Objective: To assess the alterations in human donor corneal tissue induced by Q-switched erbium (Er):YAG laser corneal trephination.

Methods: Thirty human corneoscleral donor buttons unsuitable for transplantation were placed in an artificial chamber on an automated rotation device. Corneas were trephined with a Q-switched Er:YAG laser (wavelength, 2.94 µm; pulse duration, 400 nanoseconds) along (donor and recipient) aluminum silicate (ceramic) open masks. A spot diameter of 0.65 mm, energy setting of 50 mJ/pulse, and repetition rate of 5 Hz were used. Corneal thermal damage and cut regularity were quantitatively assessed in 24 corneas processed for light microscopy and by transmission and scanning electron microscopy.

Results: The stromal thermal damage was the highest (mean [SD], 8.0 [2.7] µm) at a 150-µm cut depth and decreased downward. Cut regularity was very good and did not significantly differ between donors and recipients. Scanning electron microscopy confirmed that the cuts were highly regular; transmission electron microscopy revealed 2 distinctive subzones within the stromal thermal damage zone.

Conclusions: Thermal damage induced by Q-switched Er:YAG nonmechanical corneal trephination was low, and the regularity of the cuts was very good.

Clinical Relevance: The Q-switched Er:YAG laser may have the potential to become an alternative to the excimer laser for nonmechanical penetrating keratoplasty.

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In our department, laboratory investigations of nonmechanical corneal trephination for penetrating keratoplasty with the free-running Er:YAG laser were performed on porcine eyes and human donor corneas. A pilot study of Q-switched Er:YAG laser corneal trephination in porcine eyes has been performed by our group and, subsequently, a larger series of porcine eyes were trephined with different laser settings (M.K., M.S., B.S., A.L., A.V., and A.V., unpublished data, 2001). The purpose of this study was to assess corneal tissue alterations induced by Q-switched midinfrared laser corneal trephination of human corneas in vitro to compare them with the reported alterations induced by similar infrared lasers and the 193-nm excimer laser as the “gold standard” for nonmechanical corneal trephination in penetrating keratoplasty.

**METHODS**

Thirty human donor corneoscleral buttons were obtained from the local corneal eye bank for Q-switched Er:YAG laser corneal trephination. The donor corneoscleral buttons were inadequate for penetrating keratoplasty because of nonsterility and/or longer than optimal storage time (varied from 12 to 36 days) in Optisol GS (Bausch & Lomb Surgical, Rochester, NY) at 4°C. The long storage time of most of the corneas (28 of 30 corneas from 31 to 36 days) caused loss of all or of a significant portion of endothelial and epithelial cells so that no meaningful analysis of laser interactions with these corneal layers could have been done. The corneas were placed in an artificial anterior chamber centered and fixed on the rotating plate of an automated rotation device with a constant rotation speed (1 rotation per minute) to produce a “dense overlap of hits” expected to provide a smooth transition of laser-tissue interactions. Previous to corneal placement in the artificial chamber the 1 drop of a viscoelastic solution was placed on the posterior corneal surface. The intracameral pressure was maintained at 21 mm Hg (Schiøtz tonometer) on average. The corneal surface was wetted with a single drop of isotonic sodium chloride solution and immediately after this an aluminum silicate (ceramic) open mask was placed on the cornea (centered according to the central hole at the bottom of the artificial chamber).

Open donor and recipient ceramic masks were used. Donor masks had 8 peripheral orientation teeth of 0.15 mm and the recipient masks had corresponding notches. Donor ceramic masks had an overall diameter of 7.1 mm, a central opening of 3.0 mm, and a weight of 0.23 g. Recipient ceramic masks had an overall diameter of 12.9 mm, a central opening of 7.0 mm, and a weight of 0.46 g. The difference in opening diameters of 0.1 mm between donor and recipient masks was chosen to compensate for expected discrepancy donor button diameter(s) and recipient bed diameter(s) caused by thermal shrinkage of cut edges. The masks with 7.0-mm and 7.1-mm openings were the only masks available from the manufacturer at the time of the experiment. However, from the theoretical point of view equal amounts of thermal damage were expected with larger openings in recipient masks and even lower thermal damage with donor masks because of the larger distance between the cornea and the mask edge, that is, more ob-
lique direction of the laser beam to the corneal surface at the trephination outline. The position of the masks on the corneal surface is determined only by gravitation and adhesive forces created by 1 drop of an isotonic sodium chloride solution on the corneal surface. No compression and no obvious change of corneal contour were noted under the microscope when masks were put on the corneal surface. However, an analysis of topography of the corneal segment visible inside the opening of the masks with and without the masks applied to the corneal surface could solve the problem of quantifying possible fine contour changes.

The laser delivery system consisted of a 2.94-µm wavelength Er:YAG device (NWL Laser Technologie GmbH, Ottenssoos, Germany) with a 670-nm diode laser as an aiming beam (Figure 1). This laser emits fluences up to 60 mJ at 400 nanoseconds’ duration. A spot diameter of 0.65 mm and pulse energy setting of 50 mJ/pulse were used. The laser equipment was fixed by a metal stand in a 90° angle to the surface of the cornea. The artificial chamber was placed under the laser arm and the guiding beam was directed to the rim of the mask with about half of the spot on the mask and half on the corneal surface (Figure 1 and Figure 2). With the rotation device in action, the trephination was performed under visual control and halted immediately on the observation of aqueous humor in the trough, which is a sign of focal corneal perforation.

**HISTOLOGICAL AND ULTRASTRUCTURAL EXAMINATIONS**

Twenty-four corneas were fixed in a 10% buffered paraformaldehyde solution and macroscopic analysis was performed 24 hours after fixation. Corneas were bisected, embedded in paraffin, cut in 8-µm sections, and stained with periodic acid–Schiff. All slides were analyzed by light microscopy and the regularity of the cut edges and the extension of thermal damage were recorded in a masked fashion (slides were randomly numbered for measurements and the corresponding laser parameters for all slide numbers were revealed after all measurements were performed). Regularity was semiquantitatively graded from 0 to 3 with 0 indicating a regular cut edge; 1, a mildly irregular cut edge; 2, a moderately irregular cut edge; and 3, a highly irregular cut edge.

Stromal thermal damage was defined as the thickness of the dark-staining zone present in contiguity with the cut edge. Measurements were taken at the following 3 stromal depth levels: upper (150 µm), intermediate (300 µm), and lower (600 µm). The perpendicular extensions of the dark-staining thermal damage zone into the stroma, so-called spikes, were counted in each specimen and their maximum and minimum heights were measured.

Randomly selected cases were submitted for transmission electron microscopy (TEM) (n = 3) and scanning electron microscopy (SEM) (n = 3). Specimens were postfixed in 2% buffered osmium tetroxide, dehydrated in graded alcohols, and embedded in epoxy resin (Epon 812; FLUKA, Buchs, Germany) for TEM. Semithin sections were stained with toluidine blue; ultrathin sections were stained with uranyl acetate–lead citrate and were examined with TEM (model EM9A; Carl Zeiss, Oberkochen, Germany). A quantitative analysis of thermal effect at the cut edge as well as an analysis of cellular changes of keratocytes in the vicinity of the cut were performed. For SEM, specimens were dehydrated in acetone graded 50% to 100%, dried to critical point, sputtered with gold, and examined using SEM (CAM SCAN, Dortmund, Germany).

**STATISTICAL ANALYSIS**

All data were entered in a common database system (Microsoft Access 2.0; Unterschleissheim, Germany). For statistical analysis, SPSS/PC Release 9.0 (Windows NT; Microsoft, Mountain View, Calif) was used. Measurements of variables in different groups of laser settings were described with a mean (SD) value and minimum and maximum values. Comparisons between groups or variables were performed with nonparametric tests (Mann-Whitney test for unpaired samples and Wilcoxon test for paired samples). For bivariate correlation analysis, Spearman rank correlation coefficient was used. A P < .05 was considered statistically significant.

**RESULTS**

Trephination time until focal perforation was 778 (106) seconds in the recipient mask group and 640 (62) seconds in the donor mask group. The mean (SD) total energy used for perforation was 194.5 (26.5) J in the recipient mask group and 160.1 (15.5) J in the donor mask group.

**MACROSCOPIC FINDINGS**

Regularity and smoothness at the cut surface were excellent (equal with donor masks and recipient masks). Retraction of the cut edges occurred progressively with rotation of the artificial chamber. The incisions were measured, but tissue shrinkage did not cause significant re-
recipient bed–donor button discrepancy in size (M.K., M.S., B.S., A.L., A.V., and A.V., unpublished data, 2001). The zone of stromal thermal effect contiguous with the cut edge was not clearly visible macroscopically after globe bisection at the side of the cut protected by the mask. The excellent regularity of the superficial outline of the cut, with sharply defined teeth and corresponding notches, is visible on low-magnification SEM (Figure 3).

HISTOLOGICAL FINDINGS

Stromal Damage

Thermal Damage Zone (Band). Thermal changes were represented by a dark band of violet staining in contiguity with the cut edge (Figure 4). Thermal injury was maximal at a cut depth of 150 µm (mean [SD], 8.0 [2.7] µm) and became less at greater cut depths within the laser excision ($P < .01$), measuring 6.5 (1.5) µm at the 300-µm cut depth and 5.8 (1.2) µm at the 600-µm cut depth when all samples were analyzed together. The values of stromal thermal damage at the 150-, 300-, and 600-µm cut depth intercorrelated with a high statistical significance ($P < .01$). In donor series the lowest width of the thermal damage was 5 µm at all 3 cut depths. In recipient series the lowest value of thermal damage was also 5 µm at all 3 cut depths. The highest width of the thermal damage zone with donor mask perforation was 15 µm in superficial portions of the cut edge while it was 10 µm in superficial and middle portions of the cut edge for the recipient mask trephination. No statistically significant differences in stromal thermal damage between the corneas trephined with donor and recipient masks were found (Table 1).
Spikes. Spike-shaped dark-staining areas identified by light microscopy that represent the lateral expansion of the thermal effect band and are perpendicular to the cut edge and parallel to corneal lamellae (Figure 4) had the lowest maximum value of 15 µm and the highest maximum value of 50 µm in the donor mask group. The lowest value of maximum spike height in the recipient group was 20 µm while the highest value of maximum spike height in this group was also 50 µm (Table 2). Mean (SD) values of maximal spike heights were 35.0 (10) µm in the donor mask group and 40.2 (8) µm in the recipient mask group. No significant differences between maximum spike heights in donor and recipient trephination were found (Table 2).

The number of spikes per cut ranged from 1 to 23 in the donor mask series and from 3 to 20 in the recipient mask series. The mean (SD) number of spikes was 10.4 (6.3) in donor trephination and 11.9 (5.1) in recipient trephination. No significant differences were found between the number of spikes in donor and recipient trephination.

Cellular Damage. In all examined specimens vacuoles were observed between collagen lamellae and inside the keratocyte cytoplasm at the cut edges and were located more frequently in superficial and midzones of the ablation. Keratocytes within the violet-staining zone or at the interface between the damaged and nondamaged collagenous fibers showed a vacuolated and bulked cytoplasm with the nuclei displaced to the periphery. However, only the keratocytes nearest to the cut edge were damaged (first-line keratocytes). The second-line keratocytes and all others were spared from damage in all examined specimens.

Cut Regularity and Orientation Teeth Outline

Cut regularity was (mean [SD]) 0.2 (0.5) on a scale from 0 (regular) to 3 (very irregular) in donor corneas while in recipient corneas cut regularity was 0.3 (0.5) (Table 2 and Figure 4). There was no statistically significant difference between cut regularities in donor and recipient trephination. In 22 of 24 cases cuts were either parallel or only slightly convergent or divergent. In 2 corneas in which intracameral pressure was more than 26 mm Hg, the cuts displayed marked divergence on periodic acid–Schiff-stained histological slides. Cut regularity displayed highly significant correlation with the number of spikes per cut and spike maximums (P<.01) and a significant correlation with the thermal damage at the 150-µm cut depth (P<.05). All of the previous and of the following observations on the quality of cut edge are only applicable to 1 side (edge) of the incision (the one protected by the mask).

TEM Findings

Transmission electron microscopy showed 3 distinct zones of thermal effect (Figure 5). The first one is a “carbonization zone” (0-2 µm thick), discontinuous, closely in contact with the laser irradiation. This zone consisted of homogenously electron-dense material distributed along the cut edge in linear, granular, or, occasionally, flocculent arrangement. Within this zone no keratocytes could be observed.

The second zone (or coagulation zone) located farther away from the cut edge showed greater variability in thickness (4-15 µm) and consisted of regions of medium electron density with “fuzzy” collagen fiber con-
tours embedded in an amorphous matrix (Figure 5 and Figure 6) as well as regions of occasional disarrangement and separation of the lamellae or fiber bundles (vacuoles in the extracellular matrix) (Figure 6).

These zones noted on TEM correlated well with light microscopic findings. A very thin more intensely stained albuminate discontinuous line can be noted with some difficulty even in light microscopic specimens and corresponds to the carbonization zone. The rest of the thermal damage band, including the spikes, corresponds to the coagulation zone. Keratocytes present in this zone showed changes in intracellular organelles such as pyknosis or vacuolization of the nucleus and vacuolization of the cytoplasm. Cellular changes analogous to those from the coagulation zone were noted also at the border with the normal collagen zone. However, only the keratocytes in the first keratocyte row (nearest to the cut edge) were occasionally damaged; that is, cellular damage was not evident in any of the keratocytes farther away from the cut edge.

SEM Findings

Scanning electron microscopy revealed very good cut edge regularity, both in recipient and in donor corneas (Figure 3 and Figure 7). In a single specimen in which a continuous epithelial layer was present, epithelium occasionally folded near the edge of the cut at the orientation teeth tips. Epithelial damage was minimal on the side under mask protection but invariably present on the side unprotected by the mask. Stromal superficial tissue elevation was unremarkable. Significant vertical separation of stromal lamellae was not noted both in the orientation teeth regions and the rest of the cut. In addition, no horizontal disarrangement (“slipping”) of the lamellae was found. The orientation teeth and notches were occasionally slightly rounded at the tip (Figure 7). No vertical grooves and/or protrusions were observed on the cut edge. Invariably (in donor and recipient specimens), a series of very thin and low (mutually parallel) horizontal ridges was seen at the edge of the cut (Figure 7). The ablation profile was maintained throughout the cut depth.

COMMENT

In our present study, the thermal damage zone was thinner (approximately twice) in Q-switched Er:YAG laser trephination in both donors and recipients in comparison to the free-running mode study. The width of the thermal damage zone observed in our study with the Q-switched laser decreased from the surface to the deep regions.

There are 2 possible explanations for this finding: First, shortly after this deep region of the cut was reached, the focal perforation occurred at one point of the circumference and the laser energy delivery was stopped. Thus, it was less probable for the laser energy to be repeatedly supplied to the entire circumference of the deep region of the cut, than to the middle and superficial portions of the cut depth. Second, in in vitro conditions, such as in our experiments, the cornea could have a gradient of water molecule concentrations across its corneal depth. The water content is probably higher in the posterior stroma in comparison to the anterior stroma because of constant passive influx of water from the artificial anterior chamber and the superficial drying caused by laser-energy–induced evaporation. This would make the cutting process more efficient because of the higher absorption of the infrared radiation by more numerous
Transmission electron microscopy analysis revealed that the alterations in cellular components under direct laser exposure were confined to the coagulation zone or the first row of keratocytes within the normal collagen zone. They are, probably, of no major consequence for the healing process because of the few keratocytes involved, even if the damage leads to keratocyte necrosis or accelerated apoptosis. Furthermore, it has been estimated that most fibroblasts (65%) that contribute to the wound repair in the cornea are not derived from keratocytes but from circulating monocytes. The overall extent of tissue damage corresponded well to the previously mentioned light microscopic findings.

The regularity of the cut edge in all of the corneas in our group was very good (mean [SD], 0.3 [0.2]), approaching the values obtained with the excimer laser. Values of more than 1 (less than very good cut regularity) were not ascribed to any of the cuts.

The spot diameters in our series were very small making precise spot centering on the mask edge somewhat more difficult to obtain than in the case of larger spots with the free-running mode. Thus, some variability is expected in the amount of energy supplied by the laser to different corneas and parts of the cut circumference. If this is the main reason for the slight remaining irregularity of the cut edge, then it could be avoided with the larger spot size supplying the same amount of energy. This is technically possible to achieve and one such device is in final phases of development.

In this study, the number of spikes, their height, and especially, the regularity of the cut were much more favorable than in porcine corneas in both the free-running and Q-switched modes. This could also be because of better centering with the artificial anterior chamber in comparison to the globe holder device (used for fixation of porcine globes during nonmechanical corneal trephination).

Since macroscopic as well as light microscopic analysis of the cut angle revealed divergent cut angles when high intracameral pressures were used, it is tempting to suggest that intracameral (intraocular) pressure should be harmonized in the donor and recipient to obtain complementary cut profiles. Further studies are needed to elucidate the relationship between the intracameral (intraocular) pressure and the cut direction (angle) in different trephination procedures.

The SEM analysis of selected specimens displayed occasional vertical separation of the lamellae in the regions of orientation teeth in donor trephination comparable to the separation seen in the free-running mode. However, the horizontal separation of the lamellae was small with the Q-switched mode. This could be related to the high repetition rate of the Q-switched Er:YAG laser in our study.

To the best of our knowledge, there is no information in the literature about whether the stromal thermal changes after corneal trephination with an Er:YAG laser could lead to greater than acceptable degrees of postoperative inflammation. The inflammation could lead to the formation of denser and wider scars that could induce irregular astigmatism. In vivo animal study of these processes is needed to elucidate the long-term effect of Q-switched Er:YAG laser corneal trephination on stromal corneal tissue (ie, scar formation). So far, it is only known that when monkeys were subjected to lamellar keratoplasty, the minor thermal changes in corneal stroma did not adversely affect the wound healing process and the outcome of corneal ablation in general.

Previous studies on the effects of lasers with shorter-pulse duration than the duration used in free-running Er:YAG laser (micosecond order) showed that the decrease in tissue damage is not significant until the microsecond pulse duration changes into the order of nanoseconds (Q-switched mode). Our study findings agree with this conclusion since the thermal damage with the use of a Q-switched Er:YAG is lower than the one with the free-running Er:YAG laser.

In this study, an area of 150 to 200 µm of endothelial layer destruction from the cut edge was observed. However, the method used may not be appropriate since the endothelium was already damaged (vacuolated) by the autolytic processes in the excessively old corneoscleral buttons that were used. By a more reliable method (vital staining of flat preparations of corneal endothelium in fresh porcine globes) an area of 120 µm width of 40% endothelial cell damage was noted after Q-switched Er:YAG laser corneal trephination.

Epithelial changes in our specimens were low and comparable with those that were reported by our group for porcine corneas.

In our study of Q-switched Er:YAG laser corneal trephination, stromal thermal damage was low and reproducible and cut regularity was very good. The values of these 2 factors of cut quality were clearly more favorable than the values reported for the free-running Er:YAG laser. The cut edge regularity in our series approached gold standard cut regularity obtained with the 193-nm excimer laser. Although the zone of laser irradiation–induced rearrangement of stromal architecture at the cut edge was about 10 times wider than with the 193-nm excimer laser, it seems as if this several-micrometer wide damage may still be considered low and acceptable out of the optical zone in penetrating keratoplasty.

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