The potential of an infrared hydrogen fluoride (HF) laser (3.0 μm) for corneal surgery

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Abstract

The histopathology of incisions in the rabbit cornea by a hydrogen fluoride (HF) laser is described and compared to that of incisions obtained utilizing excimer systems. The histological appearance of the acute HF incision is similar to those created by 248nm excimer laser radiation but not as smooth as ablations at 193nm. This finding may relate to the beam parameters in our HF system and particularly to the quality of the optics we employed as considerations of the respective absorption depths of 3 μm and excimer radiation would suggest that HF should produce incisions similar to that induced by 193nm. The implications of this infrared laser for corneal surgery are discussed.

Introduction

Over the past few years there has been a growing interest in the possible use of lasers for corneal surgery. The intention in such surgical applications is not to burn corneal tissues as this would have serious consequences for transmission. Therefore the concept implicit in all these preliminary investigations is the removal of corneal tissue by a very rapid transformation of biological materials to the gaseous state. It should be obvious that such processes should be termed laser keratotomy rather than laser keratectomy. A review of the confusion in current nomenclature has recently been prepared by Waring.¹⁶

To date two different types of lasers have been used for experimental laser keratotomy. These are the ultrafast pulsed carbon dioxide laser (CO₂)⁴ with a wavelength of 10.6 μm and an excimer laser, argon fluoride (ArF), with an emission wavelength at 193nm.¹⁵ It is important to have a clear understanding of the totally different target sites at which these systems operate on in biological tissues. In essence the carbon dioxide laser light is primarily absorbed by water molecules within the tissue, whilst that of the excimer laser interacts with the P bonds of macromolecules of tissue components. It is also of importance to note that the depths to which these radiations penetrate in the cornea varies significantly with wavelength. In water the 10.6 μm radiation emitted by the CO₂ system will penetrate to about 20 μm in depth.¹ In contrast radiation at 193nm will penetrate in the order of millimeters in aqueous media.¹¹ In biological tissues the penetration depth of the CO₂ generated radiation will be almost identical to that to which it

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penetrates in pure water. However, the penetration of excimer laser radiation at 193 nm is dramatically different in that the macromolecules present in biological tissues now limit its penetration depth to about 1 μm.

As well as a differing absorption site these two radiations also produce their tissue damage in different ways. The CO₂ laser incision is thought to be related to ultrafast thermal processes in which the energy absorbed in the water molecules induces a state change. This process results in loss of tissues but because of its speed is accompanied by little thermal conduction to the surrounding cells. In excimer laser irradiation the individual photons falling on the tissue are of sufficiently high photon energy that each photon is capable of producing a chemical change in the tissue. Such changes may cause biological macromolecules to fragment. For a detailed discussion of these mechanisms see Garrison and Srinivasan.²

The high photon energy (e.g. 6.4 eV for ArF) and photochemical nature of tissue ablation with excimer lasers has given rise to some concern in relation to actinic damage to surrounding cells. During photoablation there is always a blueish haze surrounding the area of irradiation. This blueish light indicates that photon energy is being emitted from the target area as a result of fluorescence or luminescence. The secondarily emitted radiation is always at a longer wavelength than the initiating wavelength. Ito and colleagues³ reported that in the waveband 220–260 nm, the induction of gene conversion and DNA damage occurs. Such transmutations were caused at extremely low fluences such as a total cumulative dose of 10 mJ/cm². These considerations have provoked a number of authorities to pay particular attention to the putative mutagenic effects of excimer laser keratectomy.

The CO₂ system also has potentially hazardous complications. In this case the relatively large penetration depth of the CO₂ laser light may result in spread of the thermal image outside the area of the incision. It should be apparent that the more confined the volume in which energy is absorbed, the more rapid the temperature rise generated for any given input energy. It follows therefore that the greater the absorption depth, the less steep the temperature profile and therefore the greater the conductive heating for adjacent tissues. In relation to corneal incisions this means that with CO₂ systems the incised area will always be bordered by a zone of thermally damaged but non-removed tissue. Ultrastructural studies have demonstrated these zones to be in the order of 25–30 μm in extent and these empirical results relate almost perfectly to the absorption depth at this wavelength.

Given the above complications associated with each of the two laser modalities used for corneal surgery to date, we considered that it would be useful to investigate other laser wavelengths. Thermal problems and thermal damage to tissue are less serious than the putative mutagenic properties induced by photochemical processes. For this reason we have investigated lasers in the infrared region of the spectrum. Optimally we required a laser with an emission near either one of the two regions of maximal absorption of water (Fig. 1). Two laser systems were available to us with emission lines around 3 μm, the hydrogen fluoride (HF) laser and the color center laser.¹⁰ To date it is only possible to obtain the optimal pulse geometry and pulse energies with the HF laser. This paper is the first account of the use of such an infrared system in making experimental incisions in the cornea.

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**Fig. 1.** Figure to illustrate the emission absorption peaks of water in the infrared region of the spectrum.
Materials and methods

The HF laser was first described by Kompa and Pimentel. In this system laser light is obtained by the loss of photons associated with the return of excited hydrogen fluoride molecules to their ground state. The laser produces radiation at a number of lines between 2.6 and 3 μm as shown in Fig. 2.

In this study a multiline, multimode stable cavity HF laser was used (Helios Longmont, Colorado). The laser output depended upon the pulse repetition rate, and pulse frequencies up to 25 Hz were available. At 1 Hz a pulse energy of 9 mJ was obtained but this decreased to 6.5 mJ at 25 Hz. The pulse duration was typically 50 nanoseconds. The laser beam was rectangular with dimensions of 10 mm x 5 mm. The effective irradiance was increased by focusing with a cylindrical lens of f = 65 mm. After passing through the lens the beam was cropped by a slit aperture (5 mm x 150 μm) which was placed 2 mm from the corneal surface. This lens/aperture system resulted in an increase in irradiance by a factor of 8, such that the fluence at the cornea was maximally 140 mJ/cm². By varying the flow of hydrogen into the cavity, the pulse energy could be reduced to any value between zero mJ and 9 mJ.

Albino rabbits of 2–3 kg were sacrificed with an intracardial injection of T61 and the eyes were immediately enucleated. The eyes were mounted corneal surface uppermost using a vacuum ring to secure the posterior globe. The corneas were irrigated with normal saline and then exposed to the above laser delivery system. Immediately after exposure they were fixed by immersion fixation in 3% glutaraldehyde in phosphate buffer. The maximum time between enucleation and fixation was five minutes. Five to ten minutes after initial immersion in fixative, a transscleral incision was made posterior to the equator with a razor blade. After overnight fixation the corneas were isolated by a circumferential incision at the limbus and each cornea was hemisected at right-angles to the line of the laser ablations. In each case one half of the exposed area was processed for scanning electron microscopy and the other half for light and transmission electron microscopy. SEM specimens were processed as previously described before being viewed in a Hitachi scanning electron microscope. For light and electron microscopy specimens were post fixed in 2% osmium tetroxide before being processed as previously described.

Results

Owing to problems with the transmission of our lens system it was difficult to ensure a uniform radiant exposure over a number of different experiments. Therefore our inadequate optics rendered a detailed correlation between depth and incision and pulse energy somewhat obscure. However some approximations can be made. With a pulse energy of 6.7 mJ (fluence approximately 100 mJ/cm²) an incision depth of 1.9 μm/pulse was achieved. In contrast, pulses with an energy of 4.3 mJ (fluence approximately 65 mJ/cm²) only resulted in damage to the corneal epithelium and negligible penetration of the stroma (Fig. 3a).

On gross observation these incisions were easily seen and were bordered on both edges by a distinct narrow white band. This clearly defined white band was the only perturbation in corneal transparency and outside this band and inside the center of the incision there was no hazing or changes in the transmission or reflection of light.
On scanning electron microscopy the problems that we had encountered with focusing the beam were immediately apparent in that two distinct surface topographies were observed (Fig. 3). In some incisions a broad V-shaped trough was perceived in which the walls of the trough were lined by highly distorted deposits of cell debris (Fig. 3b). In contrast in other incisions a relatively narrow but deep trough was apparent and in such cases the superficial margins of the incisions were relatively clean with fewer and far less disorganised deposits of debris (Fig. 3c).

In the broad V-shaped incisions the debris was undulating and uneven with an extremely complex irregular surface topography (Fig. 4). On the outermost margin of this zone of debris it overlapped what appeared to be viable surface epithelial cells (Fig. 4a). In some instances the floor of the trough of these V-shaped incisions were clearly not denatured by thermal transients emanating from the absorption of laser radiation but resulted from stretching and release of elastic tension within the corneal tissue (Fig. 4b).

In those areas where the surface of deep incisions were viewed the absence of this complex deposit of cell debris resulted in a surface view of the epithelial cells bordering the incision. These appeared to be relatively normal and gave no indication that they had suffered any significant effects from a transitory coating of surface debris. It could well be that in such areas an initial build-up of surface debris has been removed by subsequent pulses in the pulse train.

The two extremes of topography seen on the scanning electron microscope are also seen in sections prepared for the light microscope. In those exposures that have resulted in broad superficial V-shaped grooves there was a significant amount of damaged or denatured tissue associated with the epithelial margins of the incision. At the light microscope level this material was seen as an amorphous darkish staining zone which extended over both the epithelial and stromal course of the incision (Fig. 4a). In the epithelial region the zone was approximately 50 μm in extent, was relatively smoothly bordered where lining the incision, and followed cell margins on its distal surface. High power examination showed that part of the debris was dead epithelial cells and part of it seemed to be acellular ribbons of debris. In the stroma this dark zone extended from 5–20 μm in thickness and was frequently vacuolated on its proximal edge. The distal edge of the dark zone in this case seemed to undulate in relation to the stromal lamellae. Occasionally pyknotic keratocytes could be seen extending from the distal edge of the dark zone. A similar morphology was seen in the deep or penetrating incisions but in these cases the epithelial debris was often less extensive than that described above. In many of these incisions the lateral extent of the debris was in the order of 35 μm or approximately 3 basal corneal epithelial cells in extent. A disturbing feature of the deep incisions were the corrugations of the margins (Fig. 5). These frequently undulated in relation to microanatomy of stromal lamellae and possibly arose as a consequence of exposing post mortem corneas. One further consideration should be taken into account at this point and that is relative movement of the globe pulse to pulse caused by expulsion of surface material.

Within the line of the incisions in some areas fragments of debris could also be seen, and this tended to be less densely stained and more floccular in appearance (Fig. 5a).

On transmission electron microscopy the dark zone in the epithelial region was seen to be composed of two elements (Fig. 5c). On the border immediately adjacent to the incision it seemed to be

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Fig. 3. Scanning electron micrographs of rabbit corneas showing areas irradiated with 3.0 μm radiation generated by a hydrogen fluoride (HF) laser. The bar marker is 100 μm.

a. Damage to the surface epithelial cells induced by a low energy exposure (fluence 65 mL/cm²).

b. Incision through the epithelium and superficial anterior stroma produced by a medium energy exposure.

c. Deep incision penetrating two thirds of the stroma caused by a high energy exposure (fluence 100 mL/cm²).
Fig. 4.
formed of a ribbon of extracellular debris reminiscent of that seen after irradiation with excimer lasers.8,9 In the stroma the dark zone was seen to consist of denatured collagen fibers which were swollen and disorganised in comparison with un-irradiated tissue (Fig. 6). It was quite clear that both the collagen fibers themselves and the glycoprotein matrix around them were denatured. The numerous vacuoles seen under the light microscope were seen as holes both within and between lamellae at the electron microscopic level, and also some apparent holes were areas which contained relatively unchanged collagen fibers (Fig. 6b, c). All keratocytes with a significant cell volume within the area of the incision were seen to be pyknotic and electron dense throughout their entire cytoplasmic extent.

The floors of the incisions showed the same changes as the walls but were usually less extensive in terms of the distance at which tissue changes occurred from the region of tissue loss. In some incisions the floor did not show a dark zone but there appeared to be slight tearing or disorganisation of the stromal lamellae which seemed to indicate that if sufficient changes were induced in the body of the stroma to release elastic forces then the incision could be further extended by elastic recoil or stretching (Fig. 4b). With the exception of penetrating incisions we did not see any changes or cell loss in the endothelial layer.

Discussion

In any design concept for a laser system to be utilised in corneal surgery, there are certain fundamental prerequisites. Ideally the system should comply with the following conditions:

1. It should not induce mutagenic effects.
2. The radiation should have a limited penetration depth such that tissue not removed is not irradiated.
3. It should have the ability to remove tissue with submicron accuracy.

Mutagenic effects are thought to be related to the ability of individual high energy photons to bring about changes in the chemical structure of biological macromolecules. Such effects are related to photon energy and for the most part are a problem of ultraviolet radiation.

Whilst the current experience with long-term effects of excimer radiation on animal corneas is somewhat limited there have been no reports of any significant post operative complications.5, 8, 9, 12, 14 Further, although no histopathological studies have been directly orientated towards examining mutagenic effects no observations have yet been reported of atypical cells in studies on corneal healing (Puliafito, personal communication). In contrast to these observations however, a preliminary series of experiments investigating photoreactivation utilizing yeast has shown that some DNA damage is induced by irradiation at 193nm (Seiler, personal communication). As yet we are not sure of the relevance of these studies to exposure of mammalian cells, nor are we able to state whether the observed results are due to direct interaction with the induced fluorescence or related to indirect chemical causes induced by free radical cascades.

Lasers which emit radiation in the wavelength region of 3 μm produce photons whose energy is insufficient to produce direct breakage of bonds in macromolecules. Theoretically therefore, there is no valid reason why mutagenic effects should be caused by irradiation using HF lasers. The above consideration only holds true for direct interaction of the radiant energy with the genetic macromolecules. However, if the fluences experienced during irradiation are sufficiently high even infrared radia-
tion has the ability to generate free radicals within tissues. Such radicals, although short-lived may well have a mutagenic potential. To date there have been no reports of indirect mutagenic effects induced by infrared lasers and although this is the first report of irradiation of ocular tissues at 3 μm our peak powers do not approach those commonly used in clinical YAG laser irradiations of ocular tissues.

The requirement for a limited penetration depth of radiation is important both in terms of the removal of all irradiated tissue and in relation to the mechanism by which tissue is removed. Both the excimer laser radiation at 193 nm and HF at 3 μm have an absorption depth in the region of 1 μm.17 As previously stated the absorbing medium differs with wavelength with the former being absorbed in macromolecules and the latter in water. The subsequent degradation of absorbed energy may result from two processes whose relative predominance may vary with wavelength. Currently the ablative process induced by these lasers are explained by two theoretical mechanisms, photoablation and ultrafast thermal events. In the photoablation theory it is claimed that the high photon energy results in selective interaction of photons with specific chemical bonds in the target tissue. In the case of biological macromolecules it is postulated that the breaking of valency bonds results in an increase in volume of the released molecules with the net result that their rapid expansion ejects them from the tissue surface.2 The second hypothesis is not necessarily contradictory and postulates an ultrafast non-linear increase in absorption of the target media during high power irradiation. In this case the higher the initial absorption coefficient of the target material the easier the generation of non-linear processes becomes. When sufficient photons have been absorbed and resulted in a massive increase in vibrational energy in the system then again intramolecular bonds break and material is ejected from the surface. In this hypothesis vibrational modes may involve molecules adjacent to the ablated area and may well result in mild damage outside the area of irradiation. In principle therefore any of the absorption peaks of corneal tissue may be utilised to activate the process of photoablation. The requirement in clause (2) above is therefore satisfied by both ArF and HF laser systems (Fig. 1).

Whatever the implicit mechanism both the above hypotheses demand that tissue must be removed from the target area. It is clear therefore that a threshold value of fluence must exist in order to enable a phase transition. Although we have not determined an absolute threshold for HF it is apparent from our reported observations that it lies somewhere between 65 mJ/cm² and 100 mJ/cm² at a pulse rate of 10 Hz. We found this value surprising as it is extremely similar to the values established for 193 nm (40–60 mJ/cm²) and 249 nm (100–140 mJ/cm²) determined for excimer systems.6 These values are much lower than those reported for 308 nm (450–550 mJ/cm²) and 351 nm (about 1000 mJ/cm²).6 This apparent increase in threshold with 308 and 351 may well be erroneous and due to a net loss of photons from the zone of ablation due to deep penetration through the tissue. If this is the case then the shorter the absorption depth in the tissue the more effective the ablation process. In the future it may well be worth experimenting with lasers with emissions at 17 μm as this coincides with a massive absorption peak in water.

The underlying mechanism by which the ablation process occurs is of practical importance in terms of pre-determining the dimensions and depths of surgical incisions. The greater the accuracy with which tissue can be removed, the higher the subsequent surgical potential of the system. In our preliminary experiments it seems that both the excimer and HF have the potential for removing tissue with an accuracy better than currently available with a dia-

Fig. 5. Lightmicrographs of deep incisions in the corneas of rabbits, induced by either a hydrogen fluoride laser (3.0 μm) (a, c); or by an excimer laser (248nm) (6). Both lasers result in incisions with somewhat ragged borders and a zone of densely staining material. Incisions produced by the HF system had a slightly more extensive dark zone, and usually displayed more undulating margins. The dark zone consists of denatured collagen in the stroma, and both denatured cells and deposits of debris in the epithelial layer. Normally staining cells (arrowed) were encountered within 4 to 5 cell dimensions of the cut edge. The bar markers are (a, b) 100 μm; (c) 25 μm.
Fig. 6.
mond knife. Our histopathological studies demonstrate that given the inadequacies of the optics of our delivery system, our HF induced incisions are almost exactly comparable in terms of tissue damage to those induced by an excimer laser at 248nm (Fig. 5a, b). It is also of interest that the nature of sub-cellular and cellular tissue damage induced by excimer laser radiation at 248nm and our current HF system are comparable in terms of type and dimensions. In the current series of experiments we were not able to remove tissue as cleanly as has been our experience with 193nm. We are hopeful that our reported observations will be improved upon using optical systems specifically designed to handle radiation at 3 μm.

There are two further practical advantages of utilising the HF laser for surgical purposes. The first of these is that in contrast to vacuum ultraviolet, optical fibers are available that transmit 3 μm radiation. The use of optical fibers makes the construction of clinically useful delivery systems far easier than those currently being designed to handle ultraviolet radiation. Fiber optics also open up the possibility of intraocular surgery with lasers in this wavelength domain. The other major advantage of the HF laser is that they do not require the presence of highly toxic gases in the clinical environment. The absence of fluorine from a clinic is a positive advantage as it avoids any problems associated with safety of both staff and patients. Currently HF systems require a large amount of space in order to house the pumping systems; however, future developments remove these problems by using YAG systems in conjunction with stimulated Raman scattering processes.

In conclusion we feel that laser keratectomy with a 3 μm laser is a promising procedure and may well offer an alternative to the clinical introduction of excimer lasers. This infrared system removes the risk of mutagenesis and in future forms may well be more convenient in terms of a laser in the clinical environment. As yet however the incisions we have made are not as smooth as those induced by the excimer laser at 193nm and we could not avoid the conduction of thermal damage to adjacent tissues. This conductive damage is not more significant than that perceived with excimer irradiation at 248nm. Obviously we need to carry out further experiments on the pathogenesis of wound healing and the clinical effectiveness of this laser modality.

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References


Fig. 6. Lightmicrograph (a) and transmission electronmicrographs (b, c) of the dark zone bounding the margins of HF incisions in rabbit cornea. The irregular extent and undulating course of the zone is seen in (a), together with the distribution of vacuoles (arrowed) adjacent to the proximal edge. In the low power electron micrograph (a) the junction between the distal edge of the dark zone (arrowed) and normally staining tissue is seen to correspond with the site at which the collagen fibers retain their normal dimensions. All keratocytes (K) with cytoplasmic components extending into the dark zone seem to become electron dense or pyknotic. The bar markers are (a) 50 μm; (b) 5 μm; and (c) 2 μm.