Acute Ultrastructural Changes of Cornea After Excimer Laser Ablation

Serdar A. Özler, Lih-Huei Liaw, Joseph Neev, Dan Raney, and Michael W. Berns

Corneal ultrastructural changes induced by an argon fluoride excimer laser using different parameters were investigated. Twenty-eight rabbit corneas were ablated at light doses per pulse and repetition rates ranging from 25-800 mJ/cm² and 1-100 Hz, respectively, at four different total light doses (25-150 J/cm²). Transmission electron microscopy showed that corneal ablations done at subthreshold light doses per pulse with repetition rates higher than 30 Hz and with an exposure more than 100 sec caused significant surface coagulation and an increase in pseudomembrane thickness. These changes were not observed in ablations done above threshold light doses per pulse, regardless of repetition rate and exposure time. However, repetition rates as high as 80 Hz caused damage to the endothelium and Descemet’s membrane at the same ablation depth that did not cause such damage using repetition rates under 40 Hz. It appears that high repetition rates used during excimer laser corneal surgery may cause irreversible damage to the cornea.


The argon fluoride excimer laser has been under investigation since it was used first to ablate bovine cornea in 1983. The optimal ablation parameters have been studied extensively, and it has been found that high light doses per pulse may result in acoustic shock waves and secondary radiation, whereas higher repetition rates may cause thermal or resonance-induced side effects. Thermal loading may be a major side effect during excimer laser corneal surgery. The mechanism of excimer laser corneal surgery is predominantly photochemical; however, conversion of ablated tissue to a gaseous state may involve heating of adjacent tissues. In a previously published study, subthreshold light doses per pulse (23 mJ/cm²), with a long exposure time (100 sec) and a high repetition rate (40 Hz), were used for phototherapeutic keratectomy in rabbits. Theoretically, use of this laser under threshold levels causes little, if any, ejection of corneal material. In this case, the delivered energy is transformed into heat energy that can cause thermal damage to tissue. Although use of higher repetition rates may be beneficial by decreasing the duration of surgery, shock wave-induced tissue resonance, heat deposition, and blockage of the next pulse by the material ejected from the cornea can occur. Furthermore, it has been shown that increasing the repetition rate to over 50 Hz causes less tissue ablation per pulse.

To characterize the mechanism more specifically by which the 193-nm excimer laser exerts its tissue ablation effects and to determine the optimum dosimetry for corneal surgery, we did a quantitative and ultrastructural study. The ablation mechanism was studied by measuring the amount of corneal tissue removed at various light doses per pulse, repetition rates, and total light doses and by electron microscopic examination of corneal tissue from the area immediately adjacent to the region of irradiation.

Materials and Methods

All investigations involving animals conformed to the ARVO Resolution on the Use of Animals in Research.

A Lambda-Physik (Acton, MA) model EMG 103 MSC excimer laser with argon fluoride was used in the stable-resonator configuration. The laser beam was attenuated to achieve the desired energy and focused on the corneal surface. The laser-pulse duration was 15 nsec, and the maximum energy of the laser output was 200 mJ per pulse. The spot size of the excimer laser and actual profile were measured with a linear photodiode array to within 25 μm (Reticon [Sunnyvale, CA] K-Series with 25-μm center-to-center spacing). The most uniform part of the laser beam was used during these experiments. The energy per pulse at the irradiated surface was measured with a joulemeter (ED-500; Gentec, Quebec, Canada).
through a storage oscilloscope (model 7834; Tektronix, Portland, OR).

Both eyes of 14 New Zealand rabbits (weight range, 2–3 kg) were used in this study. All animals were anesthetized with an intramuscular injection of 0.75 ml/kg of ketamine hydrochloride (100 mg/ml) and xylazine hydrochloride (20 mg/ml) in a 1:1 solution. The animals were killed by an intracardiac injection of 1 ml of Eutha-6 (389 mg/ml pentobarbital sodium; Western Medical Supply Co., Inc., Arcadia, CA) per rabbit just before the experiment. The eyes were enucleated, and the epithelium scraped with a no. 64 Beaver (Waltham, MA) blade. The eyes were placed immediately in a paraffin holder that was located on a rotational mount. Intraocular pressure was maintained at 10–20 mm Hg with a hydrostatic column. The corneas next were ablated with a 0.3–0.4-mm rectangular focal spot in four different areas around the optical zone. The ablation zones were approximately 4 mm from each other, and the same laser settings were used in the four different areas.

The corneal ablation parameters are summarized in Table 1. Because one major objective was to complete the ablation procedure in a limited amount of time, we increased the repetition rate sequentially as we decreased the light doses per pulse, maintaining the same total light dose level. In the first four groups, total light doses were changed by increasing exposure time. In the fifth group, however, light dose per pulse and total light dose were kept constant, and only repetition rate and exposure time were changed.

Immediately after ablation, the corneas were fixed with Karnovsky’s fixative (paraformaldehyde 2% and glutaraldehyde 3%). The corneas were excised and stored in 0.1 M cacodylate buffer at 4°C until processing for light and electron microscopy was done. The tissue was postfixed in osmium tetroxide 1% in 0.1 M cacodylate buffer for 1 hr, then rinsed with double distilled water and stained en bloc for 2 hr in Kellenberger’s uranylacetate. Dehydration was done with progressive concentrations of ethanol in water in 10-min steps (30%, 50%, 70%, 90%, 100%, and 100%) and in progressive ethanol in propylene oxide, also in 10-min steps. Infiltration was started with propylene-oxide–epoxy resin substitute (Poly/Bed 812 embedding media; Polysciences, Warminster, PA) in steps of 30 min each (30% and 50%), overnight (70%), and 60 min (100%). The samples were embedded in flat molds, placed at 37°C overnight, and then at 60°C in a vacuum oven for 48 hr. The blocks were trimmed, sectioned (500 nm), and stained with Richardson’s stain for light microscopy. The thin sections (60 nm) subsequently were examined with a Jeol 100 C electron microscope at 80 kV (JEOL USA, Inc., Peabody, MA). Transmission electron microscopy was done on group 3, and samples of all the eyes ablated with light doses per pulse of less than or equal to 100 mJ/cm².

**Results**

A summary of the experimental parameters and groups is presented in Table 1. The light dose per pulse was varied from 25–800 mJ/cm², and total light dose was varied from 25–150 J/cm². Repetition rates were varied from 5–40 Hz for groups 1–4. All exposures in group 5 were at a total light dose of 25 mJ/cm² and a light dose per pulse of 100 mJ/cm². The repetition frequency for this group was varied from 1–100 Hz.

The ablation data are summarized in Figure 1. There were four corneal perforations at total light doses of 100 and 150 J/cm². As seen from the slopes of the graphs, there was a distinct decrease in the percent of ablation depth with an increase in light dose per pulse above 200 mJ/cm². The most efficient ablation occurred at 100 mJ/cm². Generally, there was little or no ablation at 50 and 25 mJ/cm², although we saw a

### Table 1. Corneal ablation parameters

<table>
<thead>
<tr>
<th>Total energy density‡</th>
<th>Fluence*/repetition rate†</th>
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<tr>
<td></td>
<td>800/5</td>
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<tr>
<td>Group 1: 150</td>
<td>38</td>
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<tr>
<td>Group 2: 100</td>
<td>25</td>
</tr>
<tr>
<td>Group 3: 50</td>
<td>13</td>
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<tr>
<td>Group 4: 25</td>
<td>7</td>
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<tr>
<td>Group 5: 25</td>
<td>100</td>
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* Fluence: millijoules per square centimeter (mJ/cm²).
† Repetition rate: Hertz (Hz).
‡ Total energy density: Joules per square centimeter (J/cm²).
§ Time: seconds.

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perforation at 150 J/cm² using a light dose per pulse of 50 mJ/cm².

Because we found corneal swelling in a previous study, careful attention was given to this phenomenon in this study (Fig. 2). In the four corneas that were perforated, edema was greater than 20% of the corneal thickness. In nonperforated corneas, edema was observed to be between 5–10% for ablations that were made at the higher light doses per pulse (400 and 800 mJ/cm²) and the higher total light doses (100 and 150 J/cm²). Swelling was less than 5% of the corneal thickness in samples that were exposed to lower light doses per pulse (50–200 mJ/cm²) and total light doses (25–50 J/cm²). An increase in corneal swelling was observed also when the pulse frequency was increased to 80 Hz for a total light dose of 25 J/cm² at 100 mJ/cm² per pulse (Fig. 3A). In this example, there was also a substantial increase in ablation depth for the 80-Hz specimen compared with the 20-Hz specimen (Fig. 3B), even though the energy parameters were identical for both specimens.

An attempt was made also to characterize the smoothness of the ablated corneal surface as a function of light dose per pulse and total light dose. Because all the pulse frequencies used were between 5–40 Hz (which is slower than the thermal relaxation time of the tissue), it was thought that this parameter probably did not play a role in the observed tissue response. A smoothness scoring system of 1–5 was used: 5, no irregularity at the base of the corneal wound; 4, irregularities less than 3 μm in height at the base of the wound and along the sides of the wound; 3, irregularities greater than 3 μm in the base and along the edges of the wound; 2, jagged and irregular edges to the wound with numerous pits greater than 3 μm in depth at the base of the wound; and 1, obvious thermal necrosis at the base of the wound or along its edges. A summary of these data are presented in Figure 4. In general, there appeared to be an increase in wound smoothness after increasing either the light dose per pulse or the total light dose. At the preferred clinical ablation parameters of 100 mJ/cm² and 50 J/cm², the corneal wounds appeared very smooth.

Careful attention was given to the appearance of the pseudomembrane and additional structural damage to the stroma and Descemet’s membrane. Transmission electron microscopy showed an electron-dense pseudomembrane that increased from 0.1–0.35 μm in thickness as the light dose per pulse increased from 100–800 mJ/cm², even though the total light dose was kept constant at 50 J/cm² (Fig. 5). An increase in pseudomembrane thickness from 0.15–0.25 μm also was observed when the light dose per pulse was increased from 25–150 J/cm². When light doses per pulse of 50 mJ/cm² or less were used (which are generally under ablation thresholds) at total light dose of 100–150 J/cm², a thicker pseudomembrane (0.2–0.5 μm) and zone of thermal necrosis (0.3–0.9 μm) was observed (Fig. 6).

When 80 Hz was used at the light dose per pulse of 100 mJ/cm² and a total light dose of 25 J/cm² or higher, no obvious thermal damage was detected, but disruption of Descemet’s membrane and the endothelium and cracks in the ablation bed were observed (Fig. 7). These structural changes were not observed for pulse frequencies less than 40 Hz.

**Discussion**

Two main theories have been proposed to explain argon fluoride excimer laser corneal ablation as follows: (1) photon-phonon interactions, which are the result of ultrafast thermal events, and (2) photon-in-
duced molecular decomposition, which is not thermal, but photoablative. During the latter type of ablation, the molecular fragments are expelled as effluent. However, excimer laser-exposed polymers undergo a substantial temperature rise of more than 1000°K, and it is reasonable to assume that the ejected corneal fragments attain comparable temperatures. Thermal camera images of the ablated material have demonstrated a substantial temperature rise. With a light dose per pulse level of 150 mJ/cm², the upper limit of repetition rate that does not induce a corneal temperature higher than 45°C is reported to be 82 Hz. In a previous study, we found a thermal spread out of the ablation site with light doses per pulse of 400 mJ/cm² and repetition frequencies of 50 Hz. In addition, slight stromal ablation can be detected with repetition rates as high as 40 Hz and light doses per pulse at or below the molecular decomposition threshold levels. When threshold or below-threshold light doses per pulse are used with high repetition rates, the amount of ejected material is small or even undetectable. Because the ejected material would not remove the excess energy (heat), a rise in temperature would be expected. Our study showed no adjacent tissue damage at the ablation surface caused by high frequencies above threshold levels. Most of the delivered energy should have been converted through photochemical mechanisms. The coagulation necrosis of the superficial layer in 0.3-0.9 μm at the ablation bed supports a thermal mechanism in the ablation process using high repetition rates at sub-threshold light doses per pulse. Therefore, these parameters should be avoided when nonthermal ablation is desired.
Using the 193-nm excimer laser, it was found that the most efficient ablation was at the light dose per pulse level of 200 mJ/cm² using a 10-Hz repetition rate. In our study, the most efficient ablation was at 100 mJ/cm² and 20 Hz. This observation, in addition to the deeper ablation with higher repetition rates at the same total light dose, suggests that, at higher repetition rates, accumulated heat and mechanical stress contribute to tissue ablation.

When we increased the light dose per pulse while maintaining the same total light dose, we observed a smoother surface. At light doses per pulse above 600 mJ/cm², irregularities in the beam profile may not result in differential ablation, and the irregularities will not be imprinted on the cornea. This finding is similar to the results of others. In addition to the increased smoothness with an increase in light dose per pulse, we also observed increased surface smoothness with increased total light doses at each light dose per pulse. This might be explained by spatial jitter in the laser beam. Because beam inhomogeneities average out over time, the ablation of tissue may become smoother.

The pseudomembrane, an electron-dense material, is a characteristic of 193-nm excimer laser ablation of the cornea. We observed a slight increase in pseudo-
Fig. 6. Ablation with subthreshold levels in association with higher repetition rates (over 30 Hz), and longer exposure (over 100 sec) caused surface coagulation of the corneas. Transmission electron photomicrograph demonstrates the surface coagulation in 1-μm thickness. Bar = 1 μm.

membrane thickness as we increased the light dose per pulse or total light dose. At subablation thresholds, in addition to tissue coagulation, an increase in the thickness of pseudomembrane was observed along the walls of the ablation crater, possibly as a result of an increase in temperature.

When we compared the 80-Hz and 100-Hz samples at the same total light dose, significantly less tissue ablation was observed with the latter. Others reported that repetition rates greater than 50 Hz cause less tissue ablation per pulse. This may be caused by absorption of succeeding pulses by the laser ejected plume.

When corneal tissue was ablated to almost the same ablation depth using a constant light dose per pulse (100 mJ/cm²), with variation in repetition frequency (either 80 Hz or 20 Hz), different tissue damage was observed. Epithelial damage was found when lower repetition rates (10 Hz) were used and when ablation was as close as 40 μm to the endothelium. In our study, the higher repetition rate (80 Hz) produced damage to Descemet's membrane and the endothelium and cracks in the surface of the ablation bed. This damage could be a result of secondary fluorescence or acoustic shock waves.

Our observation of corneal swelling further confirmed that it might occur if inappropriate laser parameters are used. However, the amount of swelling probably is insignificant if light doses per pulse are kept below 200 mJ/cm² and the energy delivered to an ablation site is less than 50 J/cm².

In conclusion, we described our findings in a series...
of 193-nm excimer laser ablations of the cornea. As more clinical systems are developed and applied to the refractive and therapeutic treatment of the human cornea, it is important to elucidate the mechanisms of ablation and define further (quantify) the optimal parameters for laser exposure. Our data should contribute to the further refinement of these parameters.

**Key words:** cornea, laser–tissue interactions, refractive surgery

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**References**