.

Sterile transport swabs were used to swab the surface of the corneas. In each case, tissue was warmed to ambient temperature and removed from the storage solution; the tip of the swab was moistened with inoculation media (saline), and the swab was placed in direct contact with the surface of the cornea prior to laser processing. The swabs were then inserted into a sterile transport tube, capped and placed into a specimen transport bag and shipped under ambient conditions to the testing laboratory.

All processing performed at eye bank number one was performed in a validated laser suite using an IntraLase femtosecond laser (Abbott Medical Optics), following a well-defined and written protocol. The laser suite was equipped with a dedicated (HVAC) system with air filtration via high-efficiency particulate air (HEPA) filters. Following the FLAK processing, a second processing culture swab was obtained and prepared for shipment in the same manner as the first.

Ten sterile, soft hydrophilic, balafilcon A contact lenses (PureVision, Bausch + Lomb) were also used in this study to simulate a sterile cornea. The contact lenses were not actually cut but all normal processing steps (swabbing, mounting on the artificial chamber, laser docking, etc.) were followed.

In each case, two settling plates were placed at the upper left and right corners at the edge of the sterile field where the tissue and contact lenses were processed. The settling plates were 90-mm-diameter with trypticase soy agar (TSA) medium. A total of forty-six plates were used: two for each of the twenty-three FLAK sampling events. The cover was removed, making sure not to touch the agar, and the plates exposed throughout the procedure, including preparation time before and after the procedure. After one hour, the settling plate was covered with the lid, labeled with necessary identification information and shipped in ambient temperature to the environmental testing laboratory.

EK Study

At Eye Bank number two, 10 sterile contact lenses were processed for EK using an automated Moria CBM microkeratome, following well-defined SOPs. Each step of

the procedure was followed using the contact lens as a surrogate including direct contact of the tonometer tip and pachymetry probe with the contact lens. A sterile field was established under a laminar flow hood with HEPA filtration, as per the eye bank's SOP. The contact lens surface and the top of the chamber cover were lubricated with irrigation solution. The microkeratome head was activated and passed over the contact lens to simulate the sectioning of a donor cornea. The pachymetry procedure was repeated to simulate obtaining the thickness of the remaining posterior portion of the graft bed. The contact lens was placed onto the sterile field and cultured a second time. After each procedure, the settling plates were closed. In all, twenty process culture swabs and 10 environmental settling plates were performed during the EK portion of this study.

Laboratory Analysis

The settling plates and swabs from both the FLAK and EK studies were sent to LABS Inc. in Centennial, CO for microbiologic analysis. Upon receipt, the plates and swabs were incubated aerobically at 30-35°C for 2-4 days, followed by incubation at 20-25°C for the remaining days for a total incubation time of seven days. All visible colony-forming units (CFUs) were counted. Spreading colonies were counted as one CFU. If growth was detected, the isolates were gram stained and observed for macroscopic colonial morphology. The results were reported as CFUs per swab or per settling plate based on exposure time.

Bacteriostasis and Fungistasis (B/F) testing were included in the laboratory analysis to ascertain if residual Optisol GS present on the cornea during processing would inhibit the bacterial growth that might be present or introduced during the corneal swabbing procedure. The results of the B/F test demonstrated that there is no bacterial growth retardation and that sufficient nutrients are present in the test media to promote growth of any organism that may be present on the swabbed surface regardless of residual Optisol GS. The test was performed by inoculating low levels of bacteria and fungi into the test media along with sample swabs. Once inoculated with the challenge organisms, the samples were incubated for five days. The growth of the challenge organisms and Optisol GS was compared to the growth in control vessels containing only growth media and the challenge organisms. The B/F testing performed demonstrated that trace amounts of Optisol GS present on the cornea during processing would not affect the microbiological tests performed. This B/F testing was performed by LABS Inc. in Centennial, Colorado.

RESULTS

The FLAK study culture results presented in Tables 1a and 1b, show that there was no microbial growth on any of the twenty-six corneoscleral rim or twenty contact lenses pre-and post-processing swabs. Colonies were identified and cultured from 4 of the forty-six settling plates. All CFUs were coagulase-negative staphylococci (CoNS). The mean environmental contamination of the settling plates was 0.4 CFU/hour of exposure. The EK study culture results are presented in Tables 2a and 2b. There was no microbial growth on any of the twenty-contact lens swabs pre-and post-processing, or on any of the 10 settling plates.

***Table 1a**: FLAK study settling plate culture results*

Settling Plates in FLAK study	
Left (90mm) n=23	Right (90mm) n=23
3 CFUs	1 CFU
all CFUs are of coagulase-negative staphylococci (CoNS)	
Colonies (90mm)	Hours
4	34.5
0.1 CFU/hour of 90mm	

***Table 1b**: FLAK study corneal swab culture results*

Swab of Cornea/Lens	
(pre-process) n=23	(post-process) n=23
0	0
Colonies per swab	
0	0
0.0 CFU/procedure	

***Table 2a**: EK study settling plate culture results*

Settling Plates in EK study	
Next to field (90mm) n=10	
0 CFUs	
Colonies (90mm)	Hours
0	7.5
0.0 CFU/hour of 90mm	

***Table 2b**: EK study soft contact lens swab culture results*

Swab of Soft Contact Lenses	
(pre-process) n=10	(post-process) n=10
0	0
Colonies per swab	
0	0
0.0 CFU/procedure	

When the Bacteriostasis and Fungistasis testing was performed it showed that the residual Optisol GS present on the cornea would not interfere with potential bacterial growth that may be present on the cornea and captured by a swab during corneoscleral rim culturing. It was demonstrated Optisol GS would not mask the

growth of bacteria even with presence of the antibiotics in Optisol GS. The swabs of the donor cornea were taken from two different locations to prevent sampling error.

DISCUSSION

The culture results of the corneoscleral rims demonstrated no growth, even though the presence of normal ocular flora on the non-sterile prepped donor corneas might have been expected. The Bacteriostasis and Fungistasis testing showed that residual Optisol GS present on the cornea at the time of processing would not interfere with potential bacterial growth. The swabs of the donor cornea were taken from two different locations to prevent sampling error.

There was minimal growth on the settling plates; settling plates were not exposed to corneal storage media. The total contamination of the settling plates, 0.4 CFU/hour of exposure, was well below the EBAA standard of < 25 CFUs per 90-mm settle plate per 1-hour exposure for processing of tissue. This study confirmed that a variety of environments including processing suites and laminar flow hoods are acceptable environments for processing.

The use of environmental settling plates in this study are an important component in evaluating the potential presence of bioburden due to possible contamination as a result of the movement of operators, work activity, and the environment during endothelial keratoplasty processing. Passive sampling with settling plates has previously been shown to be an appropriate, easy and inexpensive way to obtain a representation of the contamination that could be expected to settle from the air at the sampling site.[15, 16]

The use of sterile soft contact lenses as a substitute for non-sterile tissue was also an important element in this study. Starting with a sterile contact lens rather than a non-sterile cornea is an effective way to identify potential bioburden introduced by the eye bank's processing procedures. This study could potentially be repeated with corneas that have been irradiated making them sterile[17]. The future use and validity of this approach may need additional investigation.

This study also demonstrated that eye banks can successfully process tissue for contemporary keratoplasty techniques using mechanical microkeratomes or lasers without introducing an unacceptable bioburden to the donor tissue. As keratoplasty techniques continue to advance, it is important that surgeons and eye banks perform procedures that ensure tissue quality that is free of contamination.

CONCLUSIONS

This study demonstrates that when appropriate pre-recovery decontamination is performed, and strict adherence to aseptic technique is maintained, eye banks can successfully process tissue following approved methodologies and techniques such as mechanical microkeratomes or femtosecond lasers in a number of processing environments without introducing an unacceptable bioburden to the donor tissue.

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