Human Excimer Laser Keratectomy: Short-Term Histopathology

BY FRANCIS A. L’ESPERANCE, JR, MD, DANIEL M. TAYLOR, MD, AND JOHN W. WARNER, Ph.D

ABSTRACT: The first human trial using the argon fluoride excimer laser to produce a superficial keratectomy in the initial three of a series of ten human eyes is described and includes the clinical appearance and short-term histopathologic evaluation of these three eyes. During an interval of less than 64 days, all three eyes reepithelialized completely, showing absolute clarity of all layers of the cornea, while the patients experienced minimal discomfort despite gross abnormalities of other structures and components of these eyes.

Histopathologically, at an average ablation depth of 30 to 40 microns below the surface of Bowman’s membrane, the epithelium appeared to be slightly hyperplastic, but firmly adherent to the underlying stromal fibers in the ablated areas. There was a slight electron-dense border at the epithelial-stromal interface, but no evidence of inflammation or abnormal keratocyte activity was noted during this interval. Further pathophysiologic and pharmacologic investigations will be required to perfect this new procedure.

The potential of far-ultraviolet laser radiation as a tool in ophthalmic surgery was initiated by reports from scientists in both the health sciences and the materials and electronics fields. It was reported that ultraviolet radiation emitted by excimer lasers could be used to etch submicron-sized patterns into the surface of plastics and other polymers with a remarkable degree of accuracy and with no degradation of nearby unirradiated areas. This type of radiation was also found to interact in a similar manner with animal tissue. The enormous potential of this unique laser-tissue interaction, if applied to human corneal tissue, led to research investigations of the incisional and, more importantly, the overall photoetching capabilities of far-ultraviolet laser spectral emissions for the purposes of establishing a reconstructive superficial keratectomy and/or a new refractive curvature on the anterior surface of the cornea (L’Esperance FA Jr, Method and Apparatus for Ophthalmic Surgery, and Method for Performing Ophthalmological Surgery, U.S. Patents 4,665,913 and 4,669,466.) We describe the clinical and histopathologic appearance of the first three human eyes treated by argon fluoride excimer laser irradiation to produce a controlled superficial lamellar keratectomy.

Our efforts in the application of far-ultraviolet laser radiation were based on the following three important assumptions:
1. That radiation from a far-ultraviolet laser would, upon impacting the anterior surface of the cornea, remove a specific volume of anterior corneal tissue.
2. That the laser could be manipulated (preferably under computer control) in such a way as to perform a reconstructive superficial keratectomy or to sculpt, reprofile, or recontour the cornea so as to correct myopic, astigmatic, and hyperopic refractive errors.
3. That the keratectomy or recontouring procedure, which would remove superficial scarred corneal tissue or sculpt a new curvature on the anterior surface of the cornea, could ablate Bowman’s membrane partly or completely; and, most probably, ablate the anterior stroma as well without creating minute opacifications or scarring of the remaining stroma or that portion of Bowman’s membrane that had not been removed by the process of photoablative decomposition.

Classical teachings from 1979–1983 suggested that...
any impact, incision, or manipulation of Bowman's membrane and the underlying anterior stroma would, in most cases, result in a moderately dense opacification that would hinder the passage of light rays through the cornea toward the retina. By the nature of this new technique, the purposeful volumetric removal of a portion of the cornea's anterior surface in such a way as to remove opacified anterior corneal tissue or create a new curvature for the correction of various refractive errors would necessarily involve the optical zone of the cornea. Therefore, considering the critical corneal area involved, our third assumption (that clarity could be maintained if the removal process is extremely delicate, as can be provided by the excimer laser) was a considerable departure from the classical teachings of corneal physiology and appeared to be the most monumental obstacle to the success of the project.

During this interval in the early 1980s, the work of Srinivasan et al\textsuperscript{2,3} at the IBM Thomas J. Watson Research Center demonstrated that high energy ultraviolet photons from excimer lasers, primarily the argon fluoride excimer laser at 193 nm, could photothermoplastics such as polyethylene terephthalate, polyimide, and polymethylmethacrylate with great precision without apparent photothermal effects. Soon thereafter, Linsker, Srinivasan, Wynne, et al\textsuperscript{14} in early 1983 showed that hair, aorta, and cartilage could be etched, incised, or contoured with great accuracy, partially confirming our original concept. In late 1983, argon fluoride excimer laser incisions into enucleated bovine eyes were produced by Trokel et al\textsuperscript{13} and later by Puliafito\textsuperscript{10} and Marshall\textsuperscript{8} et al. The processes of corneal area ablation, excision of the superficial cornea, and repilling of the anterior surface of the cornea were later tested in rabbits and monkeys, and found to be technically feasible.\textsuperscript{6}

During most investigations concerning this procedure, the argon fluoride excimer laser, with an emission wavelength of 193 nm, was used, producing results in animals that were similar in many ways to those created previously in plastics and described extensively by Srinivasan et al.\textsuperscript{2,15}

Materials and Methods

The Questek Model 1020 TOPS excimer laser combined with a delivery system to the eye consisting of enlarging apertures (myopia correction) or peripheral annuli (hyperopia correction) or enlarging rectangular slits (astigmatism correction) have been used throughout the human corneal surgical investigation (Figure 1). This system was used for both primate investigations at the University of Connecticut, for clinical studies at the Danbury Hospital at Danbury, Connecticut, and at the New Britain General Hospital at New Britain, Connecticut. All systems were designed by the Taunton Technologies, Inc., group; the surgical procedures currently under development are called Laser Reconstructive Keratectomy or Laser Refractive Keratectomy (LRK).

The patient's eye is viewed binocularly through an optical microscope system so that the scarred corneal zone or the central portion of the cornea to undergo keratectomy is aligned with the laser beam axis for excision or for the recontouring procedure. A light-emitting-diode keratoscope, an integral part of the system, captures the reflected corneal image, digitizes and analyzes it, and provides a printout. Patient positioned precisely by optical microscope (right) and side viewing microscope (left).
for the laser’s optics and the eye to be perfectly aligned throughout the procedure. Anesthesia of the cornea and anesthesia and akinesia of extraocular muscles is produced by a retrobulbar or peribulbar injection of 2% lidocaine with Wydase added. Because the vacuum ring could become less securely attached to the limbal area due to the distorted conjunctiva, care is taken not to inflate the conjunctival area with excessive amounts of lidocaine.

The actual excisional, superficial keratectomy or recontouring corneal procedure is performed as follows: Once the eye has been stabilized by the vacuum fixation device, the epithelium is removed with a no. 57 “hockey stick” Beaver blade to a distance approximately 1 mm outside of the proposed impact site, thereby creating a denuded area 5 to 8 mm in diameter depending on the intended keratectomy zone size. When all fragments of the epithelium have been removed from Bowman’s membrane, the eye is aligned with the laser beam axis, as represented by a crosshair reticle in the binocular microscope. Once aligned, photoablation begins immediately, at a repetition rate of approximately 10 Hz and with a pulsed energy density or fluence of 80 to 125 mJ/cm² at the cornea. The procedure requires 35 to 45 seconds to create, from the surface of Bowman’s membrane into the anterior stroma of the patient’s eye, an ablation to a depth of 30 to 40 microns.

All eyes were treated with energy densities (fluences) in the range of 80 to 125 mJ/cm² with a pulsed repetition rate of 10 Hz. The diameter of all impact areas was 3 or 5 mm, and the approximate tissue removal measured from Bowman’s membrane was 30 to 150 microns in depth.

In most cases, the impact site is photographed and analyzed by the digital keratoscope immediately after exposure. Balanced salt solution is placed on the ablated area, erythromycin ointment is placed in the conjunctival cul-de-sac, and the upper eyelid is drawn down over the cornea and taped to the lower eyelid. An eye pad is placed over the eye, and the procedure is considered terminated at that point.

The upper eyelid is kept taped to the lower lid until the epithelium is healed (usually 48 to 72 hours) and
various eye drops or ointments are placed in the conjunctival cul-de-sac during this interval. An eye pad is used only to absorb excessive tearing.

**Case Reports**

Presented below is a review and summary of the first three of ten human eyes that have been treated by the argon fluoride excimer laser at 193 nm. In order to reduce the length of the manuscript and to emphasize the acute short-term histopathology, we do not discuss all ten cases.

The ten cases fall into three groups: three enucleated eyes (eyes 1, 2, 3), an eye that received a corneal transplant, and six blind eyes that will be followed for the long-term effects of excimer laser surgery. The corneas from the enucleated eyes underwent histopathologic studies and those results are reported.

**CASE 1.** A 29-year-old construction worker developed a large choroidal malignant melanoma in his blind right eye for which conservative treatment failed and enucleation was recommended (Figure 3). His cornea was within normal limits for his age. After a 30 micron laser ablation of a 3-mm central corneal area, his eye healed well with full re-epithelialization approximately 64 hours after surgery. The patient experienced no discomfort during the postoperative period, and the eye was enucleated 3½ days after the laser procedure.

**CASE 2.** A 68-year-old man had a malignant melanoma of the iris and ciliary body in the right eye for which multiple procedures, including iridectomy and partial cyclectomy, had been performed (Figure 4). His eye was blind and not salvageable. His intraocular pressure was 51 mm Hg, and he had a hyphema. We performed an approximately 35 micron ablation of the central cornea in an area 4 mm in diameter, which re-epithelialized within 64 to 68 hours. The cornea remained perfectly clear 12 days after laser irradiation, at which time enucleation was performed. The healing process was not affected by the high intraocular pressure and blood in the anterior chamber.

**CASE 3.** A 44-year-old man had a large malignant melanoma impinging on the optic nerve and growing far into the vitreous from the nasal portion of the retina of his right eye, thereby permitting 20/20 macular vision (Figure 5). A 4-mm circular ablation, approximately 35 to 40 microns in depth, was performed. The epithelium regrew completely within 70 hours. The eye was enucleated seven days following laser irradiation, and the cornea was perfectly clear without stromal speckling or without any reaction to the interface between the stroma and the epithelium.

**Results**

**Clinical.** The eyes exhibited the following features during the short interval between laser treatment and enucleation.

1. All corneas healed within 64 to 70 hours with complete re-epithelialization.
2. One patient experienced no discomfort, and the other two experienced minimal discomfort relieved by aspirin or Tylenol.
3. All eyes appeared clinically clear by biomicroscopic examination (retro-illumination and direct inspection), as well as by slit-lamp photography.
4. All other layers and elements of the cornea appeared entirely within normal limits, with no indication of any endothelial or Descemet’s membrane effect.
5. There appeared to be no anterior chamber reaction with neither evidence of cells or protein accumulation (flare) nor any change in the dynamics of the iris or pupil.

**Histopathologic.** The corneal tissue obtained from these patients was studied by scanning and transmission electron microscopy, as well as light microscopy. The following histopathologic events were shown to have occurred during the healing process (Figures 6-9).

1. The irradiation of Bowman’s membrane in the human corneas by excimer laser radiation at 193
nm ablated Bowman's membrane in the target area, leaving no residue and creating a relatively smooth surface on the underlying stroma. The degree of smoothness of the stroma appeared to be in the range of 5 ± 2 microns and was uniform throughout the area of impact. The depth of the ablations was estimated to be 30 to 40 microns.

2. The smoothness of the new anterior surface of the cornea allowed the epithelium to grow across the denuded epithelial area in a rapid fashion and to be secured firmly to the underlying stroma in a manner similar to an intact Bowman's membrane. Histopathologically, there appeared to be little difference between the epithelial cells attached to ablated areas and those attached to normal Bowman's membrane. The epithelial cell process interdigitated with stromal fibers in the ablated areas.

3. The surface of the stroma in ablated areas appeared by transmission electron microscopy to have a slightly more electron-dense border than that in apposition to Bowman's membrane in the non-ablated areas.

4. There was no evidence of any inflammation in any areas of the cornea.

5. The epithelium healed over the area of ablation,
creating normal-appearing cells, although with some thickening and overproduction of the epithelium in the ablated region. The epithelium appeared to be approximately 40% to 50% thicker than that sectioned in the non-treated areas.

6. The transition from the normal Bowman's membrane to the ablated and completely removed area of Bowman's membrane and bare stroma was even and graduated.

The basic conclusions noted from the first three patients are as follows:
- Tissue removal occurred at rates that were consistent with reports in the literature;
- No adverse conditions or events occurred during the ablation process, and the patients all tolerated the procedure well;
- Re-epithelialization occurred within normal periods of time;
- The epithelium bonded well to the newly ablated surface of the stroma, and an epithelial defect did not occur;
- The new epithelium was somewhat thicker postoperatively, but the ablated area did not completely fill in with a new layer of tissue;
- The corneas remained completely clear, both grossly and under slit-lamp examination;
- The adjacent cornea was unremarkable and an inflammatory reaction was conspicuously absent;
- The deeper stroma, Descemet's membrane, and the corneal endothelium were also unremarkable.

Discussion

The introduction and initial use of human excimer laser-induced superficial lamellar keratectomy procedures has been successful, but the long-term results, long-term corneal stability and clarity, and the basic corneal pathophysiologic reaction to excimer laser irradiation remain poorly understood. The results of our human studies and animal studies by a number of investigators during the past few years have shown that our original concept for removing a precisely shaped volume of tissue by ultraviolet radiation from the anterior cornea to excise abnormal or opacified cornea or to correct various refractive errors by establishing a new radius of curvature of the anterior portion of the cornea is feasible. In the future, photoablative reconstructive lamellar keratectomy involving the precise volumetric removal of anterior corneal tissue by laser irradiation to excise areas of opacified, scarred, or abnormal cornea could be possible. Photorefractive keratectomy involving the precise volumetric removal of anterior corneal tissue by laser irradiation to create a new anterior corneal curvature to correct refractive errors may also be used in certain cases.

The properties of tissue photoablative decomposition that are fundamental to this type of surgery and that have been demonstrated histopathologically in animals in our investigations and in other studies can be summarized as follows:
- The limited penetration of the cornea by ultraviolet radiation at 193 nm and the nature of the photoablative process restricts the effect of each laser pulse to a superficial layer with only a narrow zone of conduction damage to adjacent tissue.
- The amount of tissue removed per laser pulse is energy dependent once the ablation threshold has been obtained. Tissue may thus be removed from the target surface in predictable increments.
- The ablative surface is exceptionally smooth, leaving a suitable substrate to preserve the new optical properties of the eye with regrowth of the overlying epithelium.

Physiologically, the following requirements had to be satisfied during the healing process, many of which were poorly understood:

1. It was considered that epithelial migration across the wound must proceed without significant delay or loss of adhesion to the underlying Bowman's membrane or corneal stroma.
2. The ablative process should not result in visually significant Bowman's membrane or stromal opacification from either acute thermal effects or subepithelial scarring from tissue repair.
3. The contour of the repolished or excised surface must remain constant within narrow limits following either reconstructive or refractive superficial keratectomy. It became apparent that a tendency either for the forward movement of thinned cornea or for epithelium and new stromal collagen to fill ablated or repolished areas might defeat the long-term objective of a clear corneal with a predictable or stable refractive change.

Previous animal investigations have reinforced the concept that reconstructive and refractive keratectomy procedures can be performed in a relatively safe fashion. The keratectomy results obtained from these first FDA-approved human trials, using the argon fluoride excimer laser at 193 nm, also indicate that the data obtained in rabbits and particularly in monkeys are comparable and can be related directly to those obtained in humans. The gross and histopathologic findings of this initial excimer laser study with intact living human corneas are promising, and the procedures of laser superficial keratectomy and laser refractive keratectomy appear to avoid most of the serious complications associated with other current surgical methods.

At 193 nm, the laser keratectomy procedures give excellent control over the depth of tissue removal, producing a hitherto unobtainable smoothness in the lamellar section of the stroma without the apparent introduction of thermal damage to tissues adjacent to the area of ablation. In addition, the healing process begins rapidly, because only limited tissue reorganization is required. Other parts of the human cornea

Journal of Refractive Surgery 123
Laser Keratectomy

such as the deeper stroma, Descemet’s membrane, and corneal endothelium are not damaged histopathologically by the procedure as previously noted in other animal studies.\(^8,10,12\) No other form of surgical intervention can compete with these particular attributes.

It therefore appears that the argon fluoride excimer laser-induced incisions fulfill the corneal surgeon’s strictest criteria for an ideal cutting instrument, producing infinitely smooth lamellar sections, with sealed surfaces and no damage to adjacent tissues.

As demonstrated by gross and histopathologic examination of these first human corneal area ablations, the laser procedures called phototherapeutic lamellar keratectomy for the excision of anterior corneal opacifications or photorefractive keratectomy for corneal repolishing or recontouring appear to be feasible. These keratectomy techniques create a predictable removal of anterior corneal tissue that can modify the curvature of the cornea in such a way as to clarify corneas or correct refractive errors of the eye without scar tissue formation or other deleterious effects.

The process of laser superficial keratectomy has proved to be one of the promising areas of surgical intervention for reconstructive or refractive keratoplasty in the future. Intensive investigations of the corneal wound healing process following laser ablation as well as the nature and long-term stability of the corneal excisions or induced refractive corrections are needed. \textit{It is essential that the optimal laser parameters be established for the various refractive corrections and other corneal surgical techniques, and that pathophysiological and histopathological changes that have been induced by the excimer laser-corneal tissue interaction in animals and humans be critically and extensively analyzed.}

References


