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Dental Enamel Irradiated with a Low-Intensity Infrared Laser and Photoabsorbing Cream: A Study of Microhardness, Surface, and Pulp Temperature

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Abstract

Objective: The aim of this study was to assess the effect of low-intensity infrared laser light ($\lambda = 810$ nm, 100 mW/cm^2, 90 sec, 4.47 J/cm^2, 9 J) with or without indocyanine green cream fluorinated or not fluorinated, using Knoop surface microhardness analysis. ***Background data:*** Lasers can be used as tools for the prevention of tooth enamel demineralization. ***Methods:*** The surface and pulp temperatures of the human deciduous tooth enamel were measured. For the analysis of surface hardness, a total of 48 specimens were prepared and randomly assigned into six groups ($n = 8$/group): C (+), which received laser light; C(-), which received no treatment; cream (IV); cream and fluoride (IVF); cream and light (IVL); and cream and fluoride and light (IVFL). The specimens were subjected to treatment before demineralizing challenge by pH cycling. To analyze the surface and pulp temperatures, the samples were divided into the following groups ($n = 10$): C(+), IVL, and IVFL. ***Results:*** The hardness analysis indicated that the groups that received irradiation had less hardness reduction following the demineralizing challenge ($p < 0.001$), with IVFL and IVL presenting the lowest percentages of surface microhardness loss at 3.98% and 9.3%, respectively. Surface temperature analysis indicated a maximum increase of 74°C and a mean of 45.25°C and 45.95°C for the IVL and IVFL groups, respectively. Pulp temperature analysis indicated a higher mean increase of 2.40°C ± 0.65 in the IVL group. ***Conclusions:*** These results suggest that the combination of cream and laser light possibly promoted protein denaturation of the tooth enamel organic matrix, which possibly decreased the loss of hardness without causing pulp damage.

Introduction

Despite the decline in its prevalence, tooth decay has become a polarized disease.[1] The development of dental caries is the result of a dynamic process acid mediated with the tooth structure mineral loss.[2] Caries lesions are closely related to the loss of hardness and mineral content of the normal tooth enamel.[3] This, in turn, justifies the use of microhardness tests to measure variations in the gain or loss of mineral content.[4]

In order for a laser to be biologically active, it has to interact with the tissue. The tooth enamel has little absorption in the visible (400–700 nm) and in the near-infrared (810 nm) spectra. The use of a chromophore applied to the enamel to absorb the radiation could improve the tissue interaction for these wavelengths bands, resulting in thermal and photochemical effects.[5]

High-power lasers can significantly increase the enamel acid resistance, and when combined with fluoride, a significant synergy has been demonstrated in reducing enamel solubility[6]; however, currently, the cost of acquiring devices with such configurations still makes it unfeasible to use the combined technique on a daily basis.

A possible method for preventing tooth demineralization (DE) that is relatively simple and noninvasive would be the treatment of teeth enamel with low-intensity laser, alone or in combination with fluoride treatment, resulting in reduced enamel solubility and dissolution.[7]

Given the interest in finding therapeutic approaches to prevent dental caries using lasers that are routinely available, the possibility of using light and a chemical substance aimed at changing the enamel structure to make it more resistant is in line with the pursuit of better health.

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The present *in vitro* study evaluated the effect of photo-absorbing creams irradiated with low-intensity near-infrared laser in human deciduous enamel under DE challenge on surface microhardness, and surface and pulp temperatures.

Materials and Methods

Microhardness analysis

This study was approved by Ethics and Research Committee, Cruzeiro do Sul University/# 007/2010. Caries-free human deciduous molars ($n=24$) analyzed with a stereoscopic magnifying glass were sectioned mesiodistally with a micromotor (LB100, Beltec; Brazil) and a carborundum disk (Dremel, USA) with water cooling, obtaining 48 samples. Each fragment was embedded in unsaturated polyester resin (Avipol Ltda., Brazil), and the sectioned surface was left exposed (1 mm^2 area) and polished. The samples were smoothed and polished with silicon carbide abrasive paper, 1200, 2400, and 4000 (Union Carbide do Brazil; Cubatão, SP, Brazil), in a polishing machine (Struers, PD-10, model Panambra SA; SP, Brazil) with water cooling, and then with diamond paste and $-1.25\,\mu$m tissue (Buehler Metallographic Ltda.; SP, Brazil). The samples were cleaned with ultrasound for 10 min.

Then, the specimens were randomly assigned into six groups (Table 1), stored individually, and kept in deionized water. To establish the baseline levels, the hardness of tooth enamel was measured prior to any treatment (Fig. 1).

The six groups received specific surface treatments according to the coding described (Table 1).

Table 1. Groups and Surface Treatments

Groups	n	*Treatment*
C(-)	8	• No treatment
C(+)	8	• Irradiated with infrared diode laser • Samples were washed in deionized water
IVL	8	• Indocyanine green gel cream (Acros; NJ; lot: A0232896) (0.05 g, Buenos Aires Lab, Brazil) (60 sec) • Irradiated with infrared diode laser • Samples were washed in deionized water
IV	8	• Indocyanine green gel cream (Acros; NJ; lot: A0232896) (0.05 g, Buenos Aires Lab, Brazil) (60 sec) • Samples were washed in deionized water
IVFL	8	• Indocyanine green gel cream (Acros; NJ; lot: A0232896) (0.05 g, Buenos Aires Lab/Brazil) with fluoride (2% sodium fluoride, ionization coefficient: Basic) (60 sec) • Irradiated with infrared diode laser • Samples were washed in deionized water
IVF	8	• Indocyanine green gel cream (Acros; NJ; lot: A0232896) (0.05 g, Buenos Aires Lab, Brazil) fluoride (2% sodium fluoride, ionization coefficient: Basic) (60 sec) • Samples were washed in deionized water

The near-infrared diode laser parameters (UltraBlue IV Plus II, DMC equipment; São Carlos, Brazil) were as follows: $\lambda=810$ nm, 30 mW, 90 sec, 4.47 J/cm^2, 9 J, 16 mm incident beam diameter, and continuous wave (CW) laser source. The laser beam touched the sample over 1 mm of creams when they were used, and maintained the same distance in the groups without cream.

After these treatments, the samples were subjected to induced artificial tooth DE.[8,9] The DE and remineralization (RE) solutions were alternated at room temperature without stirring. The samples were stored individually in 8 mL of DE solution, pH 4.8 for 8 h. The samples were then placed in 8 mL of RE solution, pH 7.0 for 16 h/day for 7 days to simulate 8 h of RE and DE during the day and 8 h of RE during the night. The solutions were changed daily and kept at room temperature. Between the exchanges of solutions, the samples were washed in deionized water.

After DE challenge, a new hardness measurement was performed. The enamel surface microhardness was assessed using a Shimadzu HMV-2 (Micro Hardness Tester, Shimadzu Corporation; Kyoto, Japan) and analyzed with Can-Win image analysis software (NewAge Industries, USA.). A Knoop pyramidal-diamond indenter was used, with a static load of 25*g* applied for 5 sec. In each sample, three indentations[10] were created at random, with a minimum distance of 200 μm away from the edge.[11] Knoop hardness measurements were obtained. Indentation values (final microhardness after first challenge and baseline) were used to assess the percentage of surface microhardness loss (%SML).

Surface temperature analysis

The tissue surface temperature during laser treatment was assessed. Caries-free deciduous molars ($n=15$) were sectioned mesiodistally as previously described ($n=30$). The sectioned faces were flattened with # 600 grain sandpaper (Carbimed Paper Discs, Buehler, IL) and stored in deionized water (Fig. 2).

The samples were randomly divided into three experimental groups ($n=10$) (Table 2).

The temperature was measured with a thermographic camera (Therma Cam FLIR SC 3000 Systems, Boston, MA).

The experiments were performed under a controlled temperature of 18.5°C at 50% relative humidity and a tooth emissivity of 0.91.[12] The area of interest was isolated at a focal distance of 10 cm with the aid of appropriate lenses. The images were obtained with a resolution of 0.01°C using 60 Hz frequencies to optimize image definition and detection of temperature variations.

The light was positioned perpendicularly to the samples' occlusal surface at a distance of 1 mm, tangential to the sectioned faces, to visualize heat propagation from the surface to the interior of the sample.

To obtain a characteristic curve for the decline in temperature as a function of time, irradiations were performed after application of the photo-absorbent cream (if used) at a single point for 90 sec, and temperature monitoring was conducted by recording the initial and final temperatures, in addition to the temperature drop 2 sec after the end of irradiation.

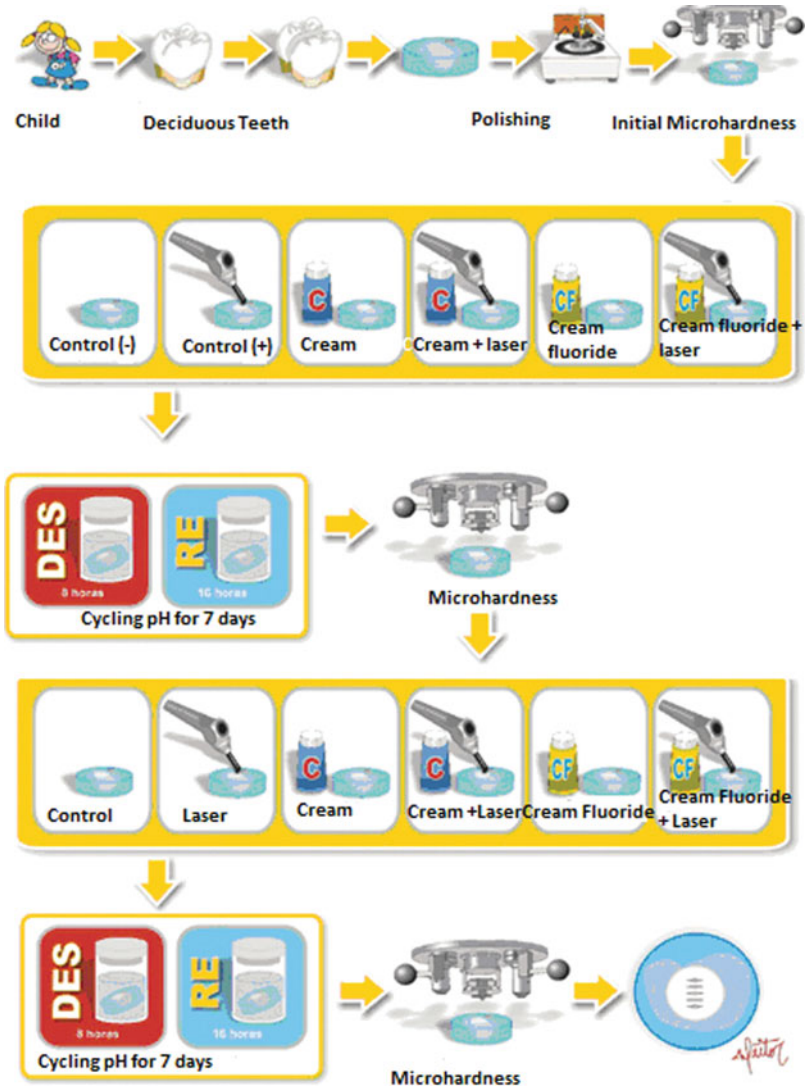

FIG. 1. Flowchart experiments - Part 1.

A point located on the surface of the enamel immediately below the light was selected for the purpose of determining the enamel surface temperature.

Pulp temperature analysis

To assess the pulp temperature, 30 caries-free human deciduous molars were stored in deionized water and distributed in three groups ($n = 10$) (Table 3). The residual pulp was removed using a curette.

A thermal paste (Implastec; Votorantim, Brazil) with a thermal conductivity of 0.4 cal s^{-1} m^{-1} K^{-1} was applied using disposable syringes. Subsequently, K-type thermocouples were introduced (Chomel-alumel - NiCr-NiAl, Omega Eng. Inc.; Stamford, CT), with a 127 μm thickness and 0.2°C resolution, so that their ends coincided with the pulp chamber ceiling. The apical portion of the tooth was sealed with utility wax (Wilson, Polidental, Cotia, Brazil). During the analyses, the room temperature was increased to 37 ± 1°C to simulate body temperature. The thermocouples were then connected to a temperature monitoring system consisting of an amplifier, a signal converter, and a recorder with a temporal resolution of 0.05 sec (Lock-in Amplifier SR510, CA).

The irradiations were performed manually under punctual stimulation for 90 sec over the entire occlusal surface to simulate a clinical procedure. Thus, it was possible to assess the possible damage that could occur during a more prolonged laser exposure. Pulp temperature was monitored throughout the irradiation period.

Statistical Analysis

The means and standard deviations assessed in the temperature tests were calculated, and one way analysis of variance (ANOVA) was performed (GraphPad Instat 4.0; San Diego, CA). For the study, confidence interval was set at 95%. For the microhardness data, an ANOVA model was created using Minitab statistical software 15.1 (State College, PA), with two factors: group and moment. In cases of statistical significance $p < 0.05$, Tukey's multiple comparison was used to determine which groups differed from each other.

Results

The samples from all of the groups demonstrated an initially similar Knoop hardness profile (290–304), denoting

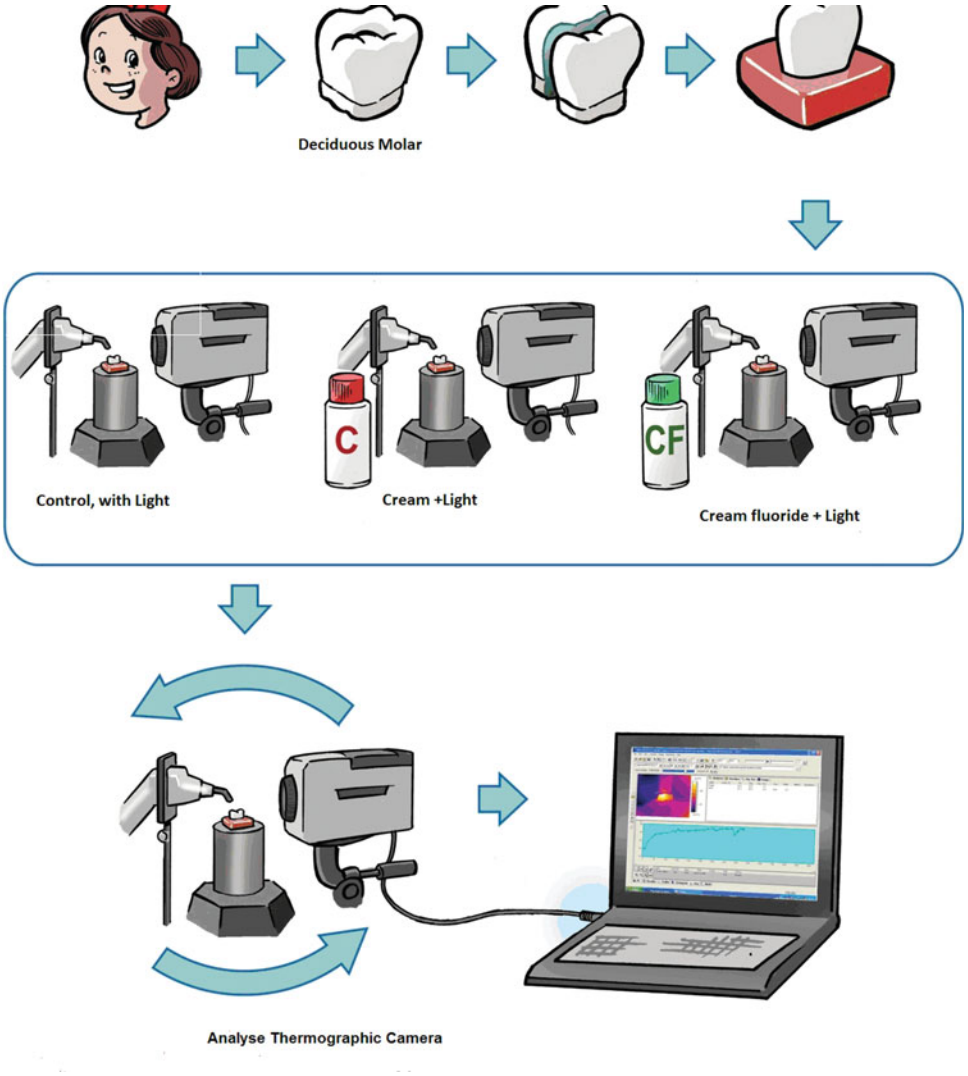

FIG. 2. Flowchart experiments - Part 2.

hardness values compatible with the studied structure, the enamel (272–440).[13] Therefore, there were no statistically significant differences among the groups prior to any treatment or pH cycling.

The mean Knoop hardness analysis after treatment and DE challenge indicated that all groups differed ($p < 0.0001$).

Table 2. Groups and Maximum Surface Temperature Experiment

Groups	n	*Treatment*
L	10	• Irradiated (infrared diode laser)
IVL	10	• Indocyanine green gel cream (Acros; NJ; lot: A0232896) (0.05 g, Buenos Aires Lab, Brazil) (60 sec) • Irradiated with infrared diode laser
IVFL	10	• Indocyanine green gel cream (Acros; NJ; lot: A0232896) (0.05 g, Buenos Aires Lab, Brazil) with fluoride (2% sodium fluoride, ionization coefficient: Basic) (60 sec) • Irradiated with infrared diode laser

After analyzing the effect of treatment and challenge in the same group, all groups presented a significant difference except the IVFL ($p = 0.06$). The smallest hardness losses were observed in the IVFL (285.92 ± 4.10), IVL (263.05 ± 4.005) and C(+) (229.00 ± 5.74) groups. The highest hardness losses were recorded in the C(−) (156.79 ± 5.39) and IV groups (158.41 ± 9.229) (Table 4).

IVF, IV, and C(−) groups presented higher percentages of %SML between the baseline and challenge, at 30.86%, 47.95%, and 47.59%, respectively (Table 5).

The IVFL group had the highest maximum surface temperature (45.95°C ± 15.39). That temperature was not significantly different from that of IVL (45.25 ± 13.81°C) ($p > 0.05$) (Table 6). However, these temperatures were different ($p < 0.001$) when compared with those of the C(+) group, both in the post-treatment temperature and in the difference between the initial and final temperature post-treatment (Table 6).

When pulp temperature is considered, IVL and IVFL presented no differences ($p > 0.05$). The highest mean temperature increase corresponded to the cream group (2.40°C ± 0.65). However, the temperatures for IVL and IVFL groups were significantly different at $p < 0.01$ and $p < 0.001$,

Table 3. Groups and Pulp Temperature Experiment

Groups	n	*Treatment*
L	10	• Irradiated with infrared laser diode
IVL	10	• Indocyanine green gel cream (Acros; NJ; lot: A0232896) (0.05 g, Buenos Aires Lab, Brazil) (60 sec) • Irradiated with infrared diode laser
IVFL	10	• Indocyanine green gel cream (Acros; NJ; lot: A0232896) (0.05 g, Buenos Aires Lab, Brazil) with fluoride (2% sodium fluoride, ionization coefficient: Basic) (60 sec) • Irradiated with infrared diode laser

respectively, compared with that from the laser group (Table 7).

Discussion

Laser therapy is a treatment option that has good prospects for caries prevention. When employed under appropriate irradiation parameters, it can modify the structure of the tooth enamel, making it more resistant to DE.[14,15] It may even enhance the action of conventional therapies.[16] In tooth decay prevention, high-intensity lasers work primarily through a thermal effect, promoting physical[17,18] and chemical changes in the target tissue.[19] Studies using high-intensity lasers suggest that the increased DE resistance is a change in the enamel surface morphology caused by its melting and resolidification, the formation of calcium phosphate compounds, or the elimination of the organic matrix.[20–22] It is believed that the decrease in enamel solubility is caused by severe structural changes, such as reduced carbonate and water content, increased amounts of hydroxyl ions, formation of pyrophosphates, and breakdown of proteins.[23]

Similar actions should not be expected with low-intensity lasers, unless the energy is concentrated on the surface in a new interaction approach using photo-absorbent creams. These creams, which have an absorbance in resonance with the light source wavelength, could absorb the energy, eventually converting it into heat inside the cream and then transferring this heat to the tooth enamel; this process would minimize the heat transfer from the surface to inside the pulp tissue, as observed when using nankin ink as a photo-absorbent in irradiations with Nd:YAG lasers.[24] Photo-absorbing substances are mainly used before irradiation with Nd:YAG and Ho:YLF lasers, because without this treatment, the energy would be mostly transmitted to the pulp.[25]

Indocyanine green, which is a biocompatible dye, is used in the medical sciences with an absorbance at 700 nm. This indocyanine green cream was used to maximize the absorption of infrared laser energy on the enamel surface.[14]

Hardness is a mechanical property of the enamel that can qualitatively predict the degree of mineralization. Hardness directly depends upon the mineral content, as well as on the crystalline arrangement of its prisms.[8] The surface hardness measure has been accepted, therefore, as a valid way to assess the mineral loss or gain in the tooth enamel.[26] The mean microhardness value obtained for the control group at baseline was 299.21, which was lower than the value obtained by Westerman et al.,[26] whose mean value was 316, but greater than the value obtained by Banda et al.,[27] which was 295.4. These subtle differences can be attributed to the use of permanent teeth and Vickers hardness system by those authors, whereas we opted for the Knoop hardness and deciduous teeth.

The results indicated reduced hardness losses in the laser-irradiated groups between baseline and challenge (Table 4). The IVFL group presented the lowest percentage of surface hardness loss during this period (Table 5). Although the interaction mechanisms are different, the results are in agreement with those of Vlacic et al.,[28] who compared five high-intensity lasers (Nd:YAG, InGaAsP, InGaAs, GaAs, and KTP) and demonstrated that treatment with laser-activated fluoride has a protective effect with the smallest hardness losses after DE challenge.

Irradiation[26] with high-intensity laser alters the lattice parameters of hydroxyapatite crystals and decreases the solubility, through changes in the carbonate, water, and organic content of the tooth mineral phases. These changes lead to microporosity, allowing re-precipitation of mobilized calcium, phosphate, and fluoride during DE.[27,29] The reduction in the mineral structure permeability caused by protein denaturation and turgescence is also attributed to laser irradiation. The laser also promotes the increased uptake of fluoride, calcium, and phosphate from exogenous sources, creating surface deposits, which under high-risk conditions, lead to a bacteriostatic or bactericidal effect on the microorganisms in the dental biofilm.[27]

Several physicochemical changes have been suggested to result from laser and fluoride treatments, including deposition of calcium fluoride,[14,30] formation of microspaces in the dental hard tissue,[31] formation of tricalcium phosphate, and transformation of hydroxyapatite to fluorapatite.[28]

The results (Table 3) indicate that the use of non-irradiated fluoride cream failed to rebalance the mineral losses during the remineralization periods of the challenge. This suggests less hardness conservation in the samples. The treatments with fluoride cream or non-fluorinated cream combined with laser irradiation maintained the stability of inorganic components during the demineralization challenge, with a greater conservation of surface hardness (Table 4). In agreement De Sant'Anna et al.,[14] using energy-dispersive microspectroscopy X-ray fluorescence (μ-EDX), observed the conservation of calcium and phosphorus in the elemental weight of enamel irradiated using the same laser with or without the fluoride creams used in this study. However in contrast, Muller et al.[30]

Table 4. Means and Standard Deviation (SD) of Enamel Hardness for Each Group and Treatment Period

Groups	*Before*	*Post-challenge*	p *Value comparison pre-treatment vs. challenge*
IVL	290.00 ± 9.445	263.05 ± 4.005	= 0.003
IVFL	297.79 ± 15.52	285.92 ± 4.10	= 0.0651
IVF	301.33 ± 6.28	208.33 ± 6.58	< 0.0001
IV	301.96 ± 10.50	158.41 ± 9.229	< 0.0001
C(+)	303.88 ± 10.89	229.00 ± 5.74	< 0.001
C(−)	299.21 ± 8.93	156.79 ± 5.39	< 0.001

IVL, cream and light; IVFL, cream and fluoride and light; IVF, cream and fluoride; IV, cream; C (+), laser light; C (−), no treatment.

Table 5. Percentage of Surface Microhardness Loss (%SML) for Each Group

Moment	*IVL*	*IVFL*	*IVF*	*IV*	*C(+)*	*C(−)*
Before X challenge	9.3%	3.98%	30.86%	47.95%	24.64%	47.59%

IVL, cream and light; IVFL, cream and fluoride and light; IVF, cream and fluoride; IV, cream; C (+), laser light; C (−), no treatment.

observed no difference in enamel hardness when using fluoride alone or in combination with laser irradiation. Those authors emphasized that after the topical fluoride application, there was a slight dissolution of the enamel surface, concomitant with the precipitation of products from the reaction of enamel with F ions, with CaF_2 as the main product.

Studies with Nd:YAG lasers[27,32] have shown a hardness increase in irradiated teeth not subjected to DE challenge. The results were attributed to the melting of the tooth enamel. However, other authors[32] claim that there is a hardness decrease in irradiated teeth when subjected to high-energy parameters. This finding was attributed to the fact that higher-power densities can produce larger cracks, which renders the enamel more fragile. In this study, both the energy density and the laser power were not high, which may have implications in maintaining the enamel structure, as observed in a scanning electron microscopy (SEM) study.[7]

Another advantage of choosing a low-intensity laser over a high-intensity device is the safety in regards to the pulpal temperature. The mean variations of the temperatures recorded on the walls of the pulp chambers were 0.6°C, 1.8°C, and 2.4°C (Table 7), which are considered below the tolerable safety range of up to 5.5°C.[33]

A study[34] suggested that a low-intensity laser does not lead to thermal effects. The results (Table 6) obtained using infrared thermography demonstrate that laser irradiation combined with indocyanine green cream, fluorinated or not, yielded the highest mean surface temperatures of ~45.25°C and 45.95°C, respectively, which were significantly different from that of the control group.

The mean temperatures obtained from IVL (45.25°C) and IVFL (45.95°C) treatments may be responsible for protein denaturation of the enamel organic matrix, which would explain a possible resistance to DE. Mukai et al.[35] related this temperature level to possible protein denaturation processes.

Subtle changes in the chemical environment, such as in pH, temperature, and ionic strength, can easily lead to the breakage of the weak bonds that stabilize the secondary and quaternary protein structures present in the enamel organic matrix, which results in its denaturation.[35]

Temperature also greatly influences protein solubility, because when there is a sufficient increase in temperature over a prolonged period of time, the protein is denatured because of the exposure of certain groups, which are initially within the protein molecules. Typically, when the temperature is between 0 and 40°C, increasing the temperature promotes solubility because heat causes an increase in the kinetic energy of protein molecules, thereby facilitating their interactions with the solvent. However, if the temperature is >40°C, the proteins start to precipitate, and the molecular motions become so intense that the chemical groups deviate beyond the allowable re-association distance, approaching other groups that they can associate with. Thus, the secondary, tertiary, and quaternary structures break down, and the protein conformation is altered, resulting in an inactive and denatured conformation.[36,37]

De Sant'Anna et al.[14] used the same treatment protocol as this study. Using X-ray fluorescence, they observed the variable organic balance (oxygen and carbon), which provides information about the organic components of the enamel and their links with water. The results demonstrated that no negative changes in the weight of the organic balance in irradiated groups occurred, except for the combination of indocyanine green cream with laser light, most likely indicating protein denaturation. A possible explanation for this finding may be found in results of the surface temperature analysis (Table 6) obtained in the present study for the groups receiving the cream and laser treatment, in that the elevated temperature may cause denaturation.

The greater energy absorption on the surfaces and the higher temperature obtained in the experimental IVL and IVFL groups may have also resulted in some water loss. The evaporation of water contained in the dental hard tissues caused by irradiation has been associated with mechanisms of increased enamel resistance.[38] Microspaces can form after laser irradiation as a result of the losses of water, carbonate, or organic substances. These losses might prevent demineralization by entrapping dissolved ions.[35]

Despite the results obtained in the present study, further studies are needed to evaluate the effect of this therapeutic approach on the depth of lesions using optical coherence tomography and transversal microhardness.

Table 6. Measurements of the Surface Temperature According to Each Treatment

Type of treatment	n	*Mean*	*SD*	p *Value*
L^A	10	21.24°C	1.105	<0,001
IVL^B	10	45.25°C	13.81	
$IVFL^{B,C}$	10	45.95°C	15.39	

Different superscript letters indicate statistical significance.
L, laser light; IVL, cream and light; IVFL, cream and fluoride and light.

Table 7. Pulp Temperature Measurements According to Treatment

Type of treatment	n	*Mean*	*SD*	p *Value*
L^A	10	0.665167°C	0.528731	<0.01
IVL^B	10	2.40886°C	0.652142	
$IVFL^{B,C}$	10	1.891944°C	0.648026	

Different superscript letters indicate statistical significance.
L, laser light; IVL, cream and light; IVFL, cream and fluoride and light.

Conclusions

The combination of laser light and cream resulted in less surface hardness loss, and this same combination was responsible for the highest mean surface temperature.

Author Disclosure Statement

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