Ablation Rates and Surface Ultrastructure of 193 nm Excimer Laser Keratectomies

Mauro Campos,* Xun Wei Wang,* Lars Hertzog,* Martha Lee,* Terry Clapham,† Stephen L. Trokel,‡ and Peter J. McDonnell*

Purpose. To determine whether photorefractive keratectomy can be performed at lower energies than are currently employed in clinical trials.

Methods. Fresh pig corneas were ablated using a clinical excimer laser to study the effects of various energy densities (100–200 mJ/cm²) and beam diameters on ablation rates and on the surface ultrastructure of the ablated cornea.

Results. A 20-mJ increase in energy density was associated with a 0.03 nm per pulse increase in the ablation rate. A nearly linear increase in the pseudomembrane thickness occurred with increasing energy densities ($r^2 = 0.83$) or decreasing ablation area diameter ($r^2 = 0.86$).

Conclusions. Our findings suggest that fluences less than those currently used in clinical trials (160–180 mJ) are capable of ablating tissue while producing thinner electron-dense pseudomembranes on the corneal surface. The relationship between pseudomembrane thickness and clinical factors such as reepithelialization and postoperative haze remains to be determined. Operating at lower fluences does have the advantages of allowing larger diameter ablations, reducing possible shockwave damage, and reducing the maintenance requirements for the laser. Invest Ophthalmol Vis Sci. 1993;34:2493–2500.

The argon fluoride excimer laser ablates corneal tissue with high precision and with minimal damage to adjacent tissue. Based on this ability, the excimer laser has been proposed as a tool for performing refractive procedures on the cornea. The success of laser keratectomy for correction of refractive errors depends on the ability to induce a smooth lamellar bed that reepithelializes with minimal stromal regrowth and epithelial hyperplasia. Variables that could affect reepithelialization and healing of the cornea have been studied in animals and in humans, and include edge profile, wound depth, size of the ablated zone, homogeneity of the laser beam, and edge of the mask used to shape the beam. Departures from a smooth surface might degrade clinical results. An irregular epithelial interface, or new collagen production with scarring of the tissue might induce scattering of the light, and thereby decrease visual acuity and cause regression of the effect. The interaction of the excimer laser and the corneal tissue, the so-called ablative photodecomposition, produces a localized removal of tissue at the laser-irradiated area. After surface ablation, the surface of the ablated area becomes covered by a relatively electron-dense layer of material, known as a pseudomembrane, the precise nature of which is undetermined. The thickness of the pseudomembrane is reported to vary between 100 and 500 nm, and the smoothness of the surface has been reported to depend on repetition rates, radiant exposures, use of expanding and contracting diaphragm.
apertures and removal of vaporized debris during laser treatment.5,8,18-20

Studies that describe variables, such as fluence or repetition rate, that might affect the thickness of the pseudomembrane and the smoothness of the ablated surface have used a laboratory excimer laser with no beam processing,1,3,13,15-17,20-22 which differs substantially from the instrumentation currently used in clinical trials.2,3,25 This report describes the surface ultrastructure and quantitative ablation rates of fresh porcine corneas treated with a beam-processed clinical excimer. The system includes beam homogenizers and rotators that provide spatial and temporal integration of the beam. The energy distribution within the diameter of interest does not vary more than 5%. Our study focuses on the possible influences of different ablation area diameters and beam fluences on the laser interaction with the cornea.

MATERIALS AND METHODS

Forty-four fresh pig eyes were obtained from a local abattoir (Farmer John, Vernon, CA). Eyes were kept on ice until surgery, which was performed within 6 hr of death. Preoperative intraocular pressures were in the range of 12–15 mmHg, as measured with a Tonopen (Oculab, Glendale, CA). All eyes were deepithelialized mechanically using a blunt Paton spatula. Immediately after deepithelialization, corneal thickness was measured using an ultrasonic pachometer (Storz Co., St. Louis, MO) and the average of three measurements was recorded. Excimer laser ablations were performed immediately after the measurements. The eyes were placed in a foam head mold and the laser beam was centered on the entrance pupil. The globes were examined from the side to ensure that the beam would be normal to the corneal surface.

Ablation Rate Calculations

Thirty-three eyes underwent a circular ablation of uniform diameter (as in phototherapeutic keratectomy) until perforation of the cornea was noted by the surgeon using the operating microscope. A commercially available excimer laser (VISX, Sunnyvale, CA) was used to perform all ablations. These eyes were divided into two groups. In Group 1 (18 eyes), six sets of three eyes were ablated using a different energy density (200, 180, 160, 140, 120, and 100 mJ/cm²; maximum of ± 5 mJ variation at each energy density used) in each set. The ablation area diameter was constant (5 mm), and pulse rate was 5 Hz. In Group 2 (15 eyes), five sets of three eyes were ablated using a different ablation area diameter (6, 5, 4, 3, and 2 mm) in each set. The energy density was constant (180 mJ/cm²), and pulse rate was 5 Hz. No nitrogen gas was blown above the plane of the cornea to use aspirate ejected debris during the ablation. The total number of pulses necessary to perforate each cornea was recorded. A brisk egress of fluid was noted at this time in all corneas, and inspection revealed a uniform diaphanous layer of residual tissue in the bed of the ablation. The ablation rate was determined by dividing the preoperative corneal thickness by the total number of pulses.

Characterization of Excimer Laser Beam

To measure the homogeneity of the excimer laser beam, we used a beam profile analyzer (StarTech Instruments, Inc., Danbury, CN) located at the corneal plane. Data were analyzed and graphically demonstrated using Beamcode, a commercially available computer program (Big Sky Software, Bozeman, MT).

Surface Ultrastructure Studies

Ablations on another 11 pig eyes were performed in identical fashion as just described, but instead of ablating the entire corneal thickness, these eyes underwent a myopic ablation designed to correct 5 diopters. Thus, six eyes were operated on under the different energy densities tested above (200, 180, 160, 140, 120, and 100 mJ/cm²) using the same ablation zone diameter (5 mm), and the remaining five eyes were operated on under the same energy density (180 mJ/cm²) but with the different diameters tested previously (6, 5, 4, 3, and 2 mm). After ablation, all eyes were immediately prepared in identical fashion for transmission electron microscopy. The whole globes were immersed in half-strength Karnovsky fixative (2% paraformaldehyde, 2.5% glutaraldehyde, and 0.1 M sodium cacodylate buffer). The globes were fixed at physiologic pressure by infusion of fixative into the vitreous cavity. After 48 hr of fixation, the corneas were trephined, bisected, and the two central specimens obtained were studied. These were washed in 0.1 M sodium cacodylate buffer, postfixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer for 2 hr, washed in 0.1 M sodium cacodylate buffer, dehydrated in a graded series of alcohol and then in pure polypropylene oxide, and embedded under vacuum in eponate (epoxy) plastic resin.

For transmission electron microscopy, 60–80 nm thick sections were cut with a diamond knife ultramicrotome. Tissue was mounted on copper grids and stained with uranyl acetate-lead citrate. Sections were examined independently by two examiners who were ignorant of the treatment protocol. The examiners analyzed two representative photomicrographs from the ablated area of each of the specimens. The thickness of the surface pseudomembrane was determined by each examiner in three different locations of the photomicrograph, and then averaged. Calipers were used to...
span the pseudomembrane thickness and then applied to a standardized scale. The presence of any discontinuities in the surface pseudomembrane was determined by examining in their entirety each of the sections with the transmission electron microscope. Documentary photographs were taken of each section (10 micrographs per quartile of ablation zone).

Statistical Analysis

The relationships of ablation rate to fluence, thickness of the pseudomembrane to fluence, and thickness of the pseudomembrane to ablation area diameter were examined by the linear regression technique. Analysis of variance was employed to test the differences in ablation rate as a function of ablation area diameter.

RESULTS

In the absence of blowing nitrogen gas over the surface of the corneas being ablated, the appearance through the operating microscope was that of a moist layer over the entire ablated surface.

Ablation Rate

The ablation rates from 33 pig eyes that underwent corneal ablations with the excimer laser are presented in Figures 1 and 2. The data show a nearly linear increase in the ablation rate with increasing fluence. A 20-mJ increase in energy density was associated with a 0.03 μm per pulse increase in the ablation rate. The eyes ablated with energy fluence of 100 mJ/cm² had an ablation rate of 0.12 ± 0.005 μm per pulse (mean ± SEM), increasing to 0.15 ± 0.03 μm per pulse at 120 mJ/cm², 0.21 ± 0.008 μm per pulse at 140 mJ/cm², 0.24 ± 0.008 microns per pulse at 160 mJ/cm², 0.27 ± 0.0004 μm per pulse at 180 mJ/cm² and 0.27 ± 0.0002 at 200 mJ/cm². Figure 2 shows the ablation rate plotted against the ablation area diameter. The ablation rate increased with increasing ablation area diameter from 2 to 4 mm (0.28 ± 0.04, 0.30 ± 0.02 and 0.31 ± 0.009 μm per pulse, respectively, for 2, 3, and 4 mm) and then decreased as the diameter of the ablated area increased to 5 and 6 mm (0.28 ± 0.03 and 0.24 ± 0.015 microns per pulse, respectively). The dif-

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933174/)

**FIGURE 1.** Graph of ablation rate in pig corneas as a function of 193 nm excimer laser ablation diameter. Error bars represent standard errors.

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933174/)

**FIGURE 2.** Graph of ablation rate in pig corneas as a function of 193 nm excimer laser ablation diameter. Error bars represent standard errors.

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933174/)

**FIGURE 3.** Graph of pseudomembrane thickness in pig corneas as a function of 193 nm excimer laser fluence. Error bars represent standard errors.
FIGURE 4. Transmission electron micrographs of pig corneas after ablation using a fluence of 200 mJ/cm² (top), 180 mJ/cm² (middle) and 160 mJ/cm² (bottom). Pseudomembrane revealed a smooth and regular appearance (original magnification, ×25,000).

FIGURE 5. Transmission electron micrograph of pig corneas after excimer laser ablation using a fluence of 140 mJ/cm² (top), 120 mJ/cm² (middle) and 100 mJ/cm² (bottom). Pseudomembrane thickness increased with increasing fluences. An increasingly irregular appearance of the pseudomembrane and an increased frequency of discontinuities or breaks in the pseudomembrane were noticed with decreasing energy fluence (original magnification, ×25,000).
Surface Ultrastructure Studies

When the surface of the ablated area was examined ultrastructurally, it was apparent that the pseudomembrane had an increased thickness and a more regular appearance with increasing fluence between 100 and 180 mJ/cm². The thickness of the pseudomembrane as a function of fluence are shown in Figure 3. The average thickness (± SE) of the pseudomembrane of the corneas operated on under 100, 120, 140, 160, 180, and 200 mJ/cm² was, respectively, 36.4 ± 21.0, 91.0 ± 10.5, 138.8 ± 20.0, 206.4 ± 12.1, 249.9 ± 24.0, and 258.0 ± 23.7 nm. The data show a nearly linear increase in the pseudomembrane thickness with increasing fluences ($r^2 = 0.83$). No discontinuities or gross irregularities were present in pseudomembranes formed with fluences from 200 to 160 mJ (Fig. 4). An irregular appearance of the pseudomembrane and an increased frequency of discontinuities or breaks in the pseudomembrane were noticed on the corneas when the operating fluences were lowered from 140 to 100 mJ/cm² (Fig. 5).

Similar measurements of pseudomembrane thicknesses as a function of the ablated area diameter is shown in Figure 6. The averaged thickness (± SE) of the pseudomembrane of the corneas having a 2, 3, 4, 5, and 6 mm ablation area diameter were, respectively, 263.3 ± 29.6, 123.0 ± 45.3, 170.0 ± 10.0, 58.3 ± 42.1, and 33.0 ± 0.0 nm. The data show a nearly linear decrease in the pseudomembrane thickness with increasing ablation area diameter ($r^2 = 0.63$). Figure 7 shows the ultrastructure of the pseudomembrane in pig corneas ablated with different diameters. Using a 2-mm ablation diameter, two distinct zones were seen in the pseudomembrane of different electron densities (Fig. 7, left). Using a 6-mm ablation diameter (currently used clinically in photorefractive keratectomy), the pseudomembrane was thinner than when smaller ablation diameters were used, but the surface presented a regular appearance, with consistent thickness of the pseudomembrane (Fig. 7, right).

Beam Profile Studies

Measurements of energy across the diameter of the beam revealed regional variability of energy intensity
FIGURE 8. Three-dimensional graphic representation of laser energy distribution in the corneal plane across the beam. Beam irradiance is uniform to within ± 5%.

on the order of 5% or less. Three-dimensional graphic display of beam energy (Fig. 8) illustrates the homogeneity of the processed beam at the corneal plane.

DISCUSSION

As in previous studies using laboratory lasers,

we demonstrated increasing depth of ablation per pulse as laser fluence was increased from 100 to 180 mJ/cm²; the depth per pulse at 200 mJ/cm² was not measurably increased over that at 180 mJ/cm². We have also demonstrated a measurable ultrastructural difference between corneas ablated at different energies, with a positive correlation between fluence and thickness of the electron dense pseudomembrane present on the surface of the cornea. Also, at lower energy levels, discontinuities in the pseudomembrane were more common than at higher energy levels. A nearly linear relationship was demonstrated when analyzing ablation rate as a function of fluence, pseudomembrane thickness as a function of fluence and pseudomembrane thickness as a function of ablation area diameter.

The term “pseudomembrane” may be an unfortunate one, because this probably is a damage zone. Intuitively, we believe it would be best for this superficial layer of the ablated cornea to be as thin and smooth as possible. We have shown previously that the number of discontinuities in the pseudomembrane differs depending on whether a constricting or expanding diaphragm is used; in a small study performed on rabbits, however, differences in epithelial healing rates and amount of stromal haze between the two groups were not demonstrable. We believe it is appropriate to consider studies to determine the exact nature of this pseudomembrane and to determine what role it might play, positive or negative, in the postoperative wound healing process.

Previous studies have not examined the possibility that ablation rate varies with the diameter of the ablation zone. Many such studies examined only very small diameter spots or slits, unlike the large-diameter ablations currently used in human clinical trials of phototherapeutic and photorefractive keratectomy. In our study, we noted very little variability in ablation depth per pulse as ablation diameter was varied in 1-mm increments from 2 to 6 mm. One possible explanation for the small amount of variability observed involves the distribution of stromal fluid in the cornea as it is being ablated. When the surface being ablated is not dried with nitrogen gas during the procedure, a wet film is observable over the surface during the treatment. As the diameter of the ablation zone is increased, the circumference of the ablated-nonablated junction increases, and this may affect how much fluid is present in the region being ablated. The distribution of fluid over the area of treatment may affect the local rate of tissue ablation.

The term “pseudomembrane” may be an unfortunate one, because this probably is a damage zone. Intuitively, we believe it would be best for this superficial layer of the ablated cornea to be as thin and smooth as possible. We have shown previously that the number of discontinuities in the pseudomembrane differs depending on whether a constricting or expanding diaphragm is used; in a small study performed on rabbits, however, differences in epithelial healing rates and amount of stromal haze between the two groups were not demonstrable. We believe it is appropriate to consider studies to determine the exact nature of this pseudomembrane and to determine what role it might play, positive or negative, in the postoperative wound healing process.

Previous studies have not examined the possibility that ablation rate varies with the diameter of the ablation zone. Many such studies examined only very small diameter spots or slits, unlike the large-diameter ablations currently used in human clinical trials of phototherapeutic and photorefractive keratectomy. In our study, we noted very little variability in ablation depth per pulse as ablation diameter was varied in 1-mm increments from 2 to 6 mm. One possible explanation for the small amount of variability observed involves the distribution of stromal fluid in the cornea as it is being ablated. When the surface being ablated is not dried with nitrogen gas during the procedure, a wet film is observable over the surface during the treatment. As the diameter of the ablation zone is increased, the circumference of the ablated-nonablated junction increases, and this may affect how much fluid is present in the region being ablated. The distribution of fluid over the area of treatment may affect the local rate of tissue ablation.
Our data on pseudomembrane thickness differ from those of Fantes and Waring, who reported no change in thickness as radiant exposures were increased in eight irregular increments from 50 to 850 mJ/cm². In their study, however, they reported that their laser had a nonuniform energy distribution. Van Saarloos and Constablereported the same problem, and noted that "a hot spot within the beam could perforate the cornea before the rest of the cut was more than half way through." A beam from an industrial laser has focal spots of high and low density that may vary markedly. The homogenizing optical elements employed in many current lasers used clinically, including the laser used in our study, reduces hot and cold spots on the corneal surface being ablated. The laser used in our study is engineered such that it achieves a uniform irradiance across the beam diameter of within 5%.

Our data suggest that it may be feasible to perform photorefractive keratectomy at substantially lower energies than are now employed in clinical trials. Operating at lower fluences offers the advantages of reducing the maintenance requirements for the laser (particularly prolonging the service life of the mirrors), allowing larger diameter treatment zones, and possibly reducing the shock wave damage to the cornea. Conversely, operating at lower fluences resulted in greater variability in ablation rates per pulse and pseudomembrane thickness and uniformity. The potential clinical implications of these findings are not known.

Our study did not examine two variables that may affect stromal ablation: repetition rate and possible variability in ablation rate between anterior and posterior stroma. Our study used only the repetition rate of 5 Hz (currently used clinically with the VISX laser). It is clear that careful quantitative studies will be necessary to determine and quantitate the multiple factors that may contribute to the amount of tissue removed by the excimer laser and the nature of the ablated surface.

**Key Words**

excimer laser, photorefractive keratectomy, pseudomembrane, electron microscopy, myopia

**References**


