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# Ultraviolet 308-nm excimer laser ablation of bone: an acute and chronic study

J. Stuart Nelson, Arie Orenstein, Lih-Huei L. Liaw, Rhonda B. Zavar, Sanjay Gianchandani, and Michael W. Berns

A comparative study was undertaken in rabbit tibiae to assess the healing of bone in response to osteotomies by a XeCl 308-nm excimer laser and a mechanical saw. The laser parameter producing the most efficient ablation rate for osteotomy was determined and shown to produce deep cuts with sharp edges. However, it was noted histologically that there was a significant delay in healing of the laser osteotomies compared with saw osteotomies. This delay was caused by thermal damage to bone adjacent to the laser cuts. It is suggested that the excimer laser at this wavelength ablates bone primarily through a photothermal as opposed to a photodissociative mechanism.

#### I. Introduction

Excimer lasers are used in photochemistry to provoke photochemical reactions in research and production processes, including the enrichment of uranium. 1,2 They are also used in photolithography—for example, to produce electronic circuits previously made by a photographic process.3 Predictable amounts of surface material can be removed, leaving etched marks with microscopically sharp edges. The depth of substrate removal is precisely controllable because, for a fixed energy per pulse (energy per unit area), there is a linear relationship between etched depth and number of pulses delivered. This feature has recently been exploited to produce well-defined, nonthermal cuts of very small widths by excimer lasers at several ultraviolet wavelengths (ArF, 193 nm; KrF, 248 nm; XeCl, 308 nm; XeF, 351 nm). This etching ability has led to the suggestion that excimer lasers could have medical applications.4

The excimer laser is currently being investigated in four main clinical research areas. One of the most promising applications of this technology is in ophthalmology for the treatment of keratorefractive disorders. Ablation of the corneal stroma with 193-nm radiation may prove invaluable because of its ability to

remove precise amounts of tissue without generating secondary heat effects. Long-term studies are needed, but the prospects for early clinical use in corrective refractive surgery seem good.<sup>5</sup>

In dermatology, the extreme precision of the excimer laser may be better than the traditional scalpel for removing bulky skin lesions. The 248-nm radiation has the ability to both produce a sharp skin incision and coagulate blood.<sup>6</sup> Other potential uses include the selective ablation of subcutaneous chromophores, such as melanin, in pigmented lesions.<sup>7</sup>

Vascular surgery is the most interesting but, at the same time, the most technically challenging application of the excimer laser. In vitro and in vivo studies have demonstrated that excimer lasers at several wavelengths can cut precise craters in vascular atheromatous plaque with minimal surrounding damage.8 Of technological importance is the development of a suitable fiber optic delivery system that can deliver the ultraviolet laser energy percutaneously to an obstructed blood vessel. It is very difficult to transmit a short laser pulse down an optical fiber: the shorter the pulse, the higher the peak pulse power and the more likely is fiber damage. This problem is compounded with the excimer laser because the shorter and more useful wavelengths are increasingly absorbed by quartz, especially in the presence of impurities. Lengthening the wavelength relaxes the fiber requirements, and it is now possible to transmit up to 60 mJ per pulse down fibers at a wavelength of 308 nm which has been shown to produce ablation of plaque in human cadaver arteries.9

A new use for excimer lasers may be in orthopedic surgery. A recent study utilizing cadaver bones has shown that 308-nm radiation can produce sharp cuts in

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bone and organic polymers, such as polymethyl methacrylate, used in bone cement. However, to date, there has been no attempt to study the healing process in vivo of cuts in bone produced by the excimer laser. The purpose of the present study is twofold: (1) to provide some insight into energy requirements necessary for excimer laser osteotomy and (2) to evaluate the extent of cell damage and subsequent healing of these osteotomies compared to conventional saw osteotomies.

#### II. Materials and Methods

## A. Laser Light Delivery System

Irradiations were performed with a Spectranetics (Colorado Springs, CO) CVX-300 excimer laser. This device excites a mixture of rare gas (Xe) and halogen (Cl) to produce UV photons at a wavelength of 308 nm. The laser is capable of producing a 120-ns pulse at a maximum energy of 100 mJ per pulse with a variable repetition rate from 0 to 100 Hz. Since this laser emits light energy in the UV range, UV grade fused silica fiber optics (600- $\mu$ m diameter) are used to deliver the beam to the operative site.

To provide some insight into the laser requirements necessary for laser osteotomy, initial *in vivo* irradiations were performed with laser energies of 5–30-mJ per pulse (17.7–106.1 mJ/mm²) at repetition rates variable from 5 to 30 Hz. Higher energies or higher repetition rates were not technically possible due to disruption of the proximal and distal ends of the optical fiber. Laser irradiation was monitored with a Spectranetics JD/000 energy meter before and after treatment. Bone samples were placed on a mobile operating stage that passed underneath the laser beam a total of twenty times at a constant velocity of 1 mm/s.

#### B. Animal Model

Adult male New Zealand white rabbits weighing 2.5-3.0 kg were used in this study. The animals were anesthetized with intramuscular injections of acepromazine (10 mg/kg), xylazine (5 mg/kg), and ketamine (10 mg/kg). Both the left and right tibiae of each animal were surgically exposed by means of a 4-cm incision on the medial aspect of each leg. Using a sterile technique, sharp dissection was carried down through skin and fascia to bone with a scalpel blade. The soft tissues were retracted circumferentially and the periosteum incised longitudinally and elevated. An osteotomy was then performed on the flat medial surface of the tibia with either the excimer laser or a Both control (untreated normal mechanical saw. bone) and acute (laser and saw osteotomies) specimens were removed for histology. In those animals participating in the healing study, the right tibia had the laser osteotomy placed 1 cm from the knee joint with the saw osteotomy 1 cm distal to the laser cut. Alternatively, on the left tibia, the saw cut was placed proximally and the laser cut distally. Both laser and saw osteotomies were performed while the surgical site was being irrigated continuously with normal saline solution. The periosteum, fascia, and skin were then closed with interrupted sutures. No external immobilization of any sort was used since the osteotomies did not completely transect the bone. Animals were returned to their cages with no activity limitations or restrictions and were fed standard ration.

#### C. Healing Study

Laser (20 mJ per pulse, 70.7 mJ/mm<sup>2</sup>; repetition rate 15 Hz) and saw osteotomies were performed as described above. After surgery, animals were sacrificed by auricular vein injections of Euthasix (Western Medical Supply, Arcadia, CA) at one, two, three, four, and six weeks postoperatively. The tibiae were removed en bloc and the soft tissues carefully dissected free from the bone and callus. Bone specimens were fixed in fresh 10% formalin for 5 days and then washed in phosphate buffered saline. For decalcification, bones were suspended in Surgipath Decalcifier II (Gravslake, IL) at room temperature for 10-12 days. Samples were then dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin. The 6-8um sections were cut perpendicular to the longitudinal axis of the bone, stained with hematoxylin and eosin, cleared of paraffin in xylene, and dried. Sections through the osteotomy site were examined with an Olympus microscope and photographed with Panatomic-X film (Eastman Kodak).

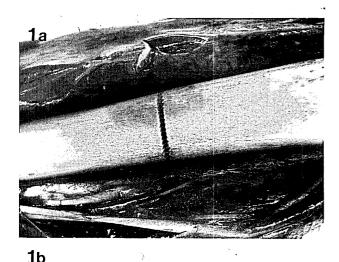
#### III. Results

#### A. Laser Osteotomy Parameter Determination

Histologic analysis of the osteotomies produced by the excimer laser revealed the most efficient ablation rate to be associated with a laser energy of 20 mJ per pulse (70.7 mJ/mm<sup>2</sup>) at a repetition rate of 15 Hz. This conclusion was based on the histologic examination of all osteotomy sections within the defined laser parameters, regardless of the way in which the sections were cut. These parameters produced deep osteotomies with sharp edges at both the gross [Fig. 1(a)] and microscopic [Fig. 1(b)] levels. There was no evidence of charring or burning of the adjacent bone. Lower energies per pulse and lower repetition rates produced shallow craters due to inadequate bone ablation. Higher energies per pulse and higher repetition rates resulted in disruption of either the proximal or distal ends of the optical fiber during the osteotomy procedure. If disruption occurred at the proximal end of the optical fiber, shallow craters were produced due to inadequate transmission of the laser energy [Fig. 2(a)]. However, if disruption occurred at the distal end of the optical fiber, osteotomies with serrated, irregular edges were seen [Fig. 2(b)]. This finding was due to the inability to focus precisely the laser output.

#### B. Bone Healing Study

Rabbit tibiae were examined immediately postsurgery and subsequently through six weeks. In the acute osteotomy specimens, both modalities produced deep cuts with sharp edges (Fig. 3). Histologic sections of



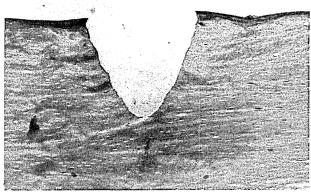
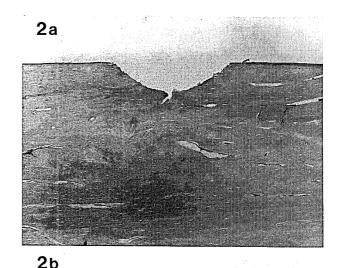


Fig. 1. Gross (a) and histologic (b) photographs of excimer laser osteotomies: 20 mJ per pulse, 15 Hz, 600-µm diam spot, 20 passes over the substrate; (a) ×16 and (b) ×100.

the laser osteotomies [Fig 4(a)] revealed a more conical pattern of ablation compared with the saw osteotomies [Fig. 4(b)]. Although there was no histological evidence of charring or carbonaceous material seen in the laser osteotomy grooves, there was a thin shear of bone disrupted by the last laser pulse but not completely ablated. However, the most notable finding observed in the laser osteotomies was a microscopic transitional zone marked by swollen empty lacunae and disorganized bone structural elements with reduced staining adjacent to the groove [Figs. 5(a) and (b)]. This zone was not visible on conventional thick (6-8-μm) sections embedded in paraffin, but was visible on thin  $(0.5-\mu m)$  sections embedded in plastic. This zone was measured by ocular micrometry to approximate 170  $\mu$ m at the periosteal surface tapering to 70  $\mu$ m at the endosteal base of the groove. No such zone was seen in the saw osteotomies [Figs. 5(c) and (d)].

The healing process is summarized in Table I. At one week, the laser osteotomies healing response was limited to the minimal formation of fibrous tissue around the bone shear in the groove [Fig. 6(a)]. In contrast, the saw osteotomies contained a well-organized plug of connective and fibrous tissue elements almost filling the groove [Fig. 6(b)].



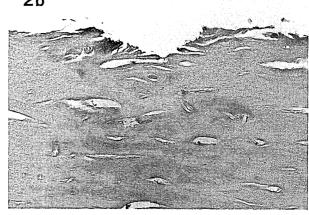


Fig. 2. Photomicrographs of excimer laser osteotomies produced after disruption of the fiber at the proximal end (a) and at the distal end (b); (a) ×90 and (b) × 90.

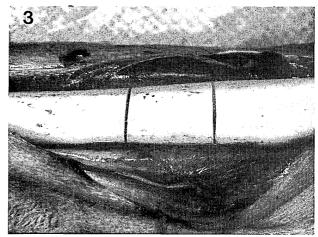


Fig. 3. Gross photograph of excimer laser (right) and saw (left) osteotomies, ×10.

At two weeks, the laser osteotomies contained a dense fibrous tissue infiltrate adjacent to the bone shear [Fig. 7(a)]. The saw osteotomies displayed osteoid (composed of fibrous, cartilaginous, and immature bone elements) formation with numerous trabeculae

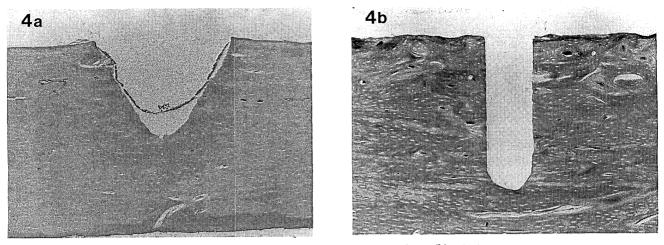


Fig. 4. Photomicrographs of acute laser (a) and saw (b) osteotomies; (a) ×90 and × 90.

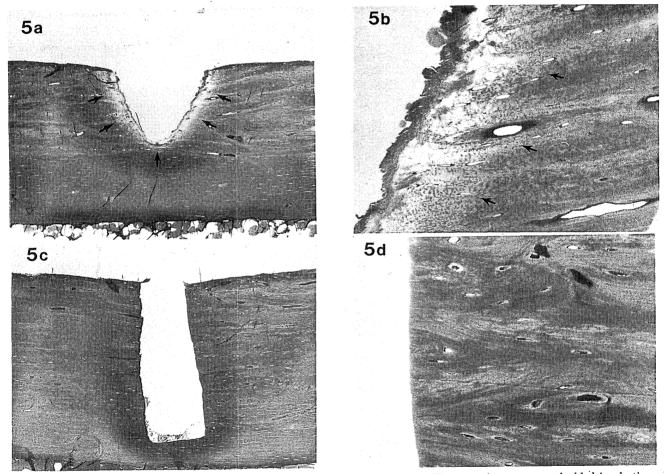


Fig. 5. Photomicrographs of (a),(b) acute excimer laser and (c),(d) saw osteotomies. These specimens were embedded in plastic and sectioned at 0.5  $\mu$ m to bring out cellular detail. Note transitional zone (outlined by arrows) marked by vacuolization and swollen empty lacunae adjacent to the laser osteotomy grooves (a),(b). No such zone was seen in the saw osteotomy grooves (c),(d); (a),(c) ×90 and (b),(d) × 490.

of immature bone forming from both the periosteal and endosteal surfaces filling the groove [Fig. 7(b)].

At three weeks, the laser osteotomies showed osteoid formation at the periosteal surface extending down into the groove. However, a large defect separated

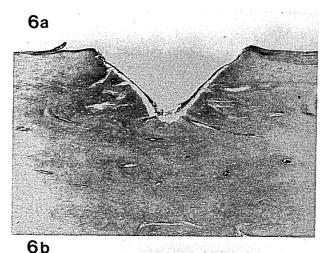
this osteoid from the preexisting endosteal bone in the base of the groove. Scanty fragments of bone shear were still visible in this defect [Fig. 8(a)]. The saw osteotomies showed maturing lamellar bone filling the groove almost completely [Fig. 8(b)].

At four weeks, the laser osteotomies showed complete periosteal bridging of the groove by osteoid which now contained islands of immature bone. However, there was still a defect between the new bone developing exclusively from the periosteal surface and the preexisting endosteal bone in the base of the groove [Fig. 9(a)]. The saw osteotomies showed complete bridging of the groove by mature lamellar bone. There was almost complete fusion of the new bone with the edges of the preexisting endosteal bone [Fig. 9(b)].

Finally, at six weeks, the laser osteotomies showed the lamellar pattern of new mature bone invading from the periosteal surface into the groove. However, the defect between this new bone developing exclusively from the periosteal surface and the preexisting endosteal bone was still present, albeit smaller than in the four-week specimens [Fig. 10(a)]. In a few areas, there

Table I. Histologic Comparison of Bone Healing Between Laser and Saw Osteotomies

Osteotomies		
Time post surgery (weeks)	Laser	Saw
1	Minimal fibrous tis- sue formation adja- cent to shear in os- teotomy groove.	Well-organized plug of fibrous and connective tissue filling the osteotomy groove.
2	Dense fibrous tissue infiltrate adjacent to shear.	Osteoid and immature bone forming from both periosteal and endosteal surfaces filling the groove.
3	Osteoid formation exclusively from periosteal surface extending down into the groove.	Mature bone from both periosteal and endosteal surfaces filling the groove almost completely.
4	Complete periosteal bridging of the groove with osteoid and immature bone. However, large defect separates new periosteal bone and preexisting endosteal bone in the base of the groove.	Almost complete fusion of new bone from perios- teal and endoste- al surface with the edges of the preexisting bone.
6	Mature bone invading from the periosteal surface. No new bone formation from endosteal surface results in a persistent large defect between the two bony interfaces.	Complete fusion of new and preexist- ing bone.



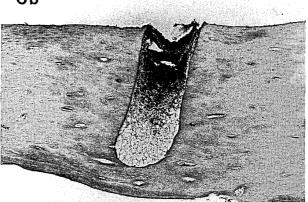


Fig. 6. Photomicrographs of one week postsurgery laser (a) and saw (b) osteotomies; (a) ×90 and (b) ×90.

was actual contact of these two bone interfaces. The saw osteotomies were completely healed with total fusion of new mature lamellar bone with the edges of the preexisting bone [Fig. 10(b)].

## IV. Discussion

Photoablation occurs when pulsed, high-energy ultraviolet photons produced by the excimer laser are absorbed on the surface of an organic substrate. Since UV radiation is absorbed intensely by most biological molecules, the penetration depths are only a few microns.11 This combination of high absorption and high individual photon energy results in the direct transfer of energy within the absorbing molecule to the bonds that hold the molecule together. When the incoming UV energy exceeds the molecular bonding energy (the ablation threshold), the substrate will undergo random bond scission and be reduced to its atomic constituents. The rapid expansion created by this excitation and bond cleavage gives rise to the actual ejection of fragments at supersonic velocities or the ablation phenomenon. $^{12}$  In contrast, the visible and infrared photons produced by lasers currently in clinical use do not have sufficient energy to dissociate molecular bonds and can therefore ablate tissue only by a thermal mechanism.

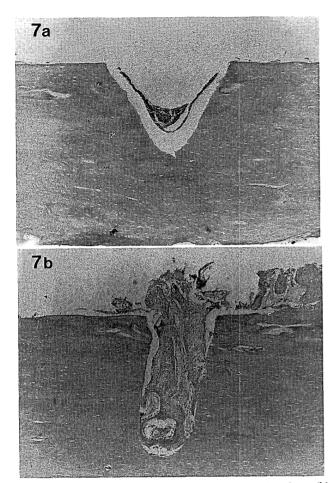


Fig. 7. Photographs of two-week postsurgery laser (a) and saw (b) osteotomies. Note presence of numerous trabeculae of immature bone forming in the saw osteotomy groove (b); (a)  $\times 90$  and (b)  $\times 90$ .

While UV laser pulses are energetic enough to break intramolecular bonds, there is still considerable controversy over whether there is also a thermal component.13 Perhaps most of the energy of the UV laser pulse goes into the thermal phase change associated with tissue vaporization. Ablated fragments ejected from the tissue surface carry most of the energy with them, leaving little energy in the form of heat to damage the surrounding tissue. This phenomenon would prevent undesirable melting or carbonization of organic molecules, a problem that exists when visible or IR laser irradiation is used. However, other studies have shown that thermal effects may also play a significant role, particularly at the longer UV wavelengths. 14,15 The latter concept is viewed as one in which the laser energy initially absorbed as electronic excitation is rapidly converted to vibrational heating of the substrate. The intense local heating results in an explosive pyrolysis since the volume occupied by the pyrolyzed material greatly exceeds that of the substrate. It is clear that a detailed understanding of the complex ablation phenomenon with excimer lasers has yet to be fully developed. It is possible that competition may exist between the photodissociative and photothermal modes. At lower fluences, photodissociation of intra-

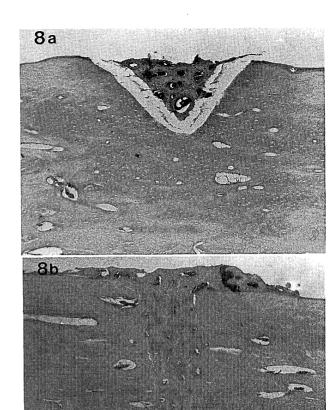


Fig. 8. Photomicrographs of three-week postsurgery laser (a) and saw (b) osteotomies. Note minimal osteoid formation from periosteal surface in laser osteotomies (a), whereas the saw osteotomies (b) are filled with maturing lamellar bone; (a) ×90 and (b) ×90.

molecular bonds may be the primary causal mechanism leading to ablation. As the fluence is increased, ablation may convert primarily to a photothermal mechanism.<sup>16</sup>

In addition to the aforementioned mechanisms, a photoacoustic effect similar to that described for Q-switched and mode-locked Nd:YAG laser destruction of pathological lesions in the eye may also play a role. <sup>17</sup> During this process, shock waves associated with a rapidly expanding laser-induced plasma cause optical breakdown inside the eye, permitting surgical photodisruption of transparent or pigmented ocular tissues. The potential role of this process during ablation with excimer lasers has yet to be fully evaluated.

In this paper, we have demonstrated that the most efficient ablation rate of bone by the excimer laser at 308 nm was associated with a laser energy of 20 mJ per pulse (70.7 mJ/mm²) at a repetition rate of 15 Hz. Higher energies per pulse and higher repetition rates resulted in disruption of either the proximal or distal ends of the optical fiber during the osteotomy procedure. It appears that fiber integrity is the limiting factor which prohibits the use of higher energies and repetition rates which could conceivably produce more efficient ablation rates in bone. Furthermore, our

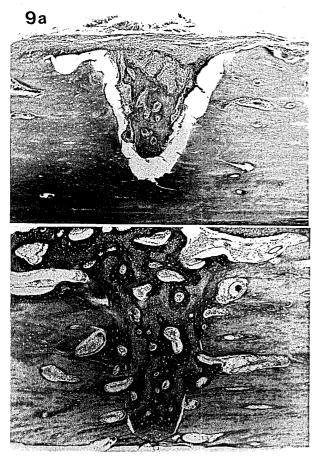


Fig. 9. Photomicrographs of four-week postsurgery laser (a) and saw (b) osteotomies. Note defect between the new bone developing

exclusively from the periosteal surface and the preexisting bone in the base of the groove in the laser osteotomies (a). The saw osteotomies (b) showed complete bridging of the groove by mature lamellar bone; (a)  $\times 90$  and (b)  $\times 90$ . results would also suggest that excimer lasers ablate bone primarily through a photothermal mechanism by an explosive pyrolysis of bone substrate. However, lateral extension of this heat energy into the surrounding tissue resulted in histologic features of thermal injury. Disorganized bone structural elements with reduced staining are seen adjacent to the groove because the tissue cannot dissipate the heat energy from the laser pulse and return to baseline temperature before the next pulse is delivered to the surgical site. The damaged zone left behind will be removed by subsequent pulses, so that only a thin zone of thermally injured bone remains no matter how deep the osteotomy. With each successive pulse, the incision deepens but the damaged layer at the bottom of the groove never gets wider. The width of this zone will be completely determined by the last pulse. In contrast, the zone of thermal injury will be larger nearer the surface due to the continued heating of the laser plume rising from the substrate with each successive pulse. Our

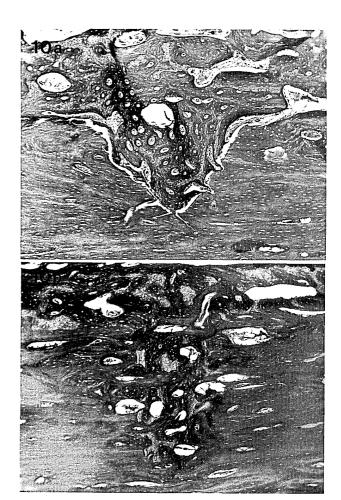


Fig. 10. Photomicrographs of six-week postsurgery laser (a) and saw (b) osteotomies. Note the persistent defect in the laser osteotomies (a), whereas the saw osteotomies are completely healed (b); (a)  $\times$ 90 and (b)  $\times$  90.

70  $\mu$ m at the endosteal base of the groove consistent with this hypothesis.

The repair of bone can be conveniently divided into two components. 18,19 From the periosteum, osteoprogenitor cells differentiate into bone-forming osteoblasts and invade the defect. From the endosteum, surviving osteocytes differentiate into osteoblasts and as these two phases of repair take place, the edges of the bone gradually become enveloped in a fusiform mass of osteoid containing increasing amounts of bone. Ultimately, over time, because of this internal and external bone formation clinical union is said to have occurred.

In the mechanical osteotomies produced by the saw, viable bone-forming cells invade the surgical site from both the periosteal and endosteal surfaces of the groove leading to rapid bone healing. In contrast, as a result of thermal damage produced by the excimer laser to adjacent bone, osteocytes on the endosteal surface of the groove are destroyed and unable to participate in bone regeneration. Therefore, in the laser osteotomies, healing is restricted entirely to the perios-

histological sections demonstrated this zone to ap-

proximate 170 µm at the periosteal surface tapering to

teal surface. This process is extremely slow and as a result, the laser osteotomies demonstrated a persistent large defect between new bone advancing from the periosteal surface and preexisting endosteal bone even at six weeks post surgery. At this time, the saw osteotomies demonstrated complete healing.

The clinical significance of this delayed healing is difficult to determine since no biomechanical strength studies were conducted. Artificial stability may need to be maintained for longer periods of time until new bone may close the defect created by nonspecific thermal injury. Despite its disadvantges, excimer lasers can effect precise and rapid removal of diseased tissue and have promise for widespread clinical applications. Clearly, there is much basic research to be done concerning the acute and chonic effects of pulsed UV laser energy prior to clinical trials.

#### V. Conclusion

Pulsed excimer laser irradiation at 308 nm effectively ablates bone by what appears to be a photothermal process. Although we cannot prove that localized photodissociation of intramolecular bonds does not occur, our study suggests that excimer laser ablation of bone at this wavelength is primarily by a photothermal mechanism. Furthermore, thermal damage to bone adjacent to the laser cuts resulted in a significant delay in healing of the laser osteotomies compared with saw osteotomies.

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