Mass spectroscopic analysis of excimer laser ablated material from human corneal tissue

Omid Kermani, M.D., Hans Joachim Koort, M.S., Eckhard Roth, M.D., M.S., Michael Ulrich Dardenne, M.D.

ABSTRACT

The clinical feasibility of photorefractive keratectomy depends on the surface structure of the ablated cornea. Two factors that influence the remodeled surface are the homogeneity of the energy distribution and the properties of the laser source (energy and wavelength). Currently, the homogeneity of the beam is difficult to control. The second factor, laser source properties, was the focus of this study. We investigated the effect of laser wavelength and energy by analyzing the reaction products of photoablation. We monitored the fragments produced by UV-laser ablation of human corneas using mass spectroscopy in the range of 0 to 100 atomic mass units. At 248 nm (KrF), average photon energy was 5 mJ/cm², increased to 90 mJ/cm² by intervals of 5 mJ/cm². At 193 nm (ArF), photon energy was increased by the same interval from 5 mJ/cm² to 80 mJ/cm². Our experiments showed that there was a fluence threshold of approximately 40 mJ/cm² at 193 nm (ArF) and 50 mJ/cm² at 248 nm (KrF). Exceeding this threshold led to sudden increases in the number and relative intensity of fragment peaks in mass spectroscopy. This indicates the onset of multiple-photon processes and effective photochemical breakdown. There was a significant difference between both wavelengths in the distribution of mass peaks, indicating higher ionization power at 193 nm.

Key Words: energy threshold, excimer laser, mass spectroscopy, multiple-photon process, photoablation, photorefractive keratectomy

Excimer lasers appear to be a suitable tool for refractive corneal surgery. They can be used to produce incisions in the corneal stroma (laser radial keratotomy)[1,2] or to ablate a lenticular layer from the corneal surface (laser shaping). After shaping procedures, the cornea is covered by a pseudomembrane that will assume the functions of Bowman's layer.[3,4] Thus, the clinical feasibility of shaping procedures depends largely on the surface structure of the photoablated cornea. Only smooth surfaces guarantee a satisfactory optical quality of the laser-treated eye. Scanning electron microscopy (SEM) studies have shown that scarring and opacification after photoablation are most pronounced in unevenly ablated areas.[3,5]

Two factors influence the smoothness of the ablated surface: the homogeneity of the energy distribution in the beam and the properties of the laser source (wavelength and energy). Currently, the homogeneity of the UV-laser beam is difficult to control. The distribution of photon energy levels in the beam profile is more or less random because of technical problems in construction of the light source. Therefore, the term "dirty beam" is widely used to describe UV lasers.

A systematic, comparative study of the effect of laser wavelength and energy on the photochemical reactions of the human cornea has not been presented. The interpretation of such effects in surface SEMs is difficult because they are contaminated by the accom-

From Lasomed Medical Laser Research of the Ulrich-Dardenne Foundation, Bonn, West Germany (Kermani, Roth, Dardenne), and the Institut für Physik, University of Mainz, Mainz, West Germany (Koort).

Reprint requests to Dr. Omid Kermani, Department of Microsurgery of the Eye, D-5300 Bonn-Venusberg, West Germany.

panying effects of the "dirty beam". Under these circumstances it is difficult to evaluate the qualities of the remodeled corneal surface by micromorphological methods alone.

The surface structure depends on the quality of the applied UV-laser beam just as the smoothness of a wooden table depends on the quality of the plane a joiner has used. This analogy helps to explain our aim: Investigation of the size, weight, and composition of the "shavings" is a good way to determine the quality of the plane. Our "shavings" are the reaction products of photoablation and from them we can determine the quality of the laser source.

We analyzed UV-laser ablated human corneal material using different laser parameters by monitoring the atomic and molecular fragments with mass spectroscopy in the range of 0 to 100 atomic mass units.

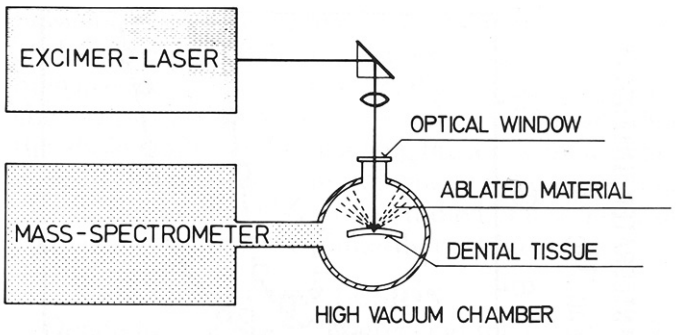

Fig. 1. (Kermani) Experimental setup: The UV-laser beam was delivered through an adjustable 90-degree quartz prism and a biconvex quartz lens (f = 30 cm) through an optical window into a high vacuum chamber in which the target material was placed. The ablated mass fragments were detected with a mass spectrometer in the range of 0-100 atomic mass units.

MATERIALS AND METHODS

Four human donor corneas (two male, two female) were used. Donor ages at death ranged from 28 to 65 years. The corneas were clear and without any pathological findings. They were not used for transplantation because the donors had died of viral infectious diseases. All corneas were obtained within 24 hours of death. They were stored in cooled, balanced saline solution for a maximum of 12 hours before usage.

The corneal tissue was placed in a vacuum chamber (500 cm^3) with optical windows (Suprasil). With a turbo pump, a final pressure of 10^{-7} Torr was reached after 30 minutes. A Lambda Physics EMG 101 MSC excimer laser was used for tissue ablation. The laser was filled with either krypton fluoride (KrF) to emit at 248 nm or argon fluoride (ArF) to emit at 193 nm. The UV-laser beam was delivered through an adjustable 90-degree quartz prism and a biconvex quartz lens (f = 30 cm) through an optical window (all Suprasil optics). The experimental setup is shown in Figure 1.

Increasing fluence levels were used at both wavelengths: At 248 nm (KrF), we started with an average photon energy of 5 mJ/cm^2 and increased it by intervals of 5 mJ/cm^2 up to 90 mJ/cm^2. At 193 nm (ArF), energy levels were between 5 mJ/cm^2 and 80 mJ/cm^2 and were increased by intervals of 5 mJ/cm^2. Energy was measured with a GenTec joulemeter. With all available excimer laser types, there is uncontrollable variation in the energy fluence level of approximately 5% to 8% per pulse. The repetition rates of laser pulses varied between 1 Hz and 2 Hz to avoid possible heating of the target material by accumulation. The mass fragments were detected with an Alcatel RGA100A mass spectrometer in the range of 0 to 100 atomic mass units and registered with an x,y plotter.

Temperature in the vacuum cell was maintained at 27°C during all measurements. One of the optical windows was used to monitor the ablative process with a video camera that was adapted to a stereo microscope (Figure 1).

RESULTS

With the onset of evacuation of the vacuum chamber, the cornea dried and opacified immediately to clear again slowly after some minutes. At this time we only registrated the peak at mass unit 18 (H_2O). Extracellular and intracellular water was outgasing.

Photoablation with 5 to 35 mJ/cm^2 (193 nm) and 5 to 40 mJ/cm^2 (248 nm) showed a small number of mass peaks with low mass intensities. There was no significant difference between the wavelengths in the distribution of the relative intensity and the number of mass peaks. Increasing energy at both wavelengths caused a significant sudden increase of the height (relative intensity) and number of mass peaks at energy levels of 45 to 50 mJ/cm^2 at 248 nm and 35 to 40 mJ/cm^2 at 193 nm. These are the tissue-specific fluence levels (energy threshold) that must be exceeded to perform effective and complete ablative photodecomposition (Figure 2).

More than 50 peaks in the mass spectrum could be observed when we reached energy levels of 56 mJ/cm^2 (193 nm) and 75 mJ/cm^2 (248 nm). The most intensive peaks ranged from 2 to 19 atomic mass units (Figures 3 and 4).

While the measurements with 248 nm (KrF) showed high peaks around the masses 55, 68, 81, and 92 (atomic mass units), those peaks were seen during 193 nm (ArF) ablation only by using the maximum amplification power of the spectrometer.

In the first range (2 to 19 atomic mass units), most of the particles could be identified (Figure 3). The fragments were basic components of organic tissue.

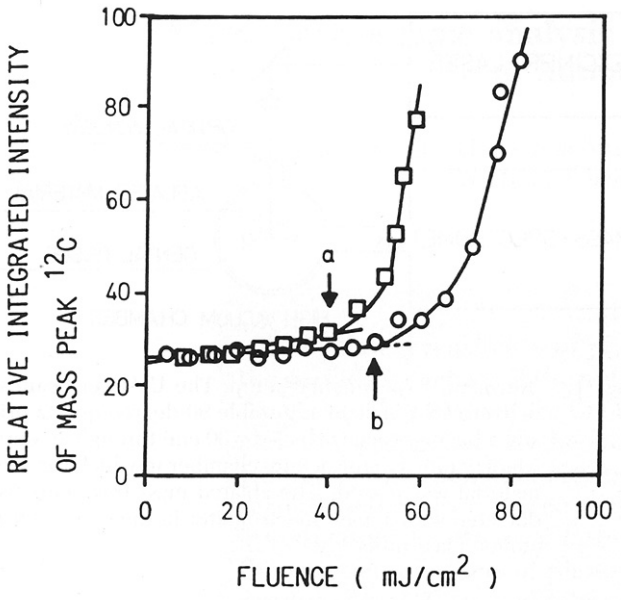

Fig. 2. (Kermani) Onset of multiple-photon process: The average photon energy versus the relative integrated intensity of the mass peak^{12}C is shown: □ = 193 nm ArF excimer laser; ○ = 248 KrF excimer laser. The energy thresholds are indicated by point *a* for 40 mJ/cm^2 at 193 nm (ArF) and by point *b* for 50 mJ/cm^2 at 248 nm (KrF).

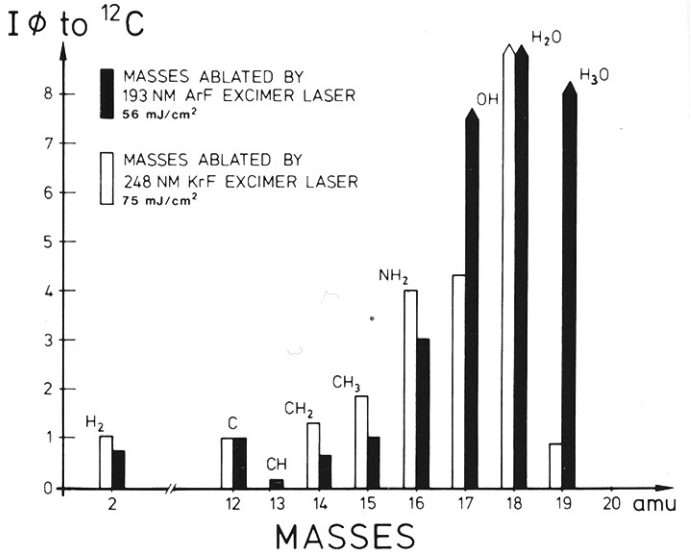

Fig. 3. (Kermani) Atomic mass units in the range 1-20: The mass intensities relate to the integrated intensity of the mass^{12}C. All masses in the shown range 1-20 could be identified.

The very high carbon monoxide peak (CO) after 193 nm ablation was striking. The second range (25 to 35 atomic mass units) caused problems in exact identification; $-COH$, $-CH_2NH_2$, $-CH_2OH$ could be correctly identified, knowing the origin of these molecules. Identification of other mass peaks in this range and in the third range (37 to 93 atomic mass units) seemed to be speculation. Yet 44 (atomic mass units) could stand for CO_2 and 45 for carboxyl ($-COOH$) (Figure 4).

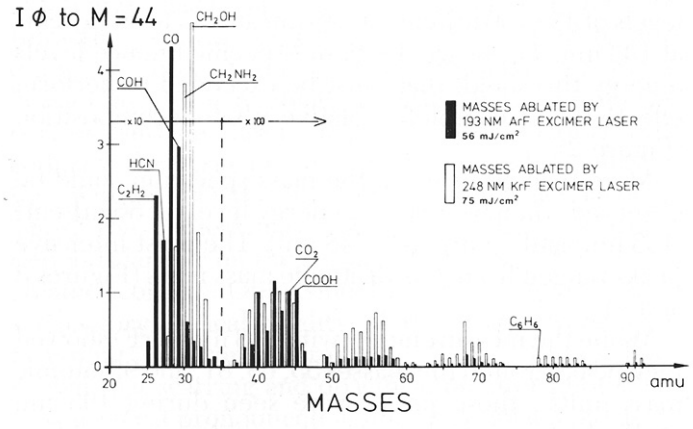

Fig. 4. (Kermani) Atomic mass units in the range 21-100: The mass intensities relate to the integrated intensity of the mass peak 44. Some of the masses could be identified. The masses 28-35 reached 10% of the height of the peaks 1-20, while the masses 37-93 reached only 1%.

At high fluences, 193 nm laser ablation produces a significantly higher number of small molecular fragments and more elementary compounds.

DISCUSSION

Ultraviolet light can be used to produce ions as well as elementary chemical compounds for mass spectroscopy. Photon energy is used to break the bonds within molecules. Analysis of the products of UV-laser ablation of the human cornea requires a detailed understanding of the mechanisms of UV-laser ablation.

The term "ablative photodecomposition" describes the effect of high energy photon application on material, inducing bond breaking and producing molecular fragments, elementary chemical compounds, bi- and triatomic particles and ions.[6,7] In previous experiments, we investigated the fluorescence of UV-laser-induced plasma of biological materials ablated by excimer laser radiation and showed that both molecular fluorescence and atomic spectra could be observed after photoablation. The photoablation process causes a high pressure gaseous phase in a small volume that rapidly expands and finally results in expulsion of fragments with supersonic velocity.[8] Photoablation is thought to be a "nonthermal" reaction. The high intensity of CO molecules after 193 nm photoablation at energy levels over 40 mJ/cm^2 suggests that temperatures higher than 1000°C can be reached although only for picoseconds. However, one should consider that hot elementary gas is cooled down during expansion. The

thermal energy of the high pressure gaseous phase is converted into kinetic energy.

The Einstein-Planck relationship for photon energy E of a given wavelength λ is

$$E = h \times c / \lambda \text{ (Joule)}$$

where h is Planck's constant and c is velocity of light. It can be concluded that photon energy depends on wavelength.

If an ionization process requires approximately 10 eV, the corresponding photon wavelength must be 124 nm. This is quite near the cut-off wavelength of optical windows such as Suprasil quartz. The 193 nm radiation (ArF) corresponds to an energy of 6.4 eV, 1.4 eV more than the 248 nm radiation (KrF) achieves. Theoretically the single-photon energy would not be sufficient to break down most of the organic molecular bonds with bond energies of 8 to 14 eV. But if there were a sufficiently great flux of photons, the ionization would occur through a multiple-photon process, whereby the energies of several photons together would raise the atoms or molecules to an ionized state.[6,7]

Photoablation by multiple-photon processes exhibits an energy fluence threshold that depends on the laser parameters and the target material. Only by exceeding this fluence threshold do pure photochemical reactions with maximum fragmentation power occur. Investigations of these threshold effects on organic material such as PMMA and polystyrene have been reported.[8]

Application of photon energy that exceeds thresholds of 40 mJ/cm^2 at 193 nm and 50 mJ/cm^2 at 248 nm on the corneal tissue leads to photoablation by multiple-photon process. Breakdown of tissue material into ions and elementary chemical compounds is induced. The reaction products can be analyzed by mass spectroscopy. Photoablation with energies below threshold energies shows much less ionization power, indicated by few detectable masses in the medium range at very low relative intensities (see Figure 2). Regarding the domination of the heavier fragments, one has to consider that chemical reactions could have occurred during gas expansion. Also, it is possible that photofragmentation of primary ablation products is produced by the subsequent phase of the laser pulse.[8] We, however, think that our results indicate general aspects of the effect of different UV-laser parameters on the human cornea. The influence of fluence density on the ablative process is not known exactly. How the ablation threshold is affected by changing the focus of the beam remains speculative. The influence of the repetition rate of pulses should also be studied. Pulse rates of 100 Hz and more are technically possible.

CONCLUSION

Depending on the UV source and the target molecule, the energy necessary to induce effective photochemical breakdown can be transmitted in a multiple-photon process.[7] Investigation of photoablation products by mass spectroscopy showed that the specific fluence threshold for effective photoablation of the human cornea induced by multiple-photon processes is 40 mJ/cm^2 at 193 nm (ArF) and 50 mJ/cm^2 at 248 nm (KrF). Analysis of mass products confirmed that 193 nm has higher ionization power than 248 nm. A known advantage of the 193-nm application is high absorption leading to low ablation depths per pulse and precise etching. The heterogeneity of the excimer laser beam, which leads to heterogeneous ablative process and uneven surfaces, remains a problem.

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