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Pulpal Effects of Enamel Ablation With a Microsecond Pulsed $\lambda=9.3\text{-}\mu\text{m}$ CO₂ Laser

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Abstract

Background and Objectives—In vitro studies have shown that CO₂ lasers operating at the highly absorbed 9.3 and 9.6- μm wavelengths with a pulse duration in the range of 10–20-microsecond are well suited for the efficient ablation of enamel and dentin with minimal peripheral thermal damage. Even though these CO₂ lasers are highly promising, they have yet to receive FDA approval. Clinical studies are necessary to determine if excessive heat deposition in the tooth may have any detrimental pulpal effects, particularly at higher ablative fluencies. The purpose of this study was to evaluate the pulpal safety of laser irradiation of tooth occlusal surfaces under the conditions required for small conservative preparations confined to enamel.

Study Design/Materials and Methods: Test subjects requiring removal of third molar teeth were recruited and teeth scheduled for extraction were irradiated using a pulsed CO₂ laser at a wavelength of 9.3 μm operating at 25 or 50 Hz using an incident fluence of 20 J/cm² for a total of 3,000 laser pulses (36 J) for both rates with water cooling. Two control groups were used, one with no treatment and one with a small cut made with a conventional high-speed hand-piece. No anesthetic was used for any of the procedures and tooth vitality was evaluated prior to treatment by heat, cold and electrical testing. Short term effects were observed on teeth extracted within 72 hours after treatment and long term effects were observed on teeth extracted 90 days after treatment. The pulps of the teeth were fixed with formalin immediately after extraction and subjected to histological examination. Additionally, micro-thermocouple measurements were used to estimate the potential temperature rise in the pulp chamber of extracted teeth employing the same irradiation conditions used in vivo.

Results: Pulpal thermocouple measurements showed the internal temperature rise in the tooth was within safe limits, $3.3\pm 4^\circ\text{C}$ without water cooling versus $1.7\pm 6^\circ\text{C}$ with water-cooling, $n=25$, $P<0.05$. None of the control or treatment groups showed any deleterious effects on pulpal tissues and none of the 29 test-subjects felt pain or discomfort after the procedure. Only two test-subjects felt discomfort from “cold sensitivity” during the procedure caused by the water-spray.

Conclusion: It appears that this CO₂ laser can ablate enamel safely without harming the pulp under the rate of energy deposition employed in this study. *Lasers Surg.*

Keywords

CO₂ laser; laser ablation; enamel; dental pulp; temperature measurements

INTRODUCTION

Studies over the past 25 years have demonstrated that lasers have the potential to be used for several unique treatment modalities in dentistry, including: soft tissue vaporization with hemostasis, removal (ablation) of carious and noncarious dental hard tissue, caries inhibition treatments of enamel surfaces by localized surface heating, and surface conditioning for improved adhesion to composite restorative materials [1–3]. Er/YAG, Er/YSGG, and Nd/YAG solid state lasers have been approved by the FDA for caries removal and it is anticipated that the carbon dioxide laser operating at the highly absorbed 9.3 and 9.6- μ m wavelengths will soon also be approved by the FDA for caries ablation and caries preventive treatments. CO₂ lasers are the most common lasers found in clinics today and have been used for soft tissue surgical procedures for three decades. The CO₂ laser can be designed to operate or “lase” at discrete wavelengths between $\lambda=9$ and 11 μ m. Early studies using continuous wave CO₂ lasers operated at 10.6- μ m reported extensive cracking and charring of surrounding enamel, dentin and bone. Based on these disappointing initial observations, many laser researchers overlooked the potential of CO₂ laser based systems for hard tissue ablation [4–14]. Recent studies using pulsed TEA and RF-excited CO₂ laser pulses of sub-millisecond duration indicate that dental hard tissues can be ablated efficiently without generating peripheral damage [15–19].

Lasers are ideally suited for the conservative and selective removal of decay in the pits and fissures of the occlusal surfaces. It is difficult to remove caries in such surfaces due to the highly localized nature of the decay without damaging the surrounding sound tissue. Moreover, such surfaces are often filled with composite sealants and we have demonstrated that lasers also have great potential for the selective removal of composite sealants and restorations without removing sound enamel [20,21]. New caries diagnostic procedures are becoming available that enable the dentist to identify early caries lesions before they have spread extensively into the underlying dentin. For lesions of this magnitude, it is too early to use conventional restorations that require the removal of large amounts of healthy tissue. As part of the more conservative approach to restorative dentistry, some experts have revised the G. V. Black cavity classification scheme to emphasize the importance of early pit and fissure sites [22]. This new conservative approach emphasizes micropreparation with minimal removal of healthy tissue. Highly absorbed CO₂ laser radiation is ideally suited for this approach since laser pulses can preferentially ablate carious tissue due to the higher volatility of water and protein that are present in carious tissue at a higher ratio than in normal tissue, and it can be tightly focused to drill holes with very high aspect ratios (depth/height) well beyond those obtainable by the dental drill that is limited by the size of the dental burr. Conservation of enamel structure is paramount for preservation of the natural dentition for retention of sealants and to limit exposure to wear. The laser can be used to open up the neck of the fissures sufficiently to allow the entrance of flowable composite to the base of the fissure. Moreover, laser irradiation does not produce a smear layer that needs to be removed, hence restorative materials can be applied directly to the ablated area without the need for further surface preparation and etching [23]. This advantage is of great importance since fissure areas are difficult to etch by conventional means due to the unique enamel morphology. The enamel on the shoulder at the entry to the fissure is often prismless and irregular and may not accept a good etch pattern, so attachment of the resin may be

tenuous. Therefore, laser preparation may be superior to the conventional acid etch in pits and fissures. CO₂ laser radiation vaporizes water and protein and changes the chemical composition of the remaining mineral of enamel and dentin, thus decreasing the solubility to acids around the periphery of the restoration site to leave a smooth surface with an enhanced resistance to secondary caries [24–27].

Excessive heat deposition in the tooth may lead to eventual loss of pulpal vitality [28]. This may be caused by mechanical friction in the case of the high-speed dental hand-piece or a high-rate of heat deposition during laser irradiation. The accumulation of heat in the tooth can be minimized by using a laser wavelength tuned to the maximum absorption coefficient of the tissue irradiated and by judicious selection of the laser pulse duration to be commensurate with the thermal relaxation time of the deposited energy [29].

Recent studies using pulsed transverse excited atmospheric pressure (TEA) and radio-frequency (RF) excited slab CO₂ laser pulses of sub-millisecond duration indicate that dental hard tissues can be ablated efficiently without generating excessive peripheral thermal and mechanical damage [15–19,30]. The peak absorption of dental hard tissues occurs near 9.3 and 9.6- μm where the incident laser light will be absorbed at a depth of under 1–2 μm . Our measurements over the past several years have shown that a pulse duration near 10–20-microsecond is optimal for the ablation of enamel, dentin and bone with minimal thermal damage [31,32]. The thermal relaxation time of the energy deposited in enamel at these wavelengths is on the order of 1–2 microseconds and stretching the laser pulse to 10–20-microseconds reduces the threshold for plasma shielding allowing ablation of enamel and dentin at rates of 10–20- μm per pulse and 20–40- μm per pulse, respectively. The use of longer CO₂ laser pulses has the advantage of raising the plasma-shielding threshold allowing higher ablation rates per pulse, however the longer pulses are more likely to produce a larger zone of peripheral thermal damage. Although ablation rates are higher for longer pulses the peripheral thermal damage caused by these longer pulses may be too extensive for practical use. Such thermal damage may result in thermal stress cracking, the accumulation of non-apatitic calcium phosphate (CaP) phases on the surface, and excessive damage to the collagen matrix [33].

In a recent clinical study, we showed that similar non-ablative 9.6- μm CO₂ laser pulses that are well suited for caries preventive treatments could be used to irradiate tooth surfaces without any adverse effects on pulpal tissues [34] at a rate of 10-Hz.

TEA lasers employ a simple high voltage discharge to excite the gas mixture. In contrast, Radio Frequency (RF) excited slab lasers use a radiofrequency source to excite the CO₂ gas. RF-excited CO₂ lasers have been used for several years for industrial applications and have recently been integrated with high-speed scanners for cosmetic dermatology applications. The RF-excited slab laser operates most efficiently with pulse durations of 60-microsecond to 5-millisecond, and repetition rates in the tens of kHz are feasible as long as a 50% duty cycle is not exceeded. Visuri investigated a RF-excited slab laser operating at 9.4- μm with a pulse duration of 300-microsecond for laser ablation of dentin and enamel [35]. He observed very high ablation rates of ~80- μm per pulse for dentin and 44- μm per pulse for enamel with a fluence of 66 J/cm² per pulse with and without a water flow rate of 11.3 ml/minute. The maximum repetition rate investigated was limited to 10-Hz and the extent of peripheral thermal damage was not investigated. Another group employed an industrial RF-excited slab CO₂ laser system operating at 10.6- μm with a pulse repetition rate of 300-Hz to ablate bone. The peripheral thermal damage zone (with water-cooling) was 50- μm [36]. That zone of damage is much more extensive than the <15- μm zone that we have observed for dentin after ablation with shorter erbium and TEA CO₂ laser pulses [33,37]. The principal advantages of the RF-slab laser over the TEA laser are small size, sealed gas mixture and the

fact that several laser systems have already been engineered and packaged for use in medicine. The primary disadvantage is the greater potential for peripheral thermal damage since these lasers can only be operated efficiently with pulse durations greater than 60-microsecond, much longer than the desired 10–20-microsecond pulse duration that we have determined to be optimal for 9.3 and 9.6- μm CO₂ laser wavelengths in enamel. One RF-excited dermatological CO₂ laser system was modified by Lumenis (Yokeam, Israel) for dental hard tissue ablation by changing the wavelength to 9.6- μm and reducing the laser pulse duration to 70-microsecond [36,38–40]. This laser was used for two small scale in vivo safety studies and those studies were completed without incident [41,42].

The purpose of this study was to measure the rate of heat conduction to the pulp with and without water-cooling and to assess any potential changes to vital pulpal tissues due to excessive heat accumulation using the stretched $\lambda=9.3\text{-}\mu\text{m}$ CO₂ TEA laser pulses of 10–20-microsecond duration, when used at ablative energies within enamel in a limited clinical study utilizing 29 test subjects with teeth already scheduled for extraction.

MATERIALS AND METHODS

Laser Irradiation

An Impact 2,500 CO₂ laser (GSI Lumonics, Rugby, UK) operating at $\lambda=9.3$ with a pulse duration of 15-microsecond was interfaced to an articulated arm delivery system equipped with our own custom designed and machined hand-pieces with air/water aerosol spray, Figure 1. The occlusal surfaces of 3rd molars were irradiated for 1-minute at 50-Hz and 2-minutes at 25-Hz using an ablative fluence of 20 J/cm² and 12–15-mJ per pulse. The laser energy was measured and calibrated using a laser calorimeter, Model ED-200 (Gentec, Quebec, Canada). The beam diameter at the position of the focus from the hand-piece was 300- μm in diameter and was measured by scanning with a razor blade across the beam. An intercavity aperture was used to reduce the laser output to a single-transverse mode and the fluence was defined using a Gaussian beam with a $1/e^2$ beam diameter which was measured using a PyrocamTM I pyroelectric array (Sprocon, Logan, UT). The same total number of laser pulses were delivered to each tooth for both repetition rates, namely 3,000 pulses for a total energy of ~36 – 45 J. There were 17 teeth treated at the highest energy deposition rate (50-Hz) that were extracted within 72 hours and 11 that were extracted after 3 months. At the lower energy deposition rate of 25-Hz, 7 teeth were treated with 72-hour extractions and 10 teeth were treated that were extracted after 3 months. The laser treatment consisted of scanning the beam by hand over the occlusal surface of the tooth to make an incision in the enamel approximately 2 mm long, using the custom-made hand-held hand-piece. A water syringe controller by (KD Scientific, Holliston, MA) was used to deliver water spray to the laser hand-piece. The rate of water delivery was 0.70 ml/minute. The air-flow with the water was 80 SCFH (Standard Cubic Feet per Hour of equivalent air) and was measured with a flow gauge (Dwyer Instruments, Michigan City, IN).

In Vitro Thermocouple Measurements and Ablation Depth Measurements

Thermocouples were placed on the inside of the pulp chamber of the extracted human teeth to measure the temperature rise in the pulp chamber. The temperature at which the heat rise saturates (T_{sat}), that is, rate of energy deposition equilibrates with rate of energy losses, was determined. Based on the seminal Zach and Cohen [28] study, a temperature rise of 5.5°C is considered excessive. A sample size of 25 measurements per group was used and the surfaces were scanned for 2 minutes over an incision distance of 3.5-mm. Measurements were obtained with and without the application of a water spray using the custom designed hand-piece, Figure 1. Incisions were made with and without water on each of the 25 teeth so that a paired *t*-test could be used (Fig. 2). Thermally conductive paste was used to adhere the

thermocouples to the inside of the pulp chamber and maintain thermal contact with the pulp chamber wall. A high-speed air turbine hand-piece was used to drill a hole into the lingual crown of each tooth into the pulp chamber. The thermocouple was placed in the middle of the ceiling of the pulp chamber. Digital X-ray images were taken of the teeth after placement of the thermocouples to confirm proper placement. Buccal-lingual and mesial-distal images of the teeth were taken with Trophy Radiologie Digital Software and sensor (Marne La Vallee, France) to determine placement of the Type K, 36 gauge, 0.13 mm in diameter thermocouples (Omega Engineering, Inc., Stamford, CT). Omega Engineering, Omegabond OB-101 thermal conductive paste was placed inside the hole and around the thermocouple and fixed to the external surface of the tooth with one drop of cyanoacrylate adhesive. A computer-controlled stage moved the tooth across the laser beam with an average speed of 3.14 mm/second and the temperature changes in the tooth were monitored over time using a SR630 thermocouple controller (Stanford Research, Stanford, CA) and Labview software (National Instruments, Austin, TX).

After the thermocouple measurements the depth of each incision was measured using optical coherence tomography. OCT scans were acquired across each incision and the image depth was measured nondestructively with an axial resolution of 9- μ m using a custom built polarization sensitive OCT system described in Ref [43]. Sample groups with and without water-cooling were compared with a paired *t*-test using Instat (Graphpad Software, San Diego, CA) for the thermocouple measurements and the depth measurements.

Clinical Procedures

Test subjects requiring removal of third molars were recruited from the local population. Patients with systemic conditions precluding routine surgical care were excluded. Each patient first underwent a screening examination to determine suitability of any present third molars for the study. A patient was included in the study if there were at least two third molars present which were not obviously carious, not restored, and accessible for laser irradiation. This included fully erupted teeth, as well as some with partial soft tissue impactions. All patient procedures including screening and laser treatment were approved by the UCSF Committee on Human Research (CHR) and informed consent was obtained.

Each patient received at least one laser treatment and had one tooth that was used for a control. Two control groups were used: one with no treatment and one with a small occlusal incision made by a #329 carbide bur in a high-speed handpiece with water spray, approximately 1 mm in depth and 2 mm in length, confined to enamel. The choice of treatments was determined randomly. At the treatment visit, just prior to irradiation, vitality of study teeth was tested with cold, heat and electrical stimulus prior to treatment.

The study teeth were subdivided into two groups to examine both long and short term effects of the treatment. The teeth in the short-term group were scheduled for extraction within 72 hours after the laser treatment and the teeth in the long-term group were extracted 90 days after the laser treatment. Table I shows the number of teeth in each group. Routine surgical procedures were used and the teeth were collected immediately after extraction. Within 30 minutes, the roots were sectioned off just below the pulp chamber and the pulps were fixed by immersion in a formalin solution.

Histological Examination

The teeth were extracted either within 24–72 hours, or 90 days after laser treatment, using standard surgical protocol. The period of 72 hours was chosen to detect reversible pulpal changes such as inflammation and the period of 3 months was selected to show permanent changes to the pulp. The teeth were collected immediately upon removal and the roots were

cut off with a water-cooled diamond band-saw within 30 minutes of extraction just below the cemento-enamel junction to allow maximum fixation of the pulpal tissues. They were then placed in a 10% buffered formalin solution for 24 hours. Following fixation, the specimens were placed in a hydrochloric acid solution (Surgipath Decalcifier II, Richmond, IL) for an additional 24 hours. The coronal and root portions were then sectioned from coronal-to-apical portion to expose the pulp chamber and canals. Sections of 5- μ m thickness were cut and stained with hematoxylin and eosin and examined using criteria for pulpal response established by Stanley [44], by a board certified oral and maxillofacial pathologist.

RESULTS

In Vitro Thermocouple Measurements and Ablation Depth Measurements

Thermocouple measurements showed a significantly higher increase in temperature of $3.3 \pm 1.4^\circ\text{C}$ without water cooling versus $1.7 \pm 1.6^\circ\text{C}$ without water-cooling, $n=25$, $P<0.05$. The temperature of the tooth drops $7\text{--}9^\circ\text{C}$ with application of the water spray. Figure 3 represents the temperature profile during a typical measurement. The ambient temperature was approximately 22°C and the tooth temperature dropped to approximately 13°C after application of the water spray and rose by 1.7°C after 2 minutes of laser irradiation to a temperature of 14.7°C which is still more than 7°C below the ambient temperature and well below the 5.5°C above ambient temperature indicated by Zach and Cohen [28] to cause pulpal inflammation. Even without water-cooling the mean temperature rise above ambient temperature was only 3.3°C which is below the 5.5°C threshold. The mean incision depth was statistically similar with or without the water-spray $938 \pm 309\text{ }\mu\text{m}$ versus 855 ± 20 , respectively. The incision craters were smooth and free of debris when produced in conjunction with a water-spray (Fig. 2). Incisions produced dry are rough and non-uniform, more-over, white asperities are present in and around the ablation crater. We have shown in previous studies using IR spectromicroscopy [45–47] that the white asperities represent non-apatitic calcium phosphate phases that are produced due to excessive overheating of the mineral phase. These undesirable mineral phases are more susceptible to acid dissolution and compromise adhesion. Therefore, even though the temperature rise was less than 5°C without water-cooling during irradiation at 50-Hz it is still necessary to use a water-spray to produce the more desirable crater morphology and chemical composition.

Clinical Procedures

The clinical procedures were completed without any significant adverse events. No analgesic was used and only two of the 29 test subjects voiced any sensation of pain or discomfort with the procedure and that discomfort was attributed to cold sensitivity brought on by the air–water spray from the hand-piece after we turned the laser off to confirm that the water-spray alone caused the discomfort. The custom made hand-piece, Figure 1 functioned in a satisfactory manner and was able to reach the occlusal surfaces of the third molar teeth, which can sometimes be a challenge for restorative procedures due to limited access.

We expect that a commercially produced hand-piece would be even easier to use and permit at least the same access as a conventional dental hand-piece.

Histological Results

Each tooth examined showed a complete lack of pulpal response in all specimens, regardless of treatment category and time extracted post treatment. Histopathologic evaluation of the decalcified specimens' sections exhibited remaining dentin covering pulpal tissue characterized an intact odontoblast cell layer immediately subjacent to a layer of predentin. Below the odontoblastic layer was a cell free zone and a cell rich zone and parietal neural

plexus below that. There was no inflammatory cell infiltrate observed in any of the specimens and the vascular pattern was normal without vascular enlargement or engorgement, Figures 4 and 5. One tooth that was irradiated at the higher rate of energy deposition (50-Hz) was a very small super-numerary wisdom tooth, Figure 6, that also manifested normal histology. This tooth is of particular interest since it had a very small thermal mass compared to the other teeth irradiated in the study and was therefore potentially more susceptible to heat accumulation than the other teeth.

DISCUSSION

The CO₂ laser operating at 9.3- μ m is capable of generating extremely precise incisions in enamel with minimal peripheral thermal damage and heat accumulation at 25 and 50-Hz. Previous studies of ablation rates indicated that the ablation rate of enamel was approximately 10–20- μ m per pulse at incident irradiation intensities of 20 J/cm² with single pulse energies of 12–14 mJ/pulse [37]. The conditions chosen for the clinical study were those that would produce incisions that are 0.3×2×3.5 mm³. Assuming an ablation rate of at least 10- μ m per shot, that is 20 pulses per irradiation spot, an irradiation time of 1-minute was chosen for 50-Hz and 2-minute for 25-Hz. The depth of the incisions are shallower than anticipated due to the highly conical nature of the holes drilled by the CO₂ laser. The highly conical nature of the holes reduces the ablation rate and efficiency with increasing depth. Therefore to most effectively employ the CO₂ laser the laser should be electronically scanned to expose a new area for each pulse. Near-IR imaging studies of drilling through enamel with this laser show that the highly conical holes result in stalling (cessation of ablation) after penetration of 2–3 mm [48]. This can be advantageous from a safety standpoint. If a very high repetition rate is used along with an electronic scanner to scan the laser beam, one concern is that possible failure of the scanner during a procedure may result in a deep hole with possible pulp exposure. However, the self-terminating nature of the highly conical hole formation would prevent excessive depth penetration and the scanner would have to be functioning correctly for efficient enamel removal. However, stalling would still cause an increase in inter-pulpal temperature but the clinician would have ample time to intervene before the heat build up became excessive. Thermocouple measurements and depth incision measurements were also carried out for higher repetition rates up to 300-Hz with water-cooling on extracted human teeth and those are reported by Pierre [49]. The higher repetition rates resulted in only slightly higher incision depths, for example, 1,130±240 μ m due to stalling even though six times as many laser pulses were delivered in the same time interval. Temperature measurements were significantly higher due to the additional heat accumulation caused by stalling and inefficient ablation. Again, rapid scanning of the laser beam should minimize the heat accumulation. We plan on repeating those measurements using an integrated scanner to scan the beam over a large area instead of a single linear incision to confirm this hypothesis.

The CO₂ laser operating at λ 9.3 has two principal advantages over other lasers when applied to ablation of tooth structure. The first advantage is that the CO₂ laser can operate efficiently at high repetition rates well into the kHz regime. The erbium lasers presently used for hard tissue ablation operate most efficiently at very low repetition rates and rates exceeding 10 Hz are difficult due to the physics of the laser crystal and even though clinical systems are in use with repetition rates of 50-Hz, they are very expensive. Therefore, in order to achieve higher cutting rates erbium lasers must deliver a larger amount of energy per pulse, in the range of 100–500 mJ. CO₂ lasers can be operated with lower single pulse energies and irradiation intensities and the repetition rate can be increased for higher cutting rates. The laser beam can also be scanned to minimize heat accumulation in one area.

The second advantage is the very high absorption by the mineral phase that can be exploited for the modification of the irradiated surfaces to produce a layer that exhibits increased resistance to demineralization. The walls of a restored cavity are susceptible to secondary caries by microleakage of acids, bacteria, and cariogenic substrate between the restoration and the walls. Cavities prepared by the CO₂ laser have been demonstrated to be more resistant to wall lesion formation in a previous in vitro study [26].

It is important to describe the limitations of this study, the principal one is the limited extent of ablation used. Only enamel was treated, with no dentin involvement in any of the incisions created by the laser. In order to ascertain that ablation of dentin is also safe for the pulp, a study will have to be carried out that involves exposing and ablating dentin. When irradiation is confined to enamel, the remaining tooth structure between the cavity preparation and the pulp is several millimeters thick, and thus provides for a considerable amount of thermal protection. The repetition rate was also limited to only 25 or 50-Hz, future clinical studies are needed at much higher repetition rates in conjunction with scanning of the laser beam.

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Fig. 1.
CO₂ laser hand-piece with integrated aerosol water spray used for in vivo laser irradiation attached to the articulated-arm delivery system.

Fig. 2.

Image of 3rd molar tooth irradiated for 1-minute at 50-Hz with $\lambda=9.3\text{-}\mu\text{m}$ CO₂ laser pulses of 14-mJ/pulse with and without water spray. The incision length was 5-mm long. A magnified image is shown below the whole tooth image that emphasizes the morphological differences between the two craters/incisions. The upper/right incision with the water spray is smooth, uniform and free of debris or peripheral thermal effects while the lower/left crater without water-cooling is non-uniform and contains white asperities.

Fig. 3.

Top: Schematic drawing of tooth cooling below room temperature ($\sim 20^\circ\text{C}$) at the beginning of water spray from laser hand-piece at T_1 . After the temperature stabilizes, irradiation starts at T_2 . Typically within 2 minutes the temperature plateaus at T_3 (typically below T_1) reflecting the balance between the energy deposited in a specific time and the energy conducted away as heat. Middle: Pulp chamber thermocouple reading during 2 minutes (120 seconds) irradiation interval without water cooling. The temperature rises almost 3°C before reaching a steady state of heat deposition and heat loss. Bottom: Thermocouple reading during 2 minutes irradiation interval after T_2 with the water spray, temperature rise is $\sim 2^\circ\text{C}$ above T_2 but well below the ambient temperature $\sim 20^\circ\text{C}$.

Fig. 4. Pulpal histology (H&E stained) of a tooth extracted 3 months after irradiation by the CO₂ at the higher rate of energy deposition, 50-Hz, shown at two magnifications.

Fig. 5. Pulpal histology (H&E stained) of a tooth extracted within 72-hour after irradiation by the CO₂ at the higher rate of energy deposition, 50-Hz, shown at two magnifications.

Fig. 6. Pulpal histology (H&E stained) of a very small super-numerary wisdom tooth extracted within 72-hour after irradiation by the CO₂ at the higher rate of energy deposition, 50-Hz, shown at 400× magnification. Inset: An image of the entire pulp chamber is shown in the low magnification image.

TABLE I

Number of Teeth Subjected in Each of the Four Test Groups

Group	<72 hours	3 months
Control	12	8
Carbide bur	8	9
Laser at 25 Hz	7	10
Laser at 50 Hz	17	11