Human Corneal Ablation Threshold Using the 193-nm ArF Excimer Laser

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PURPOSE. To determine the human corneal threshold ablation energy density for the 193-nm ArF excimer laser, approximating clinical conditions.

METHODS. The VISX Star (Santa Clara, CA) 193-nm argon fluoride excimer laser was used to ablate the cornea in human eye bank eyes under clinical conditions. Corneas were exposed to energy densities of 10, 20, 30, 35, 40, 45, and 140 to 160 mJ/cm². Corneas were fixed for light and transmission electron microscopy immediately after laser exposure.

RESULTS. Different ablation thresholds for various corneal structural elements were observed. The ablation threshold for the collagen in the corneal stroma was determined to be 30 mJ/cm². Keratocytes had ablation thresholds of 40 mJ/cm². These different ablation thresholds accounted for the production of stromal peaks and valleys, with the keratocytes atop the peaks.

CONCLUSIONS. Different corneal structural elements have different ablation threshold energy densities.

Photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK) are being performed worldwide to treat a variety of refractive problems. Although no large-scale long-term follow-up (>10 years) has occurred, and the precise mechanisms of laser interaction with the tissue are not fully understood, PRK and LASIK are both being performed as “standard of care” by refractive surgeons and general ophthalmologists.

Trokel et al.1 first suggested the use of the excimer laser for both incisional radial keratotomy and large area lamellar keratectomy. However, most of the early studies on dosimetry and laser tissue interactions (mechanisms of light interaction and nature of tissue damage) examined the application of the laser in an incisional “knife-like” mode as opposed to the more shallow wider area ablation of lamellar keratectomy. These studies focused on describing the precision and histopathology of incisional cuts.2–6 In addition to the studies on incisional mode excimer ablation, wound healing studies were conducted on incisional7–9 and wide area lamellar ablation.5–9,10 In all the early studies describing the precision, selectivity, and efficacy in treating human patients, the energy densities used were at least 80 mJ/cm² and usually were well above 100 mJ/cm².11 Currently, all U.S. Food and Drug Administration-approved ArF excimer lasers in clinical use for refractive surgery deliver energy densities above 100 mJ/cm². It has even been suggested that ArF excimer laser corneal surgery be conducted at an energy density of 200 mJ/cm² because of the etching efficiency at this energy density.12

In reviewing the basic literature on ArF excimer laser refractive surgery, two points are clear: There is an incomplete understanding of the physical processes that contribute to the ablation process (e.g., heat, molecular bond-breaking, mechanical forces), and the optimal ablation energy density is not known. Progress in resolving these two issues would be facilitated by determining the threshold energy density for ablation of the human cornea. This would be important for several reasons. First, studying the molecular and structural tissue responses below, at, and above the threshold will provide valuable information on the nature of the ablation process. Second, knowledge of the ablation threshold or thresholds under different environmental and physiological conditions will allow for a rational determination of optimal treatment dosimetry. Third, as laser systems become more compact and widely distributed around the world, it might be desirable to perform laser procedures closer to the ablation threshold. Therefore, knowledge of that value is important.

The vast majority of excimer laser threshold corneal ablation studies have been conducted on either rabbit or cow eyes. These studies have provided widely variable threshold ablation values ranging from 20 mJ/cm² to 65 mJ/cm².2,3 The wide variation between sources of eyes (animals), condition of the eyes, laser parameters, and experimental design have resulted in a published body of data that is neither consistent nor predictive.

One of the more extensive ablation threshold studies in calf eyes used excimer lasers with emission wavelengths of 193, 249, 308, and 351 nm, at three different repetition rates: 1 Hz, 10 Hz, and 25 Hz.3 The data suggested that the ablation threshold for the 193-nm ArF laser is 50 ± 10 mJ/cm² at 10 Hz, and 55 ± 10 mJ/cm² at 25 Hz. The data showed that threshold

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Supported by Grant N00014-94-0874 from the Office of Naval Research, Washington, DC; Grant DE-FG03-91ER61227 from the Department of Energy, Washington, DC; Grant RR01192 from the National Institutes of Health, Bethesda, Maryland; and a Beckman Laser Institute Endowment, University of California—Irvine.

Submitted for publication June 4, 1998; revised November 13, 1998; accepted January 8, 1999.

Proprietary interest category: N.

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ablation values might be as high as 60 mj/cm² for the 1-Hz and 10-Hz exposures and 65 mj/cm² for the 25-Hz exposure. Other corneal eye studies have demonstrated threshold ablation energy densities of 20 mj/cm² for bovine,3 and 40 mj/cm² for rabbit corneas.13 Based on the published studies, the true ablation threshold may be anywhere between 20 mj/cm² and 65 mj/cm². Because most of the ablation threshold data from the literature is in bovine and rabbit tissues, and because even these threshold values vary greatly, it is impossible to know the threshold for the human cornea from the literature.

The two published studies to determine the ablation threshold in human corneas were conducted with research Lambda Physik ArF excimer laser systems.2–19 The first study involved gross observation of the cornea under 3x magnification in one human eye bank eye. No histopathology or electron microscopy was performed. The laser was operated at energy densities between 8 mj/cm² and 60 mj/cm² per pulse at 50 Hz as opposed to the 5 Hz to 10 Hz used in most clinical laser systems. Under the conditions of this experiment, the human corneal ablation threshold was determined to be 46 mj/cm².

Based on this observation, a human threshold ablation value of 50 mj/cm² has been widely cited in the literature.14 Additionally, the second study by Kermani et al.19 was performed on four human corneas using two different excimer laser wavelengths at 248 nm and 193 nm at 2 Hz. The main focus of this study was mass spectrum analysis of the reaction products produced by the photoablation process.19 These authors described an ablation threshold of 40 mj/cm² for corneas exposed to the 193-nm wavelength while in a high vacuum chamber. They noted that on vacuum and before laser exposure, the corneas dried and opacified.

We have undertaken a light and electron microscope study using human eye bank corneas, in a controlled environment, and approximating clinical conditions, to determine ablation thresholds for the 193-nm ArF excimer laser.

Methods

Corneas

Human corneas with a small scleral rim were obtained from eye banks in an Optisol storage medium (Bausch & Lomb Surgical, Claremont, CA). They were received in cold storage and were refrigerated at 4°C (centigrade) until used. All eyes were used between 5 and 10 days from enucleation and placement in the Optisol storage solution. For each ablation, the cornea was positioned at the normal corneal ablation plane and sectioned as described below.

Excimer Laser

The ArF 193-nm excimer used in all these studies was the VISX Star system. This unit is used daily in human PRK and PTK, and it is maintained according to strict standards in a university-based outpatient refractive surgicenter. Only VISX-trained and certified nurses and technicians operate the machine. The room temperature and humidity during these experiments were identical to human treatment conditions: 68°F to 70°F and 55% to 60% humidity. Temperature and humidity were recorded before and after each individual laser procedure. Laser calibration on methacrylate plastic was conducted using the normal human protocol. In addition, the laser energy density at the corneal ablation plane was measured before and after each laser exposure using a Molectron JMAX 43 detector and a Molectron EMAX 400 (Molectron Detector, Portland, OR) energy meter. Both of these devices were purchased for this study, and care was taken to ensure that the company (before being shipped) appropriately calibrated them against a standard.

All ablation zones were 6 mm in diameter. In all corneal exposures, the laser was first calibrated to emit a normal PTK energy density of 145 mj/cm² to 160 mj/cm² at the corneal plane at 5 Hz. After these measurements, a fused silica (quartz) plate was mounted between the exit point of the laser beam and the corneal plane. By changing the angle of the quartz plate, the amount of laser energy passing to the corneal plane could be precisely controlled at 5-mj/cm² intervals. Before and immediately after each corneal exposure, energy density readings were made at the corneal plane. The mean change from pre- to postexposure was 0.87 mj/cm². A total of 13 human corneas was exposed to the excimer laser. In a preliminary dosing series all corneas were exposed to 341 laser pulses. Three corneas were exposed to energy densities of 10 mj/cm², 20 mj/cm², and 30 mj/cm², respectively. In a fourth control cornea, the ablation zone was shielded so that one half received an exposure of 40 mj/cm², and the other half received 146 mj/cm², the latter being the actual control exposure that would have an expected clinical ablation depth of 82 μm (0.24 μm etch rate per pulse). These four corneas were examined by light microscopy to determine whether ablation had occurred in the epithelial side where it might interfere with the ablations. A suture (10-0 nylon) knot was placed in the scleral rim on two opposite sides of the cornea to mark an imaginary line separating the cornea into two equal halves. The suture needles were used to tie the sclera to the underlying silicone block and to prevent movement through the procedure.

A 7.0-mm optical zone marker was then placed on the center of the cornea and used as a well to contain a few drops of 20% ethanol for 20 to 25 seconds. The marker was promptly removed and the surface irrigated with BSS to prevent ethanol dehydration. The 7.0-mm bed was then wiped free of epithelium using a Weck-Cel sponge (Solan Ophthalmics, Jackson- ville, FL). The corneal surface was wiped again with a dampened Weck-Cel sponge.

An adjustable platform had been preset to ensure that the cornea was positioned at the normal corneal ablation plane and precentered to minimize any dehydration time. The ablation was performed as a normal human phototherapeutic keratectomy and observed to be well centered throughout the procedure. At the end of ablation, each cornea was promptly fixed and sectioned as described below.
between 10 mJ/cm² and 40 mJ/cm². The control cornea permitted comparison of a standard clinical ablation energy density to a near-threshold ablation density in the same cornea. Because this particular cornea had been stored the longest in the Optisol (10 days), it also served as a control with respect to the amount of ablation depth expected for the 146-mJ/cm² control exposure dose. Based on these results, a second series of exposures was conducted on 9 human corneas that involved both light and transmission electron microscopy. Two separate corneas were exposed to each of the following energy densities: 30, 35, 40, and 45 mJ/cm². One control cornea was exposed to 146 mJ/cm². In this experiment, all the experimental corneas were exposed to 683 pulses to ensure a deeper more easily detectable ablation zone.

Gross corneal thickness was measured from histology specimens for the control corneas in both the preliminary and the second series of experiments to determine whether the storage times in the Optisol may be introducing an artifact into the experiments.

**Histopathology**

For light and transmission electron microscopy, the human corneal tissues were fixed for 24 hours in Karnovsky's fixative (2% paraformaldehyde, 3% glutaraldehyde) immediately after ablation and rinsed in 0.1 M cacodylate buffer. The corneas were postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 hour and then rinsed with double distilled water. The osmium stain displayed a different shade between the laser-ablated area in the center and the unablated area in the adjacent periphery of the cornea. Using the laser-ablated area as center, a central 7-mm to 8-mm square of the cornea was excised with a razor blade oriented with the epithelial side up. The corneal square was hemisected 0.5 mm off-center into two rectangles. On the larger rectangular piece, two cuts were made perpendicular to the long axis to produce three equal sections. The middle section contained the exact center of ablation, whereas the side sections contained the edge of the ablation and also the periphery of the unablated zone. After dissection, the samples were en bloc stained in Kellenberger's uranyl acetate for 2 hours. Dehydration was achieved with progressive concentrations of ethanol-water in 10-minute steps (30%, 50% 70%, 90%, 100%) and also in progressive ethanol-propyleneoxide in 10-minute steps. Infiltration was done with 0.15/µL Ag electron dense pseudomembrane at the ablation surface. Note the clean ablation of the collagen fibrils and the 0.05-µm to 0.15-µm electron dense pseudomembrane at the ablation surface.

The ablation threshold for stromal collagen was determined to be 30 mJ/cm². No traces of ablation were detected below this value. Note the clean ablation of the collagen fibrils and the 0.05-µm to 0.15-µm electron dense pseudomembrane at the ablation surface.

**RESULTS**

The ablation threshold for stromal collagen was determined to be 30 mJ/cm². No traces of ablation were detected below this value. Note the clean ablation of the collagen fibrils and the 0.05-µm to 0.15-µm electron dense pseudomembrane at the ablation surface (Fig. 1). The pseudomembrane does appear to be broken, with some underlying collagen fibers directly exposed. At this energy density, Bowman's membrane also was ablated.

Clean photobleaching at 30 mJ/cm² did not occur when the beam encountered a stromal keratocyte. In light micrograph images, keratocytes can be seen atop small "islands" of unablated stroma (Fig. 2). Ultrastructural images (Fig. 3) of this material reveal a distinct electron-dense region that appears to be the remnants of a keratocyte. Also visible are nearly intact membrane-bound structures that are found in the keratocyte cytoplasm. It is also of interest to note that in regions in which the keratocyte was fully ablated, ablation of the underlying...
stromal collagen was detected (Fig. 2). Keratocyte nuclei were never observed in the ablation zone, suggesting that the nucleus has a lower ablation threshold than the cytoplasm.

At 35 ml/cm² keratocytes were almost completely ablated. However, some cytoplasmic remnants were still observed at the ablation surface and extending into the surrounding collagen (Fig. 4). At 40 ml/cm² complete ablation of the stromal collagen and keratocytes were observed (Fig. 5). Except for a greater ablation depth, the ultrastructure of the 40-mJ/cm² region was indistinguishable from the control ablation at 146 ml/cm².

To examine the possibility that the storage time in the Optisol solution affected the ablation thresholds, the stromal ablation data of two replicate corneas (30.14 ml/cm² and 30.3 ml/cm²) stored for 5 and 9 days, respectively, in Optisol were carefully compared ultrastructurally. Both corneas exhibited the identical structure as depicted in Figures 1, 2, and 3. In addition, the mean of 12 ablation depth measurements on a control cornea was 80.1 μm. The measured cornea was stored in Optisol for 10 days after enucleation. This value compares favorably with the expected depth of 82 μm for an in vivo human cornea. It is equivalent to a single pulse etch rate of 0.23 μm compared with the expected 0.24 μm per pulse for the in vivo human eye. The 40-mJ/cm² ablation depth on the other half of that same cornea had a mean depth of 21.5 μm. This is equivalent to 0.06 μm of ablation per laser pulse and represents the ablation threshold etch rate.

Mean full corneal thickness measurements excluding corneal epithelium but including Bowman's membrane, Descemet's membrane, and the corneal endothelium for both control corneas were 565 μm and 616 μm.

**DISCUSSION**

This study demonstrates different corneal ablation thresholds for collagen (30 ml/cm²) and keratocytes (40 ml/cm²). The finding that the collagen ablation threshold was significantly below the published human corneal ablation thresholds of 46 ml/cm² to 50 ml/cm² and 40 ml/cm² is not surprising because the molecular components of the stromal collagen are different from those of the keratocytes. The results suggest that the keratocyte nucleus has a lower ablation threshold than the keratocyte cytoplasm. Different cellular structures and compartments with different absorption characteristics would likely have different ablation thresholds.

Although it is arguable that eye bank eyes may yield different thresholds than in vivo human eyes, care was taken in these studies to use human corneas under laser and room conditions similar to those used in clinical treatment. Two corneas were treated at each energy density—with no significant histopathologic differences observed between the replicates, despite the fact that the time in the storage medium varied from between 5 and 10 days for different eyes. In addition, the ablation depth and etch rate (material removed...
per pulse etch rate at the stromal collagen ablation threshold value, thermal loading becomes nearly constant and photoablation is achieved by conducting studies above, at, and below the thresholds.

There is considerable debate in the literature as to the mechanisms of 193-nm ArF excimer laser ablation. The mechanistic debate centers around three mechanisms: thermal (heat), photochemical (bond breaking), and shock wave-related mechanical stresses. The latter two mechanism seem to be favored by more investigators because of the lack of observed thermal damage in the ablation zone, and the fact that the energy of the 193-nm UV photon is sufficient to break molecular bonds.

However there is evidence that suggests a thermal component to the ablation process. For example, Bende et al. studied the temperature inside the cornea during 193-nm excimer laser ablation at energy densities from 10 mJ/cm² to 360 mJ/cm². Heating was observed and demonstrated to be a function of laser pulse frequency and duration. Using thin polymer films, Dyer and Sidhu demonstrated that most of the laser energy is converted to heat, resulting in a temperature rise within the matter. It was suggested that above a threshold value, thermal loading becomes nearly constant and photoablation occurs. Similar results were described for polymethacrylate, in which the threshold ablation energy density was 40 ± 10 mJ/cm². Krueger et al. determined the threshold for the calf cornea to be 50 mJ/cm².

The preceding studies emphasize two points: there probably is a thermal component to the ablation process, and at energy densities below the ablation threshold, the heat is dissipated within the tissue as opposed to being carried away as part of the ablation plume. Thus, the definition of the exact ablation threshold is important. It is likely that there is a combination of mechanisms operating at or near the ablation threshold: heat build-up in the tissue that is not ablated; heat that is carried away with the ablated fragments; and photochemical rupture of molecular bonds resulting in nonthermal ablation. In addition, there is possibly a recoil shock wave that passes through the corneal stroma as material is ejected from the surface. This would explain the observed destruction of the endothelial cells at the base of the cornea even though the intervening stroma appears normal. Stress waves passing through the stroma concurrent with the ejection of photoablated material in the laser plume have been described by Kermani et al.

Regardless of which mechanism or combination of mechanisms contributes to the ablation process, it is important to define the ablation thresholds. This study demonstrates that the human cornea is comprised of structural elements with different ablation thresholds and that the stromal collagen has an ablation threshold below what has been reported previously.

References