Confocal microscopy is a contemporary method of high-magnification imaging of the cornea.

White Light Confocal Microscopy of Preserved Human Corneas From an Eye Bank

Jacek P. Szaflik, MD

Purpose: To verify the possibility of using a commercially available ophthalmologic white light confocal microscope for imaging optical sections of donated corneas preserved at 4°C, especially for endothelial evaluation.

Methods: Sixteen corneas donated to the Warsaw Eye Bank but excluded from use in surgery for serological or morphologic reasons were examined using a Confoscan 3 confocal scanning microscope (Nidek Technologies, Padova, Italy) and a Konan Eye Bank KeratoAnalyzer specular microscope (Konan Medical, Inc.; Hyogo, Japan). Images of corneal structures were obtained, including epithelium, corneal nerves, stroma, and endothelium. The endothelial cell density was calculated for both microscopes, and the results were compared.

Results: For images obtained with the specular microscope, mean (±SD) endothelial cell density was 2168.6 ± 404.0 cells/mm²; for confocal microscope images, mean ± SD was 2090.9 ± 369.1 cells/mm². There was no significant difference between the methods.

Conclusions: White light confocal microscopy can be used for high-magnification imaging of corneas preserved in an eye bank with the 4°C method, and images obtained permit evaluation of endothelium. Although the quality of confocal microscopy images of eye bank corneas is generally lower than that achieved with in vivo examinations, and although technical adaptations are needed for easier and safer application to corneas intended for transplantation, confocal microscopy is a promising new tool for evaluation of collected corneas.

Key Words: cornea, confocal microscopy, eye banking

Corneas collected by eye banks are usually evaluated morphologically by slit-lamp and specular microscopy before surgery. The aim of this study was to determine whether confocal microscopy can be adopted for preserved corneas in eye banks and to compare endothelial cell density counts obtained with confocal and specular microscopy.

Generally, 2 types of confocal microscopes have been used for imaging corneal structures: histologic laser-scanning confocal microscopes and ophthalmologic in vivo white light confocal microscopy systems. Recently, a new laser-scanning confocal microscopy system for in vivo examinations was introduced in ophthalmology. Histologic laser-scanning confocal microscopes have been used successfully to study corneal structure, but because staining and most often physical sectioning of the cornea are needed, these microscopes are not suitable for evaluation of corneas in an eye bank.

White light confocal microscopy systems obtain real-time images of optical sections of the patient's cornea, causing no harm to in vivo tissue. In vivo white light confocal microscopy imaging of both physiologic cornea and patients with different pathologies, including dystrophies, keratitis, and others, has been described. Dawson et al applied white light confocal microscopy to postmortem corneas and showed that the technique can be efficient in imaging epithelial and stromal layers and post-laser-assisted in situ keratomileusis (LASIK) stromal deposits.

However, until now, there have been no reports of attempts to use white light confocal microscopy for endothelium evaluation of eye bank corneas, comparing results with those obtained with the technique currently most often used, specular microscopy.

Two techniques for preserving corneas in eye banks are most frequently used: storing at 4°C and organ culture. This study was carried out on corneas stored at 4°C.

MATERIALS AND METHODS

Materials for the study were 16 human corneas donated to the Warsaw Eye Bank but deemed unsuitable for transplantation because of either poor morphology, as evaluated by slit-lamp and specular microscopy (9 corneas), or positive serology for hepatitis B and/or hepatitis C virus (7 corneas). The experiments were performed between June 15, 2003, and June 30, 2004.

The corneoscleral buttons were collected from the donors and were preserved in Optisol GS medium (Bausch & Lomb, Rochester, NY) and stored at 4°C.
The donor age was from 28 to 76 years (mean, 53 years). Time from death to collecting tissue was from 2 to 8 hours (mean, 6 hours). The corneoscleral buttons were acquired directly from the donors and immediately placed in Optisol GS medium after collection; no enucleations were performed. Confocal microscopy examinations were performed 8 hours after collection of the corneas and on days 3, 5, 7, 14, and 21 after collection. In each case before the first confocal microscopy examination, specular microscopy was performed. To improve images, the corneoscleral buttons were put into a room temperature environment 30 minutes before examination. The confocal microscope used was the ConfoScan 3 white light scanning slit confocal microscope (Nidek Technologies, Padova, Italy), equipped with the standard ×40 magnifying water immersion front lens. For specular microscopy, the Konan Eye Bank KeratoAnalyzer (Konan Medical, Tokyo, Japan) was used.

The confocal microscope was set to manual working mode, allowing free control of movements of the microscope lens and in obtaining images.

The working distance between the front microscope lens and the visualized section of the cornea is ~2 mm and thus does not allow examination of the specimen in the original medium storage bottle. Therefore, at the time of examination, corneas were placed in a sterile, thin-walled, polyurethane examining chamber (bag), dimensions 50 × 30 mm, filled with the Optisol GS medium (Fig. 1). The volume of the medium used for examination was ~2–2.5 mL. The corneoscleral buttons were transferred between the medium storage bottle and the examination chamber in a sterile, aseptic way. The standard immersion gel Vidisic (Bausch & Lomb) was applied to the front lens of the microscope in the same manner as for in vivo examination. The day after the last examination, specimens were obtained from the Optisol GS medium for culture.
Endothelial cell density counts were performed manually and by the same skilled examiner for both specular and confocal microscopy at the time of the first examination (8 hours after tissue collection), and results were compared. The observers were masked as to cell counts from the first instrument. Wilcoxon signed rank test for paired samples was used to verify statistical significance in difference between methods.

RESULTS

The layers of the eye bank corneas in this study seemed similar to those viewed by in vivo confocal examination.

Eight hours after tissue collection, basal epithelium cells in most cases were seen as in in vivo studies (Fig. 2A). In corneal stroma, keratocyte nuclei were observed (Figs. 2B–D). At that time, stromal nerves were imaged in 15 (94%) corneas (Fig. 2C). Subepithelial plexus nerves, however, were successfully imaged only in 6 (38%) cases. Other findings included dark stromal striae (Fig. 2E) and Descemet membrane folds (Fig. 2F).

Visualized endothelium revealed deposits (Fig. 3A) and various endothelial lesions (Figs. 3B–D). On days 3 and 5, basal epithelium cells were hyper-reflective and the epithelium layer continuity was often disturbed. Entire keratocyte cell bodies (in place of usually imaged keratocyte nuclei) were visualized in ~40% of the corneas (6 corneas; Fig. 3E). Different stages of stromal edema were observed, from microcystic edema to general edema; imaging was similar to in vivo examinations. The corneal nerves including subepithelial fibers could be distinguished. The endothelium could be imaged in most cases, although for some corneas that developed heavy edema, it was necessary to place the cornea in the chamber with the endothelium facing the front lens of the microscope.

Stromal edema, both microcystic and general, was the main finding in examinations from days 7 to 21. However, the corneal nerves could be observed in the following examinations from 8 hours of storage up to day 21 in most cases (11 corneas; Fig. 3F).

The quality of endothelial images was found adequate with both specular and confocal microscopy (Figs. 3A–D and 4A–C). Mean (±SD) endothelial cell density counts were 2168.6 ± 404.0 cells/mm² for specular microscopy (for all 16 corneas) and 2090.9 ± 369.1 cells/mm² for confocal microscopy (for all 16 corneas), indicating no statistically significant difference between the methods (Wilcoxon test for statistical difference between endothelial cell density found with specular and confocal microscopy: P = 0.347).

DISCUSSION

The findings show that white light confocal microscopy might be an effective method for imaging optical sections of human cornea stored at 4°C, especially in the first 8 hours after tissue collection. The technique permits one to view the
Confocal microscopy could also facilitate evaluation of subepithelial and stromal opacities. This imaging technique makes easier and more certain differentiation between active inflammatory process and scar tissue or other opacities in those cases, because the inflammation is accompanied by the presence of infiltrative cells that can be clearly imaged by this method. This issue is especially important for opacities situated in peripheral cornea, which would not disturb optical performance of the graft anyway, whereas any suspicion that they can be due to active inflammation makes the corneoscleral button deemed unsuitable. In countries that have permanent shortage of cornea donors, like Poland, the possibility of additional evaluation of corneas through high-magnification microscopy has an essential value. Further extensive application of confocal microscopy to corneas from an eye bank might in the future result in the discovery of other, new factors important in transplantology.

Although no signs of microbial contamination of the corneas or medium were observed in this study and cultures proved negative, development of easier and safer technology for performing examinations, ideally with specimens remaining in the original medium storage bottle, would be helpful. Developing of special cell would be desirable to allow confocal endothelial counting sterilely, optimally with donor cornea viewed from the back for improving endothelial images. Also, the quality of stromal images would probably be improved with greater adoption of the white light confocal microscope by eye banks.

3-dimensional structure of the cornea during the examination with no need for physical sectioning or staining. Although generally of lower quality, the high-magnification, high-resolution images acquired seem comparable to the confocal images of human corneas examined in vivo. However, to the best of my knowledge, no presently commercially available corneal confocal microscopes are optimized for viewing of the corneas from an eye bank and performing endothelial cell counting as they are currently configured.

Although the endothelial cell density measurements are similar to those achieved with specular microscopy, confocal microscopy permits further assessment of corneal layers during the same examination, thus providing additional information regarding the status of corneal stroma and endothelium. Potentially, this feature might make white light confocal microscopy useful for evaluation of collected corneas before transplantation. Post-LASIK changes identifiable with confocal microscopy but often not certain during most commonly applied slit-lamp and specular microscopy examinations, might negatively influence keratoplasty outcomes.

**REFERENCES**


