Histologic Analysis of the Effect on Dental Pulp of a 9.6-μm CO₂ Laser

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Background and Objective: Both patients and dentists would like a replacement of the dental drill. During the last decade, lasers have been investigated as a possible replacement. For lasers to be accepted, studies must show that their effect on the dental pulpal tissues is equal to or less noxious than those effects caused by the dental handpiece (drill).

Study Design/Materials and Methods: In this study, two laser systems were used; the first was a breadboard CO₂ laser and the second a prototype clinical CO₂ laser system both emitted 60-μs-long pulses of 9.6-μm radiation. On the delivery system of both lasers, a scanner moved the focussed beam in a circular pattern and a water spray system served to cool the ablation site. Both lasers were used to create holes of similar dimensions in canine teeth. The treated teeth were then restored and harvested at either 4 days or 4 weeks. The teeth were decalcified, sectioned, and stained for examination via light microscopy.

Results: The histologic examination revealed normal pulpal tissues in the canine teeth treated with both CO₂ lasers. Some histologic sections showed an increase in the predentin layer, 28 days after laser treatment. While many histologic sections showed normal pulpal architecture following handpiece treatment, some sections showed total disruption of the normal pulpal histology.

Conclusions: Histologic evaluation revealed that the lasers produced no noticeable damage to the dental pulpal tissue and appear to be a safe method for removing dental hard tissues. From this study, it appears that 9.6-μm CO₂ laser does not cause damage to the dental pulpal tissues in dogs. Lasers Surg. Med. 30:261–266, 2002.

Key words: CO₂ laser; dental pulp; ablation; thermal damage; dental handpiece

INTRODUCTION

Since the development of the laser by Maiman in the early 1960s, there has been great interest in the potential use of lasers in dentistry [1,2]. Early researchers investigated the use of lasers for the removal of dental hard tissues and for the modification of the enamel surface to reduce acid demineralization. Unfortunately, the early lasers caused significant thermal damage, thereby precluded clinical applications. As the development of different laser sources progressed, research with these new lasers added to the existing body of information relating to laser dental hard tissue interactions. Of all the potential applications of lasers, the replacement of the dental drill seems to have the greatest interest among researchers. Throughout the last decade much interest has centered on the Er:YAG laser as a possible replacement. Hibst and Keller published their findings in 1989, evaluating the ablation efficiency and thermal changes of the Er:YAG laser on dental hard tissues [3,4]. Their research, and others that followed, showed that the Er:YAG laser provided a safe method for abrating dental hard tissues. Wigdor et al. and Visuri et al. showed that pulpal tissues in dogs were undamaged in teeth that were irradiated with the Er:YAG laser [5,6]. Subsequent to these research reports, the US Food and Drug Administration (FDA) cleared the Er:YAG laser for treatment of simple carious lesions in humans. The dental community, however, did not receive this clearance with great enthusiasm. It seems that this first laser for dental hard tissue treatment did not provide dentists with a tool that removed dental hard tissues efficiently.

Nelson et al. and Featherstone et al. investigated the absorption characteristics of hydroxyapatite, which is the primary component of enamel and dentin [7–9]. They suggested that if a wavelength of light could be coupled directly into the hydroxyapatite, this light would be very efficient in affecting the enamel surface. They found that radiation in the 9.3–9.6-μm range is efficiently absorbed by hydroxyapatite. Some of their reports investigated the effect of these wavelengths on the enamel; the goal of their work was to modify the enamel surface so that the mineral would be less susceptible to acid demineralization. These groups worked with subablative radiant exposures and did

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not intend to remove dental hard tissue. They also showed that the absorption of radiation by hydroxyapatite changes with wavelength. In particular, the 10.6-μm radiation emitted by the common CO₂ laser is not as well absorbed as 9.3–9.6-μm radiation. Krpachev et al. discuss the potential of the 9.6-μm radiation for ablating dentin and enamel, because these tissues have a hydroxyapatite absorption peak near 9.6-μm [10]. Ertl and Muller working in the ablative regime showed that the 9.6-μm radiation had a lower ablation threshold due to its higher absorption coefficient [11]. Wigdor et al. reported on the effects of the 10.6-μm CO₂ laser, when used to remove dental decay [12]. Their results showed that this wavelength damages pulpal tissues. Other laser wavelengths effects on dental hard tissues have been investigated. The effects of these lasers on dental hard tissues have been reported [13–15].

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Since the crystalline structure, which is the main component of teeth, appears to be efficiently absorbed by laser radiation in the 9.3–9.6-μm range, one expects that a laser emitting in this wavelength range would be a good candidate to replace the dental drill. In this work, we evaluate such a laser and present its effects on the dental pulp. The laser parameters were selected for efficient ablation of the dental hard tissues. A scanner was also employed to move the focused laser beam between pulses, and thereby, reduce noxious thermal effects on the tissues. It is well known that the pulp tissues, specifically the odontoblasts, are susceptible to thermal damage. Thus, any detrimental thermal effect would be seen in this layer. In this work, we compare the effect on canine pulp tissue of a standard dental handpiece and ablative pulses of 9.6-μm CO₂ laser radiation.

MATERIALS AND METHODS

A breadboard CO₂ laser (ESC Medical Systems, Yokneam, Israel) (laser 1) was developed. It emitted 22 mJ of 9.6-μm radiation in 60-microsecond-long pulses. By changing the pulse repetition rate, one could control the average incident power. The beam was positioned via an optical scanner that moved the 250-μm-diameter focused beam over a 2.5-mm-diameter circular region. A mist of room temperature water was sprayed over the ablation site at a rate of approximately 10 ml/min. Two adult Beagle dogs were sedated with Acepromazine (1 mg/kg), induced with Brevital (11 mg/kg), intubated and maintained with Halothane (~1%). Once in a surgical anesthetic plane, the CO₂ laser was used to ablate the buccal surface of 16 teeth. Each laser-created hole was approximately 3.0 mm in diameter and 2.0 mm in depth. Based upon previous studies, the laser power was set at 3.0 watts (W) for efficient enamel ablation, then changed to 2.0 watts, so that the dentin could be ablated without leaving significant thermally altered material on the dentin surface. Two untreated teeth and two dental handpiece holes were created as controls in each dog. After ablation, the holes were etched with 10% phosphoric acid, rinsed, coated with primer (Caulk/Densply York, PA), then restored with a composite resin (Caulk/Densply York, PA). The dogs were maintained on soft food diet for 2 days, then fed the usual kibble. At 4 days and 30 days, the dogs were sacrificed and the 20 treated teeth were harvested from each dog. The teeth were sectioned at the dento-enamel junction to allow perfusion of 10% buffered formalin into the coronal pulp tissue. After one week in the formalin, the teeth were demineralized, embedded in paraffin and sectioned. They were stained with hematoxalin & eosin and evaluated under light microscopy.

Another series of canine teeth were prepared using a prototype clinical CO₂ laser system (ESC Medical Systems, Yokneam, Israel) (laser 2). This second laser had emission characteristics identical to laser 1 except the second laser was ergonomically designed for use in a clinical practice. The handpiece resembled a dental drill and had the scanner designed to allow for easier manipulation by the clinician. The laser controls were also designed to be more user friendly. Two adult hounds were anesthetized as above and 20 of the teeth in each dog were ablated on the buccal surfaces in a manner identical to the two dogs above. Again, the teeth were harvested at either 4 or 28 days, demineralized, sectioned and stained for light microscopy.

RESULTS

Figure 1 shows a photograph of the laser ablating the tooth of a dog. Figure 2 shows a dog’s maxillary canine with a hole in the buccal surface prepared with a CO₂ laser. This hole was restored with dental composite material, until the tooth was harvested. The photographs in Figures 1 and 2 are representative of the treatment performed on all four dogs. Figure 3 shows a photomicrograph of the pulp, 4 days after irradiation with the breadboard laser. There is no apparent disruption of the pulpal tissue. Figure 4 is a higher power photomicrograph of the tooth seen in Figure 3. The odontoblasts in the photomicrograph are just beneath the laser hole. There appears to be a healthy odontoblastic cell layer beneath the laser ablation zone. Since the odontoblasts seem unaffected our results suggest that no damage is evident in the pulp. The underlying very loose connective tissue in some of the teeth appears disrupted. It must be understood that demineralization and histologic processing of dogs teeth will sometimes harm the very loose connective tissues of the pulp. We suggest that these pulpal changes are due to processing artifact and not laser damage. Figure 5 shows a photomicrograph of the pulp, 30 days after treatment with the breadboard laser. Note the significant amount of reparative dentin produced by the odontoblasts beneath the laser treatment zone. Careful examination reveals that these hypertrophic odontoblast
are located at the pulpal termination of dentinal tubules that now distally terminate at the ablation crater. Figure 6 is a higher power photomicrograph showing the compressed odotoblasts and a better view of the dentin and predentin layers beneath the laser treatment zone.

Figure 7 shows a photomicrograph from a tooth, 4 days after treatment with the prototype laser. Figure 8 is a higher power photomicrograph of the pulp seen in Figure 7, directly beneath the area of laser treatment. There does not seem to be any disruption of the odontoblastic cell layer. Figure 9 shows a photomicrograph of a tooth harvested 28 days after treatment with the prototype laser. Even though the laser appears to have come within 500 μm of the pulp, no pulpal damage is evident. Figures 10 and 11 are low and high power photomicrographs of a tooth treated with a dental handpiece with an inverted cone bur and harvested at 28 days. There appears to be some disruption of the dental pulp just beneath the area of treatment.

During preparation of the teeth with the breadboard laser, the time necessary for preparation was recorded unbeknownst to the operative dentist (HAW). In order to ablate through the enamel and prepare a cavity within the dentin, the laser-prepared teeth required 23 ± 10 seconds (mean S.D., n = 32); the dental handpiece-prepared teeth require 13.5 ± 6.6 seconds (mean S.D., n = 4). These preparation times are not statistically significantly different (\( P > 0.05 \)).

**DISCUSSION**

From a work by Wigdor in 1996 on patients’ perception of lasers, it was reported that there is keen interest among patients to develop lasers that can replace the dental drill [18]: over 67% of the patients queried felt that lasers would make the visit to the dentist easier. It is this interest amongst patients and dentists alike that has prompted researchers to undertake the quest for a laser that can fulfill the expectations of both dentists and patients. The major stumbling block has been the development of a laser that can efficiently, yet painlessly, ablate hard tissue.

Currently, the ideal laser appears to be one, whose radiation will be absorbed by the two major components
of dental hard tissues: hydroxyapatite and water. Previous research suggests that radiation in the range of wavelengths from 9.3 to 9.6-μm is strongly absorbed by hydroxyapatite. Such radiation is also strongly absorbed by water. Thus, a laser was developed to take advantage of the hydroxyapatite absorption in the 9.3–9.6-μm region. For efficient ablation of both healthy and carious mineralized dental tissues, a laser must emit sufficient energy per pulse with the correct pulse duration and sufficient average power. The energy per pulse emitted by the CO2 laser was 20 mJ. This emitted energy was focused to produce a radiant exposure of 41 J/cm², which is sufficient to ablate tissue. The pulse duration (60-microseconds) is less than the thermal relaxation time of the absorbing layer of tissue; thus the energy invested in ablation was maximized and the energy that remains in the tissue after ablation was minimized. The pulse duration was, however, selected to be long enough to minimize plasma formation. Plasmas form readily in the presence of ions, such as Ca²⁺, that are abundant in teeth. Finally, the cutting rate is directly proportional to the average power; thus, the current laser was designed to emit several watts of average power. The 2–3 W settings were used in this study, because it represents sufficient power to cut, but not so much power that enamel or dentin would be thermally damaged or that the operator could not control the ablation process. Indeed, the difference between the preparation time with the laser and the handpiece, while indicative of a longer preparation time with the laser, was not statistically significant. To minimize thermal damage to the tooth, the focussed beam was scanned over the surface such that each pulse hit a surface that had not recently been irradiated and water was sprayed on the surface to carry away residual thermal energy.

Fig. 5. Photomicrograph of tooth from dog sacrificed at 30 days irradiated with the breadboard laser. The laser hole can be seen on the top of the photo. Note that dentinal tubules that terminate in the laser-produced hole are associated with hypotrophic odontoblasts and an increase predentin layer 100 × H&E.

Fig. 6. Higher power photomicrograph of the tooth in Figure 5. Note the increased thickness of the predentin layer 250 × H&E.

Fig. 7. Photomicrograph of tooth from dog sacrificed at 4 days irradiated with the prototype laser. Laser hole can be seen in the upper right hand corner of photo, one sees normal pulpal architecture 100 × H&E.

Fig. 8. Higher power photomicrograph of the tooth in Figure 7. Note there is no disruption of the odontoblasts lining the inner dentin wall 250 × H&E.
It is essential, however, for a laser with the above characteristics to be evaluated for the effect of such a laser on the dental pulpal tissue. Our results indicate the new laser cuts teeth in vivo with pulpal effects that are equal to or better than the existing dental drill. None of the either laser or handpiece prepared teeth that were evaluated had histologically identifiable, irreversible damage to pulpal tissues. One tooth that had been treated with the dental handpiece showed some disruption of the odontoblasts in the zone immediately adjacent to the drilled hole. As seen in Figures 10 and 11, there was a loss of the integrity of the odontoblasts and no intact cells could be seen in this zone. There were no laser treated teeth that revealed a loss of odontoblast integrity even though with both the laser and handpiece, every attempt was made to cut the dentin within the same distance of the pulp.

It is interesting that some of the teeth, harvested 30 days after treated with the breadboard laser, showed a considerable amount of reparative dentin in the area adjacent to the laser treated zone. The odontoblasts appear cuboidal in this area in contrast to the palasading odontoblasts on either sides of the treated zone. The production of reparative dentin would be expected in an area where dentin had been cut; however, the amount seen adjacent to the laser-treated zone is much greater than would be expected. The teeth treated with the prototype laser showed some reparative dentin formation, but not to the extent seen in the teeth treated with breadboard laser. What might have stimulated the production of the excessive amount of reparative dentin is not known, but it is clear that the laser ablation process stimulated odontoblastic activity.

REFERENCES