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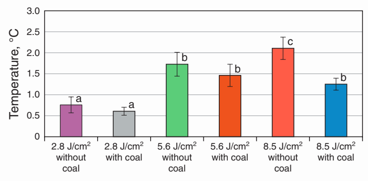

Maximum pulpal temperature rises during laser irradiation detected by thermocouples (mean and standard error). Means followed by distinct letters are statistically different by the Tukey test ($p < 0.05$)

Thermal analysis of teeth irradiated with Er,Cr:YSGG at low fluences

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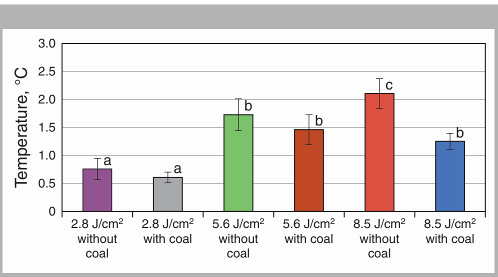

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1. Introduction

A variety of laser systems have been widely used in medicine and dentistry. In dentistry, laser systems are employed in caries removal [1], cavity preparation [2,3], root canal treatment [4,5], periodontics [6], caries prevention [7–15], surgeries [16] and other applications [17–20].

For irradiation in dental hard tissues, the most frequent laser systems used are Nd:YAG [4–6,12,14], Argon [7], Er:YAG [8,9,11,13,21,22], CO_2 [15], Ho:YLF [23], and

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Er,Cr:YSGG [1–3,8,9,11,24–26]. The Er,Cr:YSGG laser is emitted in 2.79 μm wavelength, which is better absorbed by water and OH^- contents of hydroxyapatite [10,27], and promotes surface temperatures up to 800°C at the ablation threshold [10]. Due to this fact, Er,Cr:YSGG laser is applied for cutting of enamel [2], dentin and root surfaces [4], and also for caries prevention [9,10,12].

For its application aiming caries prevention, laser irradiation must promote microstructural changes in enamel, which are commonly induced by thermal action [9,10,28]. In fact, if a laser is highly absorbed by dental hard tissue, its energy is efficiently converted into heat and induces crystallographic changes in these structures, which can result in an increase of the resistance of enamel to acids [9–11,27,28]. In order to get this effect, the smallest surface temperature necessary during laser irradiation is about 100°C [10,11,28–31].

In order to choose a parameter of irradiation for a clinical application, the fluences employed must be safe to the vitality of pulp and periodontal tissues [5,32]. The dental pulp presents a high vascularization and its viability may be compromised by thermal injury transmitted through the enamel and dentin [33]. Previous studies have indicated that temperature increments above 5.6°C can be considered potentially threatening to the vitality of the pulp and increments in excess of 16°C can result in complete pulpal necrosis [34]. Therefore, the investigation of intrapulpal heat generation during laser irradiation is the first step when choosing a laser parameter for any clinical application [32]. Excessive thermal damage has been one of the major problem associated with laser irradiation on enamel, mainly in lasers that have higher transmission into dental tissues, such as Nd:YAG and Ho:YLF. The pulpal temperature changes were also investigated with erbium lasers; however, there are no reports which describes the pulpal temperature changes when Er,Cr:YSGG laser is applied for caries preventive purpose.

In order to restrict the heat dissipation through the teeth tissues, the application of a photosensitizer is frequently applied over the enamel surface before laser irradiation [13], and this application can avoid pulpal damages even when laser is irradiated at higher fluences [35]. The application of the photosensitizer is frequently performed before Nd:YAG and Ho:YLF laser irradiation, and the Indian Ink is the most used dye. However, because of the difficulty in Indian Ink removal, it has been suggested the application of a coal paste, which does not compromise the aesthetics of teeth after laser irradiation [13].

Although the increase of acid resistance of enamel is reported to occur with higher fluences, the excessive heating produced can compromise pulp vitality due to heat dissipation. In this way, several authors have investigated intrapulpal heat generation during laser irradiation aiming caries prevention, and the best results were obtained when it was applied low fluences [9,10]. However, there are few studies which correlates the surface temperature rises and morphological findings with the potential on causing damages on pulp when Er,Cr:YSGG laser is irradiated at low fluences.

The purpose of the present study is to monitor temperature rises within the pulp chambers when human teeth are irradiated with Er,Cr:YSGG laser at low fluences, to verify the influence of a photosensitizer on heat transfer into teeth and correlate this findings with the surface temperature and the morphological findings, predicting the feasibility of Er,Cr:YSGG laser to be applied at the selected fluences for caries prevention.

2. Material and methods

After approval by the Committee on Human Research at the Energetic and Nuclear Researches Institute (Proj. 094 CEP-IPEN), University of Sao Paulo, 81 freshly extracted third molar human teeth were selected, cleaned and stored in deionized water with thymol under refrigeration to avoid the dehydration of samples and fungal growth. Teeth with cracks or irregularities of the enamel structure were excluded.

Laser irradiation was performed using an Er,Cr:YSGG hydrokinetic laser device (Millenium, Biolase Inc., San Clemente, CA, USA). This laser system operates at a wavelength of 2.79 μm, pulsed width duration of 140 μs, a repetition rate of 20 Hz and a power output ranging from 0 to 6 W. The energy is delivered through a fiberoptic system with 750 μm of spot size, bathed in an adjustable air and water spray.

Some samples had the surfaces recovered with a thin layer (approximately 100 μm) of a photosensitizer, which was composed of triturated coal (particles of ±10 μm diameter) diluted in equal parts of deionized water and 99% ethanol [13]. This mix formed a paste with fluid consistence that permitted its application with a #01 brush. The teeth selected were randomly divided into six groups, according to the laser irradiation conditions and the application of the photosensitizer:

Group 1: samples without photosensitizer application and irradiated with 2.8 J/cm^2;
Group 2: samples with photosensitizer application and irradiated with 2.8 J/cm^2;
Group 3: samples without photosensitizer application and irradiated with 5.6 J/cm^2;
Group 4: samples with photosensitizer application and irradiated with 5.6 J/cm^2;
Group 5: samples without photosensitizer application and irradiated with 8.5 J/cm^2;
Group 6: samples with photosensitizer application and irradiated with 8.5 J/cm^2.

Irradiation was performed perpendicularly to the surface of samples with the fiber end at 1 mm distance from this surface. The time of laser irradiation varied according to each experiment of this study, and all laser irradiations were performed without air-water mist.

2.1. Surface morphology

12 human teeth were selected for this experiment. From each tooth, two enamel specimens with 3×3 mm were cut from the smooth surfaces with a slow rotating diamond blade (Isomet, Buehler, IL). The 24 enamel samples were divided into the 6 groups (n = 4) described above. Samples were immobilized in optical supports and laser handpiece was coupled to a computer controlled motion control system (Newport, Irvine, CA) adjusted to a speed of 4 mm/s. Laser tip was kept at a standardized distance of 1 mm from enamel surface.

After laser irradiation, the samples were cleaned on ultrasonic bath for 1 minute and fixed with 2% glutaraldehyde solution for 2 hours. The samples were immediately perfused with a phosphate buffered solution 0.1M at room temperature, rinsed with distilled water and then dehydrated in a graded series of alcohol solutions (50, 70, 80, 90, 95, and 100%) for 10 minutes at each concentration. These samples were sputtered with a 15 μm thick gold and submitted to analysis of scanning electron microscopy (Phillips XI, Eindhover, Holland).

2.2. Surface temperature

For this experiment, it was selected 9 human teeth. Each tooth was sectioned at the mesio-distal plane with a diamond saw disk (Isomet, Buehler, IL). The 18 samples obtained were randomly divided into the six groups described above (n = 3).

The temperature changes in enamel surface during and immediately after laser irradiation were measured using a thermographic camera (ThermaCam FLIR SC3000 Systems, USA), which stores infrared images and data at rates up to 900 Hz. This experiment was performed at a room temperature of 24.6°C, 47% air relative humidity and considering teeth emissivity as 0.91. The thermographic camera was positioned at 0.1 m distance of samples and the obtained infrared images were recorded at rates of 900 Hz for later analysis.

Each sample was positioned with the sectioned side turned towards to the thermographic camera, and laser handpiece was positioned at focused beam, at 1 mm distance from the enamel surface, and tangentially to the sectioned plane of samples. This assembly was kept in optical supports and the area of interest was isolated at a focal length of 0.1 m using an internal macro lens. This apparatus made possible the recording of heat propagation from enamel surface through dentin to the pulp chamber, leading the evaluation of the temperature increasing of dental tissues at the exact moment of laser irradiation.

Laser irradiation was performed in a single point of the enamel surface at a standardized time of five seconds in order to record heat transfer and dissipation. Laser irradiation and thermal recordings were synchronized and thermal recording finished fifteen seconds after the end of the laser irradiation. For later analysis, it was chosen two standardized points on the infrared images: one located at the enamel surface immediately below the laser tip (Spot 1); and another one located at dentin-pulp chamber limit (Spot 2), at an approximated distance of 3.5 mm below Spot 1.

2.3. Pulpal temperature

It was chosen 60 human teeth (n = 10). The residual pulp tissue was removed of these teeth using endodontic type K-files introduced by apical access and root canals were enlarged with # 80 K-file in order to allow the placement of the thermocouples in the pulp chamber. At the time of the experiment, the root canals of all specimens were filled with a thermally-conductive paste (thermal conductivity of 0.4 cal s^{-1} m^{-1} K^{-1} – Implastec, Votorantim, Brazil) in order to keep thermal contact between the end probe and dentin surface. A calibrated K-type thermocouple (chromel-alumel – Omega Engineering, Stamford, USA), with a 0.05 mm diameter probe and sensitive to temperature variations between 0.1°C and 100°C assembly, was introduced into the pulp chamber. The temperature sensitive end of the probe was placed at the closest distance to the area to be irradiated, and its location was controlled radiographically for each sample. The thermocouple apparatus was connected to an analogue-to-digital converter (SR lock-in amplifier, Stamford Research System, USA) linked to a computer, and time and temperature data were recorded at sampling rate of 20 Hz, with temperature resolution of 0.1°C.

Samples were fixed and immersed in a water-filled heating circulator at standardized temperature of 37°C, with only the coronal part of the tooth not being submerged. Laser handpiece was positioned in the optical support coupled to the computer controlled motion control system (Newport, Irvine, CA), with speed adjusted to 4 mm/s. Laser irradiation was performed according to the groups described above, with end tip at 1 mm distance from the occlusal surfaces of samples, and scanning all enamel area during 30 seconds. Thermal recordings started 5 seconds before laser irradiation and ended in time with laser irradiation. After the end of the experiment, all samples were sectioned and the thickness of enamel and dentin were measured in order to assure the standardization of samples.

3. Results

3.1. Surface morphology

Following Er,Cr:YSGG laser irradiation, the surface morphology of enamel presented slight cavities, typical of ablation areas, with fissures and conical craters with sharp enamel projections. When samples were recovered with photosensitizer, laser irradiation at 2.8 J/cm^2 and 5.6 J/cm^2 promoted rough surface with the exposition of enamel rods, without any melting or cracks.

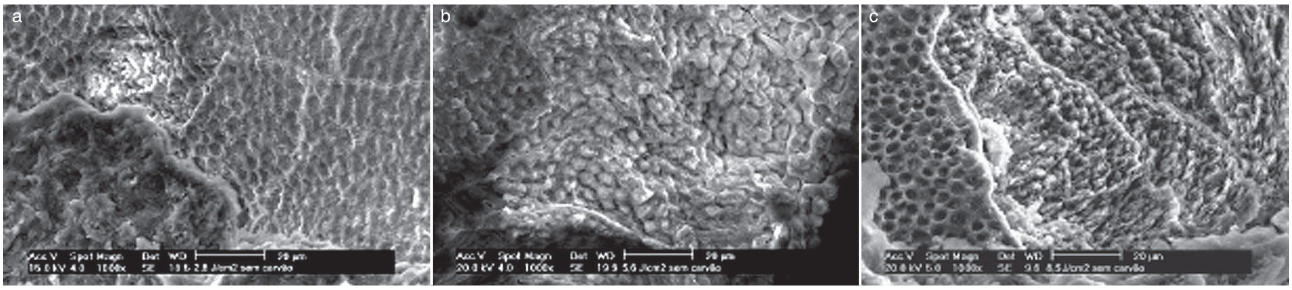

Figure 1 Scanning electron micrographs of human teeth irradiated with Er, Cr:YSGG laser with 2.8 J/cm^2 (a), 5.6 J/cm^2 (b), and 8.5 J/cm^2 (c) without photosensitizer. It can be seen ablation areas in all pictures, with sharp projections and some exposition of enamel rods. Original magnification: 1000×

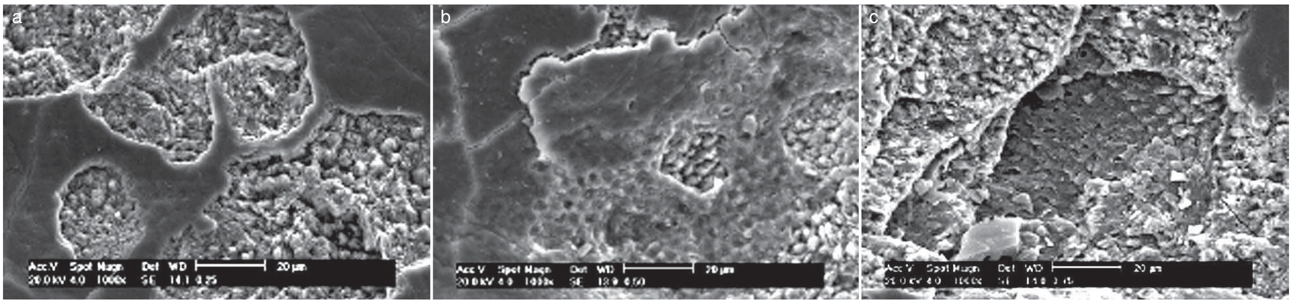

Figure 2 Scanning electron micrographs of human teeth irradiated with Er,Cr:YSGG laser with 2.8 J/cm^2 (a), 5.6 J/cm^2 (b), and 8.5 J/cm^2 (c) with photosensitizer. It can be seen ablation areas in all pictures without cracks or melting. Original magnification: 1000×

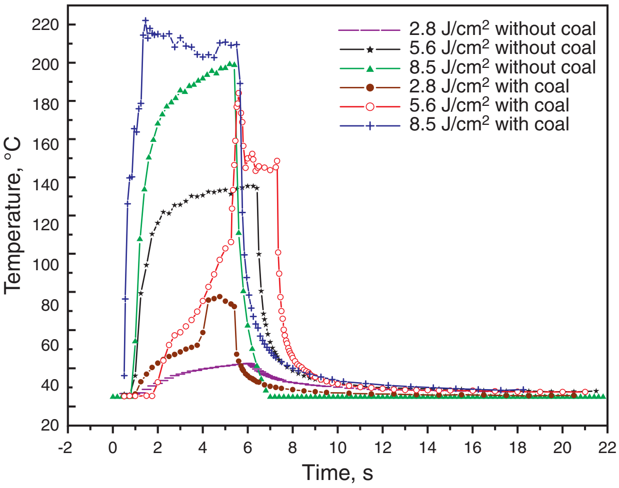

Figure 3 (online color at www.lphys.org) Surface temperature increases during enamel irradiation with Er,Cr:YSGG laser

In samples irradiated with 8.5 J/cm^2 without photosensitizer, the SEM images presented no evidence of melting or carbonization. In some areas, enamel rods were clearly exposed in ablated areas (Fig. 1c). In all samples recovered with photosensitizer, micro-irregularities and ablated areas can be seen, without melting or cracks (Fig. 2). Smooth surfaces were found between lased areas.

Even irradiated without coolant, the macroscopic observation showed some white spots when irradiated at 8.5 J/cm^2 without photosensitizer, lacking any evidence of carbonization. At fluences of 2.8 J/cm^2 and 5.6 J/cm^2, no visible changes were observed at macroscopic observation. Samples irradiated with photosensitizer showed white spots in lased areas and absence of photosensitizer vestiges. However, when enamel was lased at fluence of 8.5 J/cm^2, it was possible to evidence a slight darkness on the surface, probably due to some deposit of coal particles. This aspect, on the other hand, could be easily removed after prophylaxis with pumice.

3.2. Surface temperature

The study with thermographic camera produced infrared images that can demonstrate the pattern of temperature change along the tooth and its gradual spread to dentin. The variations of the temperature of Spot 1 and Spot 2 points are shown in Fig. 3 and in Fig. 4, respectively.

In all curves of surface temperature, the temperature increased from the beginning to the end of laser irradia-

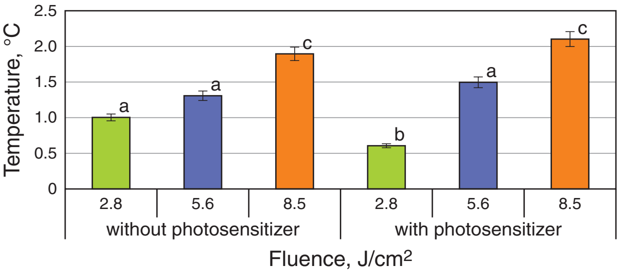

Figure 4 (online color at www.lphys.org) Intrapulpal temperature changes in samples irradiated with Er,Cr:YSGG laser recorded by thermographic camera (mean and standard error). Means followed by distinct letters are statistically different by Student t-test ($p < 0.05$)

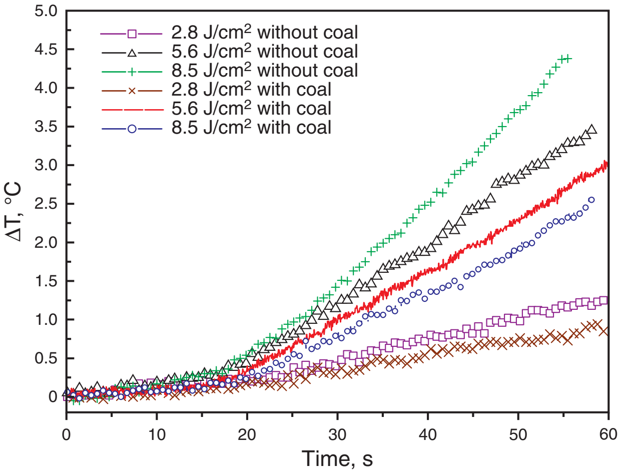

Figure 5 (online color at www.lphys.org) Pulpal temperature changes during laser irradiation detected by thermocouples

tion. The maximum variation of surface temperatures were 79.6 ± 0.8°C and 42.7 ± 1.9°C in samples irradiated at 2.8 J/cm^2 with and without photosensitizer, respectively; when lased with 5.6 J/cm^2, the maximum surfaces temperatures were 184.1±29.3°C and 136.1±13.0°C in samples with and without photosensitizer, and 247.6 ± 30.2°C and 211.8 ± 22.6°C in samples irradiated with 8.5 J/cm^2 without and with photosensitizer, respectively. After the end of irradiation, the temperatures fitted to an exponential decay, reaching nearly its initial temperature value ∼ 10 seconds after laser was turned off.

In samples recovered with the photosensitizer, the temperature peaks were coincident to the laser interaction with the coal particles. At the onset of irradiation, all photosensitizer was removed at the initial laser pulses, and the infrared images showed the ejection of the coal particles to the outer surface such as "micro-explosions".

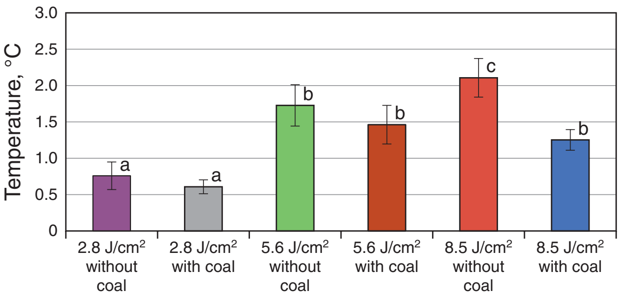

Figure 6 (online color at www.lphys.org) Maximum pulpal temperature rises during laser irradiation detected by thermocouples (mean and standard error). Means followed by distinct letters are statistically different by the Tukey test ($p < 0.05$)

The curves of dentin temperature rises (Spot 2) showed a delay in the temperature increase compared to the surface temperature, which corresponds to the time required for heat propagation. The maximum Spot 2 temperature variations were 4°C in all samples irradiated with 2.8 J/cm^2 (with and without photosensitizer). In samples irradiated with 5.6 J/cm^2, the maximum variations were 1.2°C and 2.2°C with and without photosensitizer, respectively, and 1.2°C and 2.2°C in samples irradiated with 8.5 J/cm^2 with and without photosensitizer.

Student t-test was performed for statistical analysis of the mean values of maximum temperature achieved for each experimental group, and it was shown a significant difference among means of temperature values ($p < 0.01$) considering the fluences applied in this experiment. Besides that, the photosensitizer significantly increased the surface temperature reached during laser irradiation ($p < 0.05$).

3.3. Pulpal temperature

The pulpal temperature rises when laser was applied in surfaces recovered or not with the photosensitizer is showed in Fig. 5. The temperature increments varied according to the laser fluence employed, and the differences between the temperatures were statistically significant ($p < 0.05$).

A minimal effect on the pulp temperature was noted when samples were irradiated with 2.5 J/cm^2 with or without the photosensitizer (means of 0.60 ± 0.3°C and 0.76 ± 0.6°C, respectively). With this irradiation condition, the largest temperature increase was 2.2° C. When samples were irradiated with 5.6 J/cm^2, the temperature increase in pulp was 1.46 ± 0.8°C (maximum of 2.5°C) and 1.73 ± 0.9°C (maximum of 3.0°C), with and without the photosensitizer, respectively. When applying the 8.5 J/cm^2 fluence, it was observed the highest temperature increase: 1.26 ± 0.5°C (maximum of 2.0°C) with photosensitizer and 2.11 ± 0.9°C (maximum of 3.3°C) with-

Fluence	Photosensitizer	Total thickness, mm	Enamel thickness, mm	Dentin thickness, mm
2.8 J/cm^2	yes	4.28 ± 0.77	1.55 ± 0.26	2.95 ± 0.61
2.8 J/cm^2	yes	4.00 ± 0.52	1.21 ± 0.22	2.87 ± 0.55
5.6 J/cm^2	yes	4.43 ± 0.86	1.40 ± 0.29	2.57 ± 0.47
5.6 J/cm^2	no	4.41 ± 0.59	1.28 ± 0.29	2.95 ± 0.64
8.5 J/cm^2	no	4.08 ± 0.59	1.47 ± 0.43	2.58 ± 0.48
8.5 J/cm^2	no	3.97 ± 0.48	1.37 ± 0.29	3.00 ± 0.71

Table 1 Means and standard deviation of surface-pulp distance, enamel and dentin thickness of samples used in the present study

out photosensitizer. The ANOVA's test revealed statistical significant differences ($p < 0.05$) in the intrapulpal temperature between samples irradiated with different fluences. The previous application of photosensitizer significantly reduced the pulpal temperatures reached only when enamel was lased with fluence of 8.5 J/cm^2.

The evaluation of thickness of samples showed means of 4.24 ± 0.68 mm, in which enamel presented averages of 1.39 ± 0.30 mm thickness and dentin presented means of 2.82 ± 0.59 mm. Statistical analysis showed no significant differences on thickness of samples between groups. Table 1 shows the averages and standard deviation of thickness of samples for each group.

4. Discussion

When choosing an irradiation condition in dentistry, it must be determined the potential on causing pulpal and periodontal injuries before any clinical application, as well the morphological changes should be confirmed. The present study evaluated the temperature rises on enamel surface and in pulp chamber when human teeth are irradiated with Er,Cr:YSGG at low fluences without coolant and the influence of a photosensitizer in heat transfer to the pulp. The fluences tested in the present work were described to have potential for preventing dental caries [9,10].

For application in caries prevention, laser irradiation should not promote injuries on enamel surface in order to avoid esthetic and morphological damages to irradiated enamel; therefore erbium lasers should be applied at fluences below the ablation threshold [9]. For Er,Cr:YSGG laser, the ablation threshold was determined by several authors without agreement between them. The fluence of 4 J/cm^2 was found by Belikov et al. [24] as the ablation threshold; Fried et al. [10] reported the fluence of 18 J/cm^2, and Apel et al. [25] reported the fluences of 10 J/cm^2 to 14 J/cm^2. The present work tested fluences of 2.8 J/cm^2, 5.6 J/cm^2, and 8.5 J/cm^2, in order to assure fluences that are below the ablation threshold. In all fluences tested, however, it was observed a slight degree of ablation in all samples, with the removal of outer surface of enamel and exposition of enamel rods (Figs. 1 and 2). According to the photomicrographs, Er,Cr:YSGG laser created craters with rough aspect similar than those created by Er:YAG laser [14], and these aspects became more evident as the energy per pulse was increased, which agrees with some findings reported by literature [14,21].

For erbium lasers, the water plays an important role in laser interaction with dental hard tissues, increasing this interaction and, consequently, increasing the ablative process [27,36]. In fact, the ablation process results from the higher laser absorption in water contents of enamel, which warms the water molecules and causes micro-explosions of the adjacent tissue [27], removing it. For application in caries prevention, it was already reported that the Er:YAG laser without coolant was more effective when compared with Er:YAG laser with water mist [15,37]. In that occasion, it was reported a decrease in the ablation process and also an increase in acid resistance of enamel. Besides that, it was already determined that a small layer of water can maximize enamel cutting by IR lasers [38]. Therefore, in the present study, laser was irradiated without coolant.

During laser irradiation, the surface temperatures changed according to the laser fluence. The temperature rise of 247.6°C found when teeth were irradiated with 8.5 J/cm^2 is lesser than that temperature reported by Fried et al. [10], who found approximately 400°C measured by an elliptical mirror and a HgCdZnTe detector with a time resolution of 1 μs. However, in the present study, it was detected temperature rises of 79.6°C when samples were irradiated with 2.8 J/cm^2 and 184.1°C when irradiated with 5.6 J/cm^2. These temperatures were probably higher considering the morphological changes promoted at the enamel surfaces. Taking into account that the pulse width of Er,Cr:YSGG laser is 140 μs, even the 900 Hz recording rate of thermographic camera seems to be unable to detect the highest temperature peaks during laser irradiation. In this way, the thermographic camera gives an idea of temperature increments when teeth are irradiated with high intensity lasers; however more accurate systems are required to precisely determine the maximum temperature peaks. For caries prevention, temperature rises above 100°C are required for inducing crystallographic changes on enamel that can increase it's acid resistance [11,15,23,28,30,31]. After laser irradiation, it was reported the increase in the Ca/P ratio [8,12,13], the increase the surface microhardness [12], the decrease of carbonate [23] and protein contents [12] and the change of the spectroscopic characteristics of enamel [12]. When using Nd:YAG lasers, the melted and re-solidified surface also results in a increase of resistance of enamel to a cariogenic challenge [13]. Considering this temperature range, the irradiation of enamel with Er,Cr:YSGG at the fluences of 5.6 J/cm^2 and 8.5 J/cm^2 seems to have a theoretical potential for inducing acid-resistance in enamel. Further studies are necessary to confirm this hypotesis.

The application of a photosensitizer before laser irradiation is commonly used in order to enhance tissue absorption in the near-infrared range for ablation and prevention actions in dental tissues [7]. The absorption of the laser beam is increased at the surface of the enamel and the heat produced due to laser absorption in the coating material is transmitted into the adjacent enamel. This technique certifies the deposit of a short laser pulse energy to a small volume of tissue, avoiding the excessive laser beam penetration in deeper dental structures and, consequently, with less risk of damages in dental pulp [39]. In the present work, the recovering with the coal paste promoted an increase of surface temperatures, which confirmed the absorption of laser beam at the surface. However, the coal paste significantly decreased the heat transfer into the teeth when enamel was lased with 8.5 J/cm^2, and can increase the pulpal safety when laser is irradiated for a long period of time.

Thermal effect on pulp and periodontal tissues are a major concern on the use of laser for caries prevention *in vivo*. It was demonstrated by Zach & Cohen that increments of 5.6°C are tolerable by dental pulp; however, above this threshold, the temperature rises are potentially threatening and can result in pulpitis and pulpal necrosis [34]. A study of Powel et al. [40] showed levels of 60% and 100% of pulp necrosis when pulp tissue was heated about 11°C and 17°C, respectively. The pulpal temperature rise due to laser-tissue interaction has also been investigated and most of lasers systems promoted an increase in pulpal temperature dependent on the power setting [5,6,26]. Concerning Er,Cr:YSGG laser, it was reported temperature rises of 3.0°C in pulp chamber when this laser was irradiated with fluence of 68.2 J/cm^2 in dogs and rabbits [41]. Water spray is representative in temperature rises, reducing temperatures of root canal walls from 37.1°C to 8°C when lased at fluence of 68.2 J/cm^2 [42]. At the same time, it was reported to occur any change in pulp temperature during cutting enamel and dentin of dog's teeth [26]. In the present study, even applied without coolant, the fluences chosen for Er,Cr:YSGG laser irradiation on enamel did not promote temperature rises up to 3.0°C, which was considered safe if compared to the 5.6°C threshold. Therefore, laser conditions of the present work were considered potentially safe for a clinical application; however, further histological *in vivo* studies are also necessary to confirm this hypotesis. In the present study, it was selected an irradiation time of 30 seconds in order to simulate clinical situations, allowing enough time to irradiate the entire occlusal surface of samples. However, it must be pointed out that longer applications than the 30 seconds proposed by the present work can be dangerous for pulp and periodontal tissue vitality.

The enamel and dentin thickness are important conditions that determine the generation of the intrapulpal temperature. For a future application of the parameters suggested in the present study, it must be considered that it was used non-carious teeth and, because of the great amount of water in carious lesions, the heat transfer to the pulp can be more excessive in decayed teeth. Beside that, it was chosen molar teeth that had an average of 4.43 ± 0.86 mm in the occlusal to pulp chamber distance. Considering that the human teeth present a big variation in volume and weight, and taking into account the poor thermal conductivity of dentin, the operator must judge the physical conditions of dental hard tissue in order to adequate the exposition time to avoid dangerous thermal effect on pulp.

More researches are necessary to confirm the possibility of Er,Cr:YSGG, at low fluences, to promote an efficient preventive effect on enamel. In the present study, it was confirmed that the selected parameters can be safe for pulp vitality and produces surface temperatures that could indicate crystallographic changes on enamel surface.

5. Conclusion

According to the conditions of this study, the results indicate that Er.Cr:YSGG laser irradiation at low fluences without coolant produces higher surface temperatures which could indicate crystallographic changes on enamel. The recovering of enamel surface with a photosensitizer before Er,Cr:YSGG laser irradiation can reduce the heat transfer into the tooth, decreasing the pulp temperatures and, as a consequence, increasing the pulpal safety during laser irradiation.

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