European Journal of Ophthalmology / Vol. 17 no. 6, 2007 / pp. 891-896

In vivo confocal microscopy of corneal grafts shortly after penetrating keratoplasty

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PURPOSE. *To describe the microstructural status of corneal grafts shortly after penetrating keratoplasty (PK) and to evaluate the efficacy and safety of confocal microscopy in examining corneal grafts at that time.*
METHODS. *A confocal microscope with a 40x front lens was used to examine corneal grafts in 32 patients (32 eyes) 4 days after PK. Images were analyzed, and endothelial cell density counts were compared with presurgical, eye bank values determined by specular microscopy.*
RESULTS. *Microstructural alterations of the graft included epithelial and stromal edema, epithelial degeneration in both superficial and basal cell layers, dark stromal striae, activated keratocytes, and needle-like structures in the stroma. Descemet membrane folds were visible in 31 of 32 grafts; in 1 graft, the dense stromal edema did not allow imaging of posterior layers. Stromal nerve fibers were imaged in 28 grafts (88%). Endothelial cell density ranged from 1666 to 2548 cells/mm² (mean±SD, 2125±283 cells/mm²); perioperative endothelial cell density loss varied from 0% to 29% (mean, 12%). No adverse reactions or signs of worsening of clinical condition were observed after the examination.*
CONCLUSIONS. *White light scanning slit confocal microscopy permits imaging of a graft's microstructure (including epithelium and stromal layers), as well as calculation of endothelium cell density, as soon as 4 days after PK. The most frequently observed morphologic alterations of corneal grafts shortly after PK include epithelial and stromal edema, epithelial degeneration, stromal striae, and Descemet membrane folds. Stromal nerves can still be seen in the graft 4 days after PK. (Eur J Ophthalmol 2007; 17: 891-6)*

KEY WORDS. *Cornea, Confocal microscopy, Penetrating keratoplasty, Corneal nerves, Endothelium*

Accepted: June 27, 2007

INTRODUCTION

High-magnification imaging of corneal layers by in vivo confocal microscopy has been used to visualize microstructural alterations in several pathologic conditions. Confocal microscopy findings in corneal dystrophies (1, 2), keratitis (3, 4), and various keratopathies (5, 6) have been described. Postsurgical changes, particularly after excimer laser refractive surgery, have been comprehensively described (7, 8); corneal grafts were examined (9), and changes noted in long-term follow-up (15-66 months) were recently characterized (10). Furthermore, the endothelial graft rejection process has been observed by confocal microscopy, which means that early detection is possible (6, 11). A cornea to be transplanted is collected, evaluated, and stored, and then the corneal button is trephined and sutured into the recipient bed. These mechanical, chemical, and biological factors undoubtedly affect the grafted tissue.
Despite the promising results of the new lamellar techniques (12, 13), penetrating keratoplasty (PK) remains

Presented in part at the joint 15th European Society of Ophthalmology Congress and 103rd German Ophthalmological Society Congress; September 25–29, 2005; Berlin, Germany

1120-6721/891-06$15.00/0

to be a commonly performed procedure (14).
The aim of this study was to image early postoperative alterations of corneal grafts after PK and to evaluate the efficiency and safety of white light scanning slit confocal microscopy in examining grafts shortly after surgery. We also assessed perioperative endothelial cell loss.

MATERIALS AND METHODS

The study included 32 patients (17 men and 15 women) ages 23-81 years (mean, 49.1 years) who had undergone PK in our department. The indications for PK were keratoconus in 11 patients, bullous keratopathy and Fuchs dystrophy in 8 patients, infectious ulcer resistant to pharmacologic therapy in 7 patients, trauma or posttraumatic scar in 4 patients, and granular dystrophy in 2 patients.
PK was performed by a similar method in all patients: corneal trephination using a Hessburg-Barron vacuum trephine (Jedmed Instruments, St. Louis, MO, USA) and attachment of the graft (oversized by 0.5 mm) with eight interrupted sutures and one single running suture. The grafts were 8.5 mm in 27 cases, 8 mm in 3 cases, 7.5 mm in 1 case, and 9 mm in 1 case; two therapeutic grafts were placed off-center to permit removal of the entire ulceration.
Material for transplantation was stored in OptiSol-GS medium (Bausch&Lomb, Rochester, NY, USA) at 4 ºC for 16 hours to 5 days (mean, 2.3 days). Endothelial cell density of eye bank corneas was determined using the Konan Eye Bank KeratoAnalyzer specular microscope (Konan Medical, Hyogo, Japan).
Confocal microscopy of transplanted grafts was performed using the ConfoScan 3 white light scanning slit confocal microscope (Nidek Technologies, Padova, Italy). The microscope was equipped with its standard 40x magnifying front lens (Zeiss AG, Oberkochen, Germany). A drop of immersion gel (Vidisic; 0.2% polyacrylic acid; Bausch & Lomb) was present between the front lens of the microscope and the cornea during the examination; there was no direct contact between the microscope and the cornea. The eye was carefully examined with a slit lamp directly before, and after, confocal microscopy.
Patients were administered Novesin anesthetic drops (0.4% oxybuprocaine hydrochloride; Novartis Pharma AG, Ophthalmics, Basel, Switzerland) topically before confocal microscopy examination. Because of palpebral edema and the difficulty in keeping the examined eye open, a small pediatric speculum was used during the examination. The central and paracentral (±2.5 mm from the center) area of the graft was examined. The microscope's manual examining mode was used, permitting the examiner to control the course of the examination and the acquisition of images.
For endothelial cell counts, the randomly selected images from central graft area were chosen. Endothelial cell density counts were obtained using the software provided with the microscope. All cell counts were performed automatically or manually by the same skilled person for reliable results. The automatic cell counts were checked for accurateness and corrected manually if mistakes in the automatic estimation were found (e.g., counting a cell more than once). Endothelial cell density counts were compared with presurgical, eye bank values, and the difference was calculated.
Both microscopes were calibrated: the confocal microscope by the manufacturer and the eye bank specular microscope by the user, with the manufacturer's calibration set.

RESULTS

Confocal microscopy of epithelium revealed degeneration in both superficial and basal cell layers in all cases. Irregular and elongated superficial epithelium cells were imaged (Fig. 1A). The basal layer cells were frequently enlarged, with thickened cell borders. White spots resembling cell organelles, possibly cell nuclei, were visualized within the cell matrix (Fig. 1B). They are not imaged in basal epithelium physiologically; according to the authors' unpublished data, they can often be seen in patients with severe dry eye syndrome.
Epithelial edema was another common finding. The edema was usually not intense and was limited to basal cells, but in two cases it also affected superficial epithelium, with characteristic intraepithelial bullae (Fig. 1C).
No sub-basal or subepithelial plexus nerves were imaged during the study.
Stromal alterations included dark stromal striae

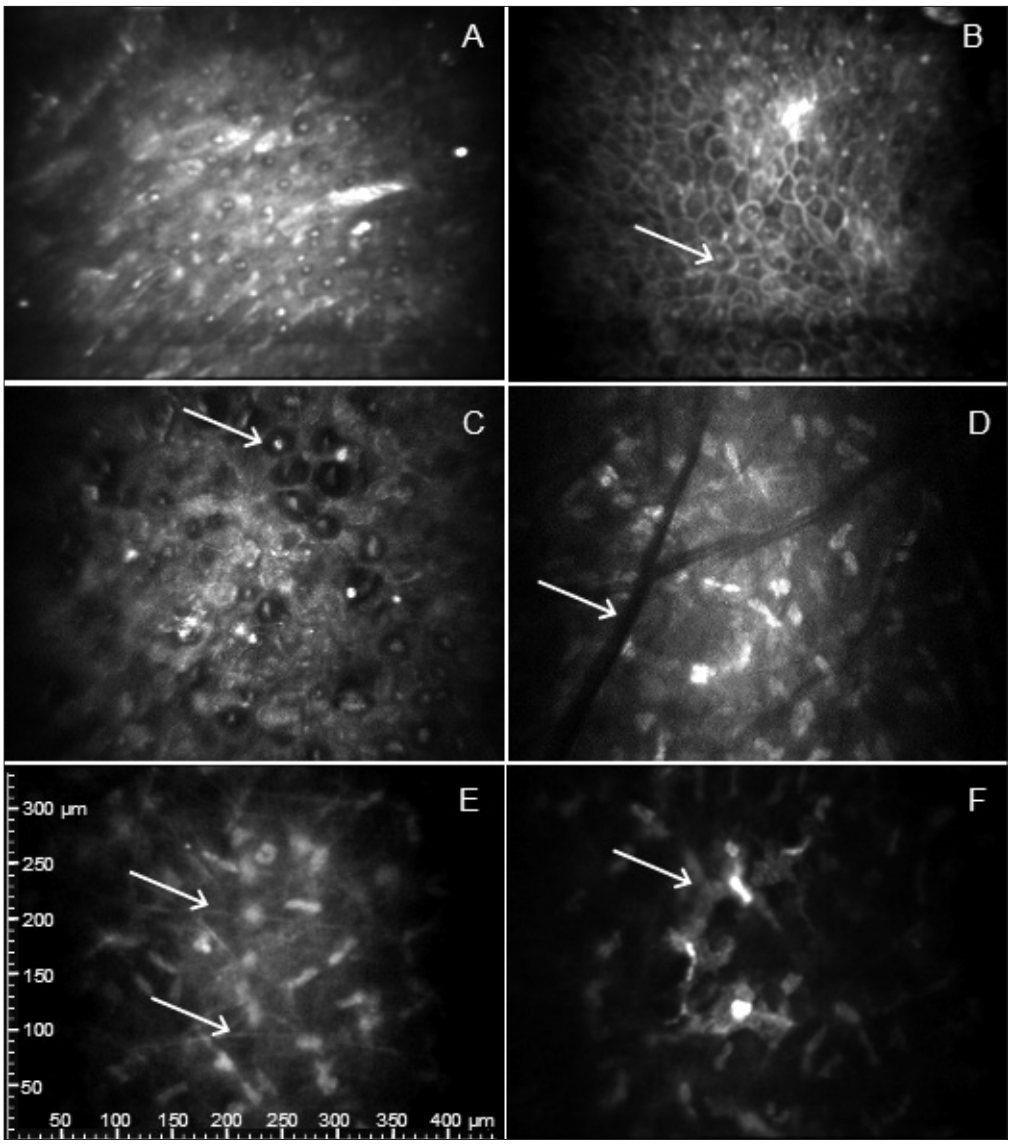

Fig. 1 - *Microstructural alterations in graft 4 days after penetrating keratoplasty.* **(A)** *Superficial epithelium: irregular and elongated cells.* **(B)** *Basal epithelium: enlarged cells with thickened cell walls. White spots resembling cell organelles (possibly cell nuclei) within cell matrix are marked with arrow.* **(C)** *Epithelial edema: superficial epithelium with characteristic intraepithelial cysts (bullae).* **(D)** *Dark stromal lines (striae).* **(E)** *Needle-like structures.* **(F)** *Activated keratocytes: cell body is visualized as H-shaped formation.*

(lines) (Fig. 1D) similar to those seen in keratoconus imaged in 97% of the examined grafts (n=31). They affected posterior and medium-posterior stroma. Needle-like structures like those imaged after keratitis or during corneal wound healing were observed locally in 19% (n=6) of the examined grafts (Fig. 1E) and activated keratocytes in 25% (n=8) (Fig. 1F). General or microcystic type of stromal edema could be observed in 50% of grafts (n=16); respectively, general type 22% (n=7) and microcystic type 31% (n=10) (Fig. 2A). Stromal nerves were observable in 28 grafts (88%) (Fig. 2, C and D), although they were often imaged thinner than in healthy cornea (Fig. 2D).

The endothelium was imaged in 31 grafts, and Descemet membrane folds were present in all of those cases (Fig. 2, D and E). In one graft, the dense general stromal edema prevented imaging of endothelium. In 5 grafts (16%), microguttae resembling those seen in early-phase Fuchs endothelial dystrophy were noted (Fig. 2B).

Endothelial cell counts were performed in 31 cases and ranged from 1661 to 2548 cells/mm² (mean±SD, 2125±283 cells/mm²). Comparison with presurgical eye bank counts revealed a difference ranging from 0 to 669 cells/mm² (decrease rate, 29%); the mean difference was 232 cells/mm² (mean decrease rate, 12%). We did not find any association between observed

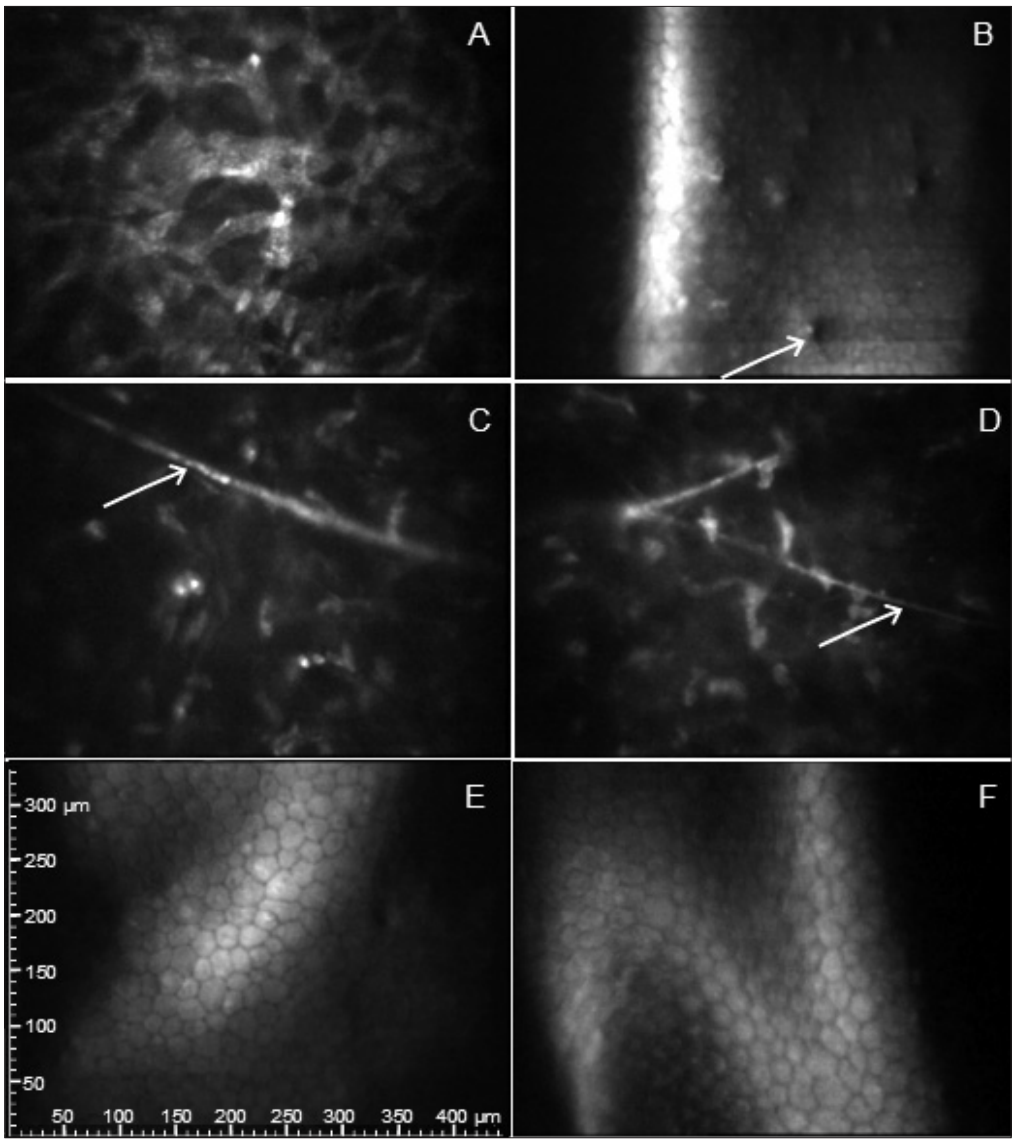

Fig. 2 - *Microstructural alterations in graft 4 days after penetrating keratoplasty.* **(A)** *Microcystic type of stromal edema.* **(B)** *Fuchs dystrophy–like microguttae.* **(C)** *Persistent donor's stromal nerve.* **(D)** *Thinned appearence of persistent donor's stromal nerve.* **(E, F)** *Descemet membrane folds.*

changes in the graft and indication for keratoplasty. Differences between pre- and postsurgical endothelial cell density did not differ with preoperative pathology (Kruskal-Wallis test to compare differences of cell density from groups of each indication for keratoplasty p=0.24).

Patients tolerated the examination well, although the eye must be kept open throughout the examination, which usually lasted about 2 minutes. The dense immersion gel (tears substitute) also had a protective function, preventing the corneal surface from drying. We did not notice any adverse reaction or signs of worsening of clinical condition after the examination or the next day. We did not notice any difference in the post surgery clinical course of the studied patients compared to our post PK patients not enrolled in the study.

DISCUSSION

For ethical reasons, studies of the microstructure of transparent corneal grafts in humans shortly after PK must be limited to noninvasive techniques. Interpretation of confocal microscopy results is therefore more difficult, because histologic correlation cannot be carried out.

The early postoperative microstructural changes observed in this study are a consequence of the varied

processes that graft tissue is subject to. These microstructural changes can be divided into three categories.

1) Degenerative: disturbances observed mostly at the cellular level and relating to changes in cell shape or cell elements, suggesting cellular or tissue degeneration. We included in this category epithelial degeneration in both superficial and basal cell layers and activated keratocytes in the stroma. The needle-shaped structures, already described as disorganized extracellular matrix and collagen lamellae (10), are a symptom of tissue deterioration.

2) Edematous: changes resulting from an excessive accumulation of fluid in the stroma and, in some cases, epithelium. Stromal edema in studied grafts was consistent with general and microcystic types of edema, as described by Mastropasqua and Nubile (6); the microcystic type was dominant.

3) Mechanical: a direct consequence of mechanical stress applied to tissue during collection of the corneoscleral button, trephination, and suturing. This category comprises Descemet membrane folds and stromal striae.

We offer two potential explanations for the endothelial lesions resembling early-stage Fuchs dystrophy microguttae: they might be an early degenerative response by endothelial cells to storage and surgery, or they could be Fuchs dystrophy microguttae transplanted from the donor. There is as yet no evidence to confirm either of these hypotheses.

In our study, no specific morphologic alterations were observed in any patients in the keratoplasty indication group; the frequency of changes and the decrease in endothelial cell density also did not vary among groups. This suggests that indication does not particularly affect the morphologic status of grafts up to 4 days after surgery.

The study showed that donor stromal nerves can be imaged as late as 4 days after surgery. The fact that the nerves were often imaged as thinner than they actually were may indicate atrophy; they nearly cannot be seen 7–8 days after transplantation (author's unpublished data). On the other hand, no subbasal or subepithelial plexus nerves were visualized at the time of examination.

Most of the observed graft structural alterations are presumably reversible. Degenerative epithelial layers are being replaced by new cells arising from host limbal stem and transient amplifying cells, stromal edema may disappear once the endothelial function is completely restored in absence of endothelial complications. Studying this case series, we were not able to perform consecutive confocal microscopy examinations in a certain time frame, because most of our patients originated from remote locations. In the future, confocal microscopy may clarify these ongoing structural changes after surgery in an extended follow-up.

The cell counts were performed using two different microscopes: a specific specular microscope dedicated for the corneas from an eye bank (Konan Eye Bank KeratoAnalyzer) and a confocal microscope for the same corneas after transplantation; nevertheless, as both microscopes were calibrated, the difference resulting from using two methods should be minimized. The specular microscope was calibrated according to the owner's manual; the confocal microscope was calibrated by the manufacturer.

To our knowledge, this study is the first direct attempt to establish the rate of perioperative endothelial cell density decrease in grafts after PK. We presume that this process can be influenced by various factors, and our results, which are based on a limited number of cases, reveal a tendency rather than serve as a precise estimation.

However, the fact that results ranged from 0% to 29% (mean, 12%) indicates the scope of the problem. Further complex studies involving large groups of patients could probably determine the possible issues playing a role in preserving endothelium cells perioperatively and might yield more knowledge on early endothelial cell loss dynamics after PK. In this regard, confocal microscopes are superior to specular microscopes, which are only seldom capable of imaging the endothelium so shortly after transplantation.

In our study, confocal microscopy utilizing the non-applanating front lens proved to be a safe and efficient method for imaging the microstructure of corneal grafts as soon as 4 days after PK.

The authors have no financial or other interest in any products used or described in this study. No financial support was received.

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