Dental Enamel Irradiated with Infrared Diode Laser and Photo-Absorbing Cream: Part 2—EDX Study

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Abstract

Objective: The effects of laser-induced compositional changes on the enamel were investigated by energy-dispersive X-ray fluorescence spectrometry (μ -EDX). After cariogenic challenge, we administered treatment of low-level infrared diode laser and a photo-absorbing cream (used to intensify the superficial light absorption). **Background Data:** Dental caries is considered the most prevalent oral disease. A simple and noninvasive caries preventive regimen is treating tooth enamel with a laser, either alone or in combination with fluoride, which reduces enamel solubility and dissolution rates. High power lasers are still not widely used in private practice. Low-power near-infrared lasers may be an alternative approach. Energy-dispersive μ -EDX is a versatile and nondestructive spectroscopic technique that allows for a qualitative and quantitative elemental analysis of inorganic enamel components, such as calcium and phosphorus. **Materials and Methods:** Twenty-four extracted or exfoliated caries-free deciduous molars were divided into six groups: 1) control group (CTR-no treatment); 2) infrared laser treatment (L) ($\lambda = 810$ nm, 100 mW/cm², 90 sec, 4.47 J/cm², 9 J); 3) infrared laser irradiation and photo-absorbing agent (CL); 4) photo-absorbing agent alone (C); 5) infrared laser irradiation and fluoridated photo-absorbing agent (FCL); and 6) fluoridated photo-absorbing agent alone (FC). Samples were analyzed using μ -EDX after two sets of treatments and pH cycling cariogenic challenges. **Results:** The CL group showed statistically significant increases in calcium and phosphorus (wt%) compared with the CTR group. The Ca/P ratio was similar in the FCL and CTR groups. There was a significant laser-induced reduction compared with the CTR group, and there was a possible modification of the organic balance content in enamel treated with laser and cream. **Conclusion:** μ -EDX may be able to detect compositional changes in mineral phases of lased enamel under cariogenic challenge. Our results suggest that with a combined laser and photo-absorbing agent (CL) treatment, there was a possible disorganization of organic content in the tooth enamel with hydroxyapatite crystal reordering and reorganization.

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Introduction

IN THE LAST few decades, there has been a documented worldwide decrease in dental caries incidence; however, caries is still considered the most prevalent oral disease during childhood and adolescence.¹⁻⁴ The disease has become polarized, with caries occurring mostly in certain groups of children who present with high caries activity.^{5,6} This transmittable bacterial disease affects more children than any other disorder and is particularly prevalent in children from families with low socioeconomic status⁷⁻⁹ and in immunocompromised children.¹⁰ An approach using combined therapies for this population might be a promising method to prevent and control dental caries.

The disease of caries originates in the dental enamel, a composite of 85% mineral, 12% water, and 3% protein and lipid by volume. The mineral component is hydroxyapatite, which has a hexagonal symmetry and the general formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$.¹¹ The structure of hydroxyapatite can be considered as being built of corner-sharing PO_4 and CaO_6 polyhedra that form channels along the crystallographic c-axis, in which the hydroxyl groups are placed. The apatite structure is very adaptive to various inclusions like carbonate, magnesium, sodium, or potassium. Dental apatite contains a substantial amount of carbonate groups, which substitute for the hydroxyl groups (A-type CO_3^{2-})¹¹ or for phosphate tetrahedral (B-type CO_3^{2-})¹¹ and are also present in relatively high quantities in primary teeth and newly erupted permanent teeth. There is a positive correlation between carbonate solubility and enamel solubility¹² once apatite diminishes in crystal stability.¹³

In populations especially affected by caries, there are common subclinical signs, like white spot lesions. These lesions are the earliest sign of a carious lesion, and they appear as chalky white spots on the surface of the tooth, indicating an area of enamel demineralization.

Prevention of dental caries, which is a complex multifactorial disease, requires dental clinics with appropriate preventive modalities and proper oral hygiene education.¹⁴⁻¹⁹ Preventive modalities include use of fluoride, reduction of dietary cariogenic refined carbohydrates, removal of plaque, and use of oral hygiene techniques and antimicrobials. A relatively simple and noninvasive caries preventive regimen is treating primary and permanent tooth enamel with low-level laser irradiation, either alone or in combination with topical fluoride treatment. This treatment results in reduced enamel solubility and dissolution.²⁰⁻²³

Since the 1960s, it has been consistently demonstrated that high power lasers, under certain conditions, can reduce the rate of subsurface demineralization in the enamel, by altering the crystallinity, acid solubility, and permeability of enamel.²⁴⁻²⁶ Nevertheless, the real mechanisms of caries inhibition by lasers remain unclear.

Due to the inherent costs of instrumentation, high power lasers are still not widely employed in private practice, particularly in developing countries. Low power red and near-infrared lasers are an alternative approach, since reports in the literature suggest that their use with or without topical fluoride, can lead to increases in the teeth's resistance against dental caries.²⁷⁻²⁹ Some authors state that for caries prevention, the laser light must be strongly absorbed and efficiently converted to heat without damage to underlying or sur-

rounding tissues. This ensures that it effectively alters the composition or solubility of dental hard tissues.³⁰

The dental literature dating back to the early 1930s contains numerous articles on the chemical analysis of human teeth and caries disease.

The X-ray fluorescence analysis is a multi-elemental, simultaneous, real-time technique that is based on the measurement of the characteristic X-ray emitted by the elements contained in a sample when irradiated. An electron beam, used as a coupled tool in scanning electron microscope (SEM) equipment, provides the most common irradiation method. The disadvantage of this tool coupled to a SEM is that, for insulating samples, the technique becomes destructive, since the sample surface needs to be covered by a conductive film. However, other sources may be used such as low-energy X-ray, gamma radiation, or synchrotron radiation.³¹⁻³³

The chemical characteristics of the sample following laser irradiation are important, and semiquantitative elemental analyses using X-ray fluorescence spectrometer provide information on the sample stoichiometry. Energy dispersive micro-X-ray fluorescence spectrometry (μ -EDX) allows for qualitative and semiquantitative elemental analyses of inorganic enamel components,^{34,35} such as calcium (Ca) and phosphorus (P), in very small areas. This is a versatile and nondestructive spectroscopic technique that allows for the accurate determination of the global chemical composition of a solid sample, with simple or no sample preparation procedures, rapid analysis, good reproducibility, and low cost.³⁴

The aim of the present study was to use μ -EDX to investigate laser-induced compositional changes on enamel using a therapy with low-level infrared diode laser and a photo-absorbing cream used to intensify the superficial light absorption. This therapy was applied after a cariogenic challenge followed by a second cariogenic challenge. We previously reported on the findings of a structural analysis of samples in which we identified specific light-induced molecular vibrations^{36,37} and investigated the vibrational modes of organic and inorganic components.³⁸

Material and Methods

Tooth selection and grouping

This study had the approval of our institution's research ethics committee (CEP – Universidade Cruzeiro do Sul, Approval protocol no. 008/07). Twenty-four extracted or exfoliated caries-free deciduous molars obtained from the Pediatric Dentistry Clinic of Cruzeiro do Sul University/São Paulo, Brazil, were divided into six groups listed in Table 1 following the study design (Fig. 1).

Selected deciduous teeth were cut (M-D) with a low speed micromotor (LB100; Beltec, Brazil) and diamond disk (Dremel, USA), under cooling, to obtain two specimens from each dental element. A rectangle of laboratory film (Parafilm® M Barrier Film; LTS, West Chester, PA; 2 mm wide and 3 mm long) was cut and positioned in the middle third of each specimen. Then, the surface was covered with two layers of acid-resistant nail varnish (Revlon, Brazil). After the varnish dried, the rectangles of the laboratorial film were removed to leave an enamel window of 2×3 mm without varnish covering that was the area to be treated.

TABLE 1. GROUPS AND TREATMENTS

Groups	n	Treatment
1. Control group	8	• No treatment was performed
2. Infra-red laser	8	• Irradiated using only infrared diode laser
3. Photo-absorbing cream + infrared diode laser irradiation	8	• Samples were washed in deionized water.
4. Photo-absorbing cream	8	• Green indocyanine (Acros, NJ, USA; lot: A0232896) gel cream (0.05 g, neutral pH; Buenos Aires Lab, Brazil) applied (60 sec)
5. Fluoridated photo-absorbing cream + infrared diode laser irradiation	8	• Irradiated using infrared diode laser
6. Fluoridated photo-absorbing agent	8	• Samples were washed in deionized water.
		• Green indocyanine (Acros; lot: A0232896) gel cream (0.05 g, neutral pH; Buenos Aires Lab) applied (60 sec)
		• Samples were washed in deionized water.
		• Fluoridated (sodium fluoride 2%, ionization coefficient: basic) green indocyanine (Acros; lot: A0232896) gel cream (0.05 g, neutral pH; Buenos Aires Lab) applied (60 sec)
		• Irradiated using infrared diode laser
		• Samples were washed in deionized water.
		• Fluoridated (sodium fluoride 2%, ionization coefficient: basic) green indocyanine (Acros; lot: A0232896) gel cream (0.05 g, neutral pH; Buenos Aires Lab) applied (60 sec)
		• Samples were washed in deionized water.

Acros, Morris Plains, New Jersey.

Treatments

After this procedure, the specimens of each group were submitted to the treatments listed in Table 1. The parameters of the infrared diode laser (UltraBlue IV Plus II; DMC Equipment, Brazil) were 810 nm, 100 mW/cm², 30 mW, 90 sec, 4.47 J/cm², 9 J, spot size diameter 16 mm and continuous wave.

After these treatments, samples were submitted to artificial induction of caries.

Artificial induction of caries

All samples were submitted to the process of superficial induction of caries lesions (pH cycling model of ten Cate and Duijsters³⁹ modified by Mendes and Nicolau⁴⁰). In this experimental model, the sample was submitted to alternating demineralizing and remineralizing solutions for 7 uninter-

rupted days at room temperature and without agitation. The specimens were put individually in plastic pots containing 8 mL of demineralization solution for 8 h and then in 8 mL of remineralization solution for 16 h, to simulate the conditions of 8 h each of remineralization and demineralization during the day and 8 hours of remineralization during the night time. The solution was changed daily and the solutions were maintained at room temperature. The solutions were prepared with distilled and deionized water.

Samples were first submitted to a cariogenic challenge, analyzed by μ -EDX, then treated again as shown in Table 1, and submitted again to a second cariogenic challenge and analyzed by μ -EDX.

X-ray fluorescence analysis

Semi-quantitative elemental analyses of calcium and phosphorus were carried out with a micro X-ray fluorescence spectrometer using a energy-dispersive, model μ -EDX 1300, Shimadzu (Kyoto, Japan), equipped with a rhodium X-ray tube and a Si(Li) detector cooled by liquid nitrogen. The equipment was coupled to a computer system for data acquisition and processing. The voltage in the tube was set at 15 kV, with automatic adjustment of the current and incident beam diameter of 50 μ m. The data from three points from each specimen were collected for each category of treatment. For statistical analyses, we analyzed the six groups, each with $n=8$, adding 288 spectra (144-Ca and 144-P). The measurements were performed with a scan rate of 100 sec per point (live time) and a dead time of 25%. The energy range of the scans was 0.0–20.0 eV. The equipment was adjusted using a certified commercial reagent of stoichiometric hydroxyapatite (Aldrich; synthetic Ca₁₀(PO₄)₆(OH)₂, grade 99.999%, lot 10818HA)⁴¹ as a reference. The measurements were collected under fundamental parameters for the characteristic X-ray emissions of calcium and phosphorus, and oxygen and hydrogen were used for chemical balance. The energy calibration was performed using internal standards for the equipment.

FIG. 1. Description of the study design.

TABLE 2. THE VARIOUS TYPES OF CALCIUM PHOSPHATES IN BIOLOGICAL SYSTEMS AND THE Ca/P RATIOS

Name of compound	Chemical formula	Occurrence	Ca/P
Dihydrate calcium phosphate monohydrogen	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	Dental calculus, decomposition bones	1.0
Calcium pyrophosphate dihydrate	$\text{Ca}_2\text{P}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$	Deposits of pseudo-drops of fluids	1.0
Octacalcic phosphate	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$	Dental and urinary calculus	1.33
Calcium phosphate	$\text{Ca}_3(\text{PO}_4)_2$	Dental and urinary calculus, salivary stones, dental caries, soft tissues calcifications	1.5
Hydroxyapatite	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	Enamel, dentin, bone, tooth and urinary calculus, stones, soft tissue calcification	1.67

In general, calcium phosphates occur in normal and pathological calcification, and the levels of calcium phosphates are measured using the molar ratio of Ca/P. For synthetic stoichiometry hydroxyapatite, the Ca/P is 1.67.³⁵ Table 2 lists the main calcium phosphates, their occurrence in biological systems, and the Ca/P ratios. A comparative quantitative assessment was conducted using the μ -EDX technique in order to determine the relationship between calcium and phosphorus element contents of the samples.

Statistical analysis

The mean and standard deviations of evaluated components from each group were calculated to plot bar diagrams of the organic balance and mineral contents using Microsoft Excel® software (Redmond, WA). The one-way ANOVA (with 95% confidence intervals) and Tukey–Kramer multiple comparisons were used to evaluate the significance of the stoichiometric variability among the groups. The tests were performed with Instat® software (Graph Pad Software, San Diego, CA). Changes in the hydroxyapatite stoichiometry in the samples before and after treatment were analyzed. Statistical analyses were performed of the differences between first and second challenges (horizontal comparisons on tables). Pair-wise comparisons were also performed to evaluate the influence of treatment (vertical comparisons on tables).

Results

The transition energy from the $K\alpha$ layer of calcium (3.691 keV) and phosphorus (2.013 keV) were the parameters used to determine the intensities of chemical elements in teeth by μ -EDX. The relatively low energy of the photons used for the analysis provides information about the elemental content of the tooth surface layers (because of the

self-absorption effect) in the enamel, which is about 2 mm thick.

The μ -EDX results for the elemental weights of calcium and phosphorus, for organic balance, as well as for the Ca/P and inorganic/organic ratios are presented in Tables 3–8 and Figures 2–6.

A reduction in % Ca elemental weight between the first and second challenge in the control group ($p = 0.0395$) was statistically significant, and this demonstrated the loss of calcium during cariogenic challenge.

However, there was an increase in this value with no significant difference between challenges in the infrared laser irradiation and photo-absorbing agent (CL) group, a sign that this inorganic element was preserved. Comparing all groups, there was a borderline statistically significant difference ($p = 0.0501$) in the control (CTR) and CL groups for calcium weight, demonstrating that there was no loss of the calcium, even with cariogenic challenge (Table 3 and 8, Figure 2).

There was a reduction in % P weight in the control group, which was of borderline significance ($p = 0.0535$) between challenges. However, the CL group showed an increase in this value, which was not significant. When comparing all groups, there was a borderline statistically significant difference ($p = 0.0509$) between the CTR group and CL group (Tables 4 and 8 and Figure 3).

For the organic balance of oxygen, carbon, and some contaminant ions, there were increases in the percentage of weight of these elements in the control group and the fluoride and cream and laser-irradiated (FCL) group, but these increases were borderline significant ($p = 0.0535$). The CL group showed no significant differences between the two challenges. However, when the groups were compared for this variable, there was a borderline significant difference

TABLE 3. MEAN AND STANDARD DEVIATIONS (SD) OF CALCIUM (WT %) FROM ENAMEL FOR EACH GROUP AND PERIOD OF TREATMENT

Groups	First cariogenic challenge	Second cariogenic challenge	% variation
1. Control	33.44** (6.77)	25.97** (3.96) a	–22.34
2. Cream	30.16 (7.82)	31.09 (5.82)	+3.08
3. Laser	30.89 (1.76)	30.81 (4.04)	–0.06
4. Cream + laser	33.88 (3.62)	34.22 (1.97) a	+1
5. Cream + laser + fluoride	29.95 (5.80)	29.01 (7.09)	–3.14
6. Cream + fluoride	30.75 (5.59)	31.10 (6.01)	+1.13

A lowercase letter following a value indicates borderline significant differences between groups.

**Statistically significant differences using paired *t* test.

TABLE 4. MEAN AND STANDARD DEVIATIONS (SD) OF PHOSPHORUS (WT %) FROM ENAMEL FOR EACH GROUP AND PERIOD OF TREATMENT

Groups	First cariogenic challenge	Second cariogenic challenge	% variation
1. Control	16.64* (2.25)	14.07* (2.13) a	-15.45
2. Cream	15.48 (3.24)	16.12 (2.18)	+4.13
3. Laser	16.02 (0.76)	16.02 (1.49)	0
4. Cream + laser	16.67 (1.75)	17.17 (0.59) A	+3
5. Cream + laser + fluoride	15.57 (2.23)	15.35 (2.64)	-1.42
6. Cream + fluoride	15.90 (2.04)	15.96 (2.02)	+0.37

A lowercase letter after a value indicates statistically significant differences between groups; an uppercase letter indicates borderline significant differences between groups.

*Borderline significant differences using paired *t* test.

($p=0.0513$) between CL and CTR groups (Tables 5 and 8; Figure 4).

The most evident reductions in the Ca/P molar ratio between challenges was in the CTR group, and this reduction was borderline significant ($p=0.0515$). All groups demonstrated a reduction in this ratio after the cariogenic challenge. However, a difference was observed between the CTR and CL groups, although this was borderline significant ($p=0.0587$). The CL group values were the highest (Tables 6 and 8, Fig. 5).

When we analyzed the data for the inorganic/organic ratio, we observed a decrease in the CTR group, indicating a more pronounced loss of inorganic constituents during the challenges, although this decrease was not quite significant ($p=0.0518$). The values for the FCL group also decreased, although this change was not statistically significant. The other groups showed nonsignificant increases in this ratio between the two challenges, indicating preservation of inorganic constituents despite the cariogenic challenges. The data for the second challenge showed borderline significant differences ($p=0.0592$) between the CTR group and the CL group (Tables 7 and 8, Fig. 6). The ratio was largest in the latter group at the second challenge.

Discussion

The reduced acid solubility of dental enamel after irradiation with high-intensity lasers has been well documented in literature,^{4,20-22,42-47} and this change is related to the physical and chemical alterations caused by photothermal and photochemical effects.⁴⁶ It is well known that intrinsic factors of the laser source (wavelength, emission mode, pulse energy, spot size, pulse duration, and application method on the

tissue) and external parameters (energy density and time exposition) are relevant to understanding the mechanism of interaction between the laser and biological tissue.⁴⁵ The absorption coefficient of tissue components determines the degree of interaction between laser and tissue.

Depending on the temperature achieved by the laser irradiation, different effects, especially on the enamel's solubility, can be produced. The smallest level of acid dissolution in the enamel is achieved after heating to 300–350°C,⁴⁸ and it was suggested that this effect is caused by the denaturation and swelling of the organic matrix that leads to the obstruction of the diffusion pathways within the enamel. Above 200°C, there is a loss of carbonate that could contribute to increased acid resistance.⁴⁹ Microspaces formed as a consequence of loss of water or carbonate and organic substances might prevent demineralization by trapping the dissolved ions.⁵⁰ An increase in pyrophosphates caused by heating to 200–400°C strongly reduces the hydroxyapatite dissolution rate.⁵¹ The laser energy from visible to infrared should be strongly absorbed and efficiently converted into heat to promote chemical or physical alterations in dental enamel,^{43,46} and the degree of absorption inside the dental enamel depends on wavelength.^{43,46} Optimal irradiation requires wavelengths be strongly absorbed by the components of the tissue such that light scattering is negligible.⁵²

In the case of laser wavelengths that are poorly absorbed by the enamel, as is the case with low-power diode lasers, a coat of an appropriate dye could absorb the light, generate heat, and then transfer this heat to the target tissue. In this study, a cream gel containing indocyanine green was used. Chromophores, like the kind in this cream, are useful as treatment agents for enhancing the photodynamic and photothermal bacterial reduction effects as well as for providing

TABLE 5. MEAN AND STANDARD DEVIATIONS (SD) OF ORGANIC BALANCE (WT %) FOR EACH GROUP AND PERIOD OF TREATMENT

Groups	First cariogenic challenge	Second cariogenic challenge	% variation
1. Control	49.91* (8.99)	59.30* (6.62) a	+18.81
2. Cream	54.35 (11.03)	52.79 (7.99)	-2.87
3. Laser	53.08 (2.48)	53.17 (5.52)	+0.16
4. Cream + laser	49.46 (5.24)	48.61 (2.55) a	-1.71
5. Cream + laser + fluoride	54.46 (8.00)	55.64 (9.73)	+2.16
6. Cream + fluoride	53.35 (7.61)	52.95 (8.00)	-0.74

A lowercase letter following a value indicates borderline significant differences between groups.

*Borderline significant differences using paired *t* test.

TABLE 6. MEAN AND STANDARD DEVIATIONS (SD) OF THE CA/P MOLAR RATIO ENAMEL CONTENT FOR EACH GROUP AND PERIOD OF TREATMENT

Groups	First cariogenic challenge	Second cariogenic challenge	% variation
1. Control	1.56* (0.12)	1.44* (0.05) a	-7.7
2. Cream	1.51 (0.09)	1.49 (0.07)	-1.33
3. Laser	1.50 (0.03)	1.49 (0.06)	-0.67
4. Cream + laser	1.60 (0.10)	1.55 (0.03) a	-3.13
5. Cream + laser + fluoride	1.49 (0.09)	1.46 (0.10)	-2.02
6. Cream + fluoride	1.50 (0.08)	1.51 (0.11)	-0.67

A lowercase letter following a value indicates borderline significant differences between groups.

*Borderline significant differences using paired *t* test.

tooth whitening effects. Indocyanine green is clinically used as a fluorescent dye for imaging purposes. Besides the dye's absorption at the 810-nm wavelength,^{23,38} its rapid circulation kinetics and minimal toxicity as well as the ease of its removal from the surface have prompted investigations into the utility of indocyanine green dyes as photosensitizers in therapeutic applications such as the application in ablating carious tissues proposed by McNally et al.⁵³

There are only a few studies that have investigated the effects of diode lasers (such as the 809–830 nm diode laser), with or without the fluoride varnish, on acid resistance.⁵⁴ Indeed, to the best of our knowledge, there have been no studies of low-level lasers for irradiating demineralized enamel. Laser irradiation of incipient dental caries or partially demineralized enamel might arrest the development of caries.³

Therefore, the treatment of white spot lesions with laser radiation for increasing the resistance to demineralization, in association with other measures such as control of diet and dental biofilms, could arrest the progression of incipient caries. Further studies with qualitative and quantitative evaluations of the inorganic substrates in incipient dental caries and such evaluations after laser irradiation, as done in this study, are required to ascertain whether such a treatment would have clinical applicability in controlling the advance of disease when caries formation has already begun.

The results of the μ -EDX measurements show that, even with cariogenic challenge, the calcium enamel content (wt %) (Tables 3 and 8, Fig. 2) was preserved and increased after irradiation in the CL group ($p=0.0501$). The phosphorus enamel content (wt %) (Tables 4 and 8, Fig. 3) was preserved or increased in all experimental groups, except for the FCL group, which showed decreases like the CTR group. Antunes

et al.⁴⁵ observed that after laser irradiation (Nd:YAG) on enamel surfaces, there were decreases in calcium and phosphorus content. They attributed the phosphorus reduction after irradiation to a volatilization caused by temperature, but this did not happen in our study.

Simmer and Fincham⁵⁵ stated that because there were 10 calcium ions per unit of calcium hydroxyapatite, the calcium activity is raised to the 10th power in the solubility product equation. As a consequence, the solubility product for dental enamel, which directly affects the enamel resistance during cariogenic challenges, is more affected by changes in calcium concentration than by changes in any other parameter.⁵⁵ The mineral solubility is linked to the stoichiometric deviations at the hydroxyapatite components.

The organic balance analyses of mainly oxygen and carbon offered information about the organic components of examined tissue and the links with inorganic components and water. The C, CL, and FC treatment groups maintained positive balances of calcium and phosphorus after cariogenic challenges, preserving the links with inorganic components. However, only the CL group presented significant differences ($p=0.0513$) in this regard compared with the control group.

There was a decrease in organic balance, demonstrating that the combined use of cream plus laser had a role in the maintenance of the inorganic content of that enamel, since a certain stability in crystal structure was maintained despite cariogenic challenge (Tables 5 and 8, Fig. 4).

As the other experimental groups treated with laser showed no negative variations in the organic balance weight, we cannot say that there was substantial water loss from the warming likely due to irradiation. However, when the cream was used, there was an intensification of energy absorption

TABLE 7. MEAN AND STANDARD DEVIATIONS (SD) OF INORGANIC/ORGANIC ENAMEL CONTENT FOR EACH GROUP AND PERIOD OF TREATMENT

Groups	First cariogenic challenge	Second cariogenic challenge	% variation
1. Control	1.08* (0.40)	0.71* (0.16) a	-34.25
2. Cream	0.95 (0.42)	0.96 (0.33)	+1.05
3. Laser	0.90 (0.09)	0.92 (0.21)	+2.22
4. Cream + laser	1.07 (0.22)	1.14 (0.20) A	+6.54
5. Cream + laser + fluoride	0.89 (0.31)	0.86 (0.35)	-3.37
6. Cream + fluoride	0.93 (0.32)	0.95 (0.30)	+2.15

A lowercase letter after a value indicates statistically significant differences between groups; an uppercase letter indicates borderline significant differences between groups.

*Borderline significant differences using paired *t* test.

TABLE 8. COMPARISONS BETWEEN THE CONTROL GROUP AND CREAM-LASER GROUP FOR VARIOUS INORGANIC AND ORGANIC COMPONENTS

Moment	Component	Comparison	p value
Second cariogenic challenge	%Ca	Control vs. cream-laser	0.0501
	%P	Control vs. cream-laser	0.0509
	% Org	Control vs. cream-laser	0.0513*
	Ca/P	Control vs. cream-laser	0.0587*
	Inorg/Organ	Control vs. cream-laser	0.0592*

*Borderline significant.

on the surfaces with probable temperature increases in the CL group, which may have caused greater water loss.

An organic matrix is present at very low concentrations (1%) in the dental enamel. This takes the form of very small peptides and amino acids distributed throughout the mature tissue and presumably represent the remnants of the original developmental matrix, perhaps retained by binding to the hydroxyapatite crystals.^{52,58} It provides the template for enamel mineralization and continues to be the means of transporting substances in the enamel. It may have great potential in controlling the diffusion pathway in enamel and thus can play a significant role in laser-induced caries prevention.^{26,56}

In contrast to a breakdown of protein matrix by proteolysis, the interaction between laser light, chromophore, and enamel can induce protein denaturation in such a way that the diffusion pathway in enamel may be blocked.²⁵

Subtle changes in the chemical environment, such as changes in pH, temperature, and ionic strength, can easily lead to breakage of weak links that stabilize the secondary and quaternary structures, which results in the denaturation of protein.⁵⁷ Indeed, the consolidation of the protein component of the organic matrix after denaturation can produce a significant reduction in the enamel crystal surface area available for acid decalcification.⁵⁸

Currently, it is believed that the decrease in enamel solubility after laser treatments is due to stringent changes in the infrastructure, such as water and carbonate content reduction, increase in hydroxyl ions, pyrophosphates formation, and protein decomposition.^{20,21,24} Another possibility would be to change and possibly destroy the organic material found in the interprismatic space. The laser irradiation can create such microspaces that can act as sites of deposition of ions⁵⁰.

In our previous study³⁸ with Fourier transform (FT)-Raman spectroscopy, there was a reduction in the integrated area of the 2940 peak. This area corresponded to the C-H stretch band, with very similar values for primary and permanent teeth. Such an area identifies links that characterize the organic part of enamel. The distribution of organic components with the CL group was similar to that found for the same group in this study, and both indicated reductions in organic balance (Table 5, Fig. 4). However, the previous study did not analyze the Ca/P ratio.

Ca/P ratio is a way to quantify the pattern of biomineralization because this value is indicative of dental hydroxyapatite mineralization. The higher the value of this ratio, the more tooth structure is mineralized with calcium. Structural changes take place when the Ca/P molar ratio departs substantially from that of stoichiometric hydroxyapatite (1.667).

In agreement with previous studies with Er:YAG laser,⁴⁴ all groups showed negative balance with the Ca/P ratio (Table 6, Fig. 5), with the Ca/P ratio being reduced after the

FIG. 2. Average and standard deviation (SD) of calcium (wt %).

FIG. 3. Average and SD of phosphorus (wt %).

second cariogenic challenge. When we analyzed the difference between the first and second challenges, there was a statistically significant reduction in the CTR group. Comparing treatment groups, the CTR vs. CL groups showed borderline significant differences ($p=0.0587$) and the CL group showed the highest average Ca/P ratios (Table 6, Fig. 5), denoting greater preservation of calcium mineral, even under cariogenic challenge. Studies with Nd:YAG⁴⁵ and ARF excimer laser⁵⁹ showed that after irradiation, the Ca/P ratios were higher when compared with nonirradiated controls; however, these samples were not submitted to cariogenic challenge. However, Muller et al.,⁶⁰ using a low-power laser, did not find a statistically significant difference in the calculated Ca/P weight (as %) after lasing treatments.

We observed reductions in the inorganic/organic ratio (Table 7, Fig. 6) for the control and FCL groups, indicating greater losses in the inorganic portions of the enamel. This reduction was significant for the control group between the first and second challenges. The C, L, CL, and FC groups showed nonsignificant increases in the inorganic/organic ratio from the first to the second challenges.

Comparing all groups, only the CL group differed from the CTR group (this difference was borderline significant, $p=0.0592$), which indicated a greater loss of the organic portion of enamel during the pH cycling cariogenic challenge. This was also demonstrated in the organic balance data (Fig. 6) and the results of inorganic enamel content preservation (Tables 7 and 8, Fig. 6). De Andrade et al.⁴⁴ state

FIG. 4. Average and SD of organic balance.

FIG. 5. Average and SD of Ca/P ratio.

that Nd:YAG laser treatments modify more of the organic components of hard tissue than the inorganic components. The highest average inorganic/organic ratio was found in the CL and FC groups, showing that fluoride or laser associated with cream presented similar efficacies post-cariogenic challenge.

Since the 1940s when fluoride started to be applied to teeth, there has been a constant search for a cost-effective method to enhance fluoride uptake and ultimately prevent dental caries.⁶⁰ Ideally, a greater fluoride preventive effect is obtained when fluoride is incorporated into the enamel structure through the formation of fluoridated apatite, which is a less soluble, more chemically stable crystal than hydroxyapatite.

Indeed, the presence of fluoride in the oral environment inhibits the formation of acids and phosphate compounds (calcium phosphate), such as tricalcic and octacalcic phosphate, which are more soluble. The concept that fluoride may be incorporated into the enamel is important for the laser-associated fluoride effect. Compared with apatite with carbonate or hydroxyl ions incorporated into its structure, fluorapatite is more resistant to both strong (corrosive/erosive) acids as well as to weaker acids.

Although the frequent use of fluoride in low concentrations¹⁶ by the patient has been the best way to control dental caries development, the professional application of fluoride in high concentrations is still needed in some clinical situations.

Currently there is a consensus that the topical use of fluoride products induces the formation of globules of calcium fluoride, considered the main enamel reaction product. This product acts as a reservoir of fluoride ions, controlled by the pH variation during cariogenic challenges. In an enamel ultrastructure study, de Sant'Anna et al.²³ reported that after using a fluoridated photo-absorbing laser-irradiated cream, the same used in our study, there was a confluent globular coverage deposition on the dental enamel, which probably represented calcium fluoride.

Calcium fluoride is a weakly linked fluoride responsible for the cariostatic effect. It is also known that its formation on the enamel is pH dependent, with more acidic conditions resulting in greater incorporation. We used a cream with a neutral pH, which probably resulted in lower calcium fluoride incorporation in the treated area.

The calcium fluoride adsorbed to the tooth surface increases the remineralization and attracts calcium ions present

FIG. 6. Average and SD of inorganic/organic ratio.

in saliva. The fluoride ions also act by adding calcium and phosphate and are involved in the chemical reactions that occur. Also, they produce a crystal surface that is much less soluble in acid than the original mineralization of the tooth.

Several studies^{20–22,43,46,47} emphasize the use of different lasers in association with various forms of fluoride for the reduction of demineralization. The laser is applied to fluoride to increase fluoride diffusibility, which promotes greater ion absorption in the enamel or favors ion linking in the adjacent area. Other studies⁶¹ recommend laser irradiation after fluoride application to promote melting as well as formation of solidified hydroxyapatite crystal layers combined with a fluoride layer formed by topical treatment prior to irradiation. In addition, this fluoride effect may be due to an increased fluoride uptake, because removing the organic matrix may render a greater crystal surface area for the binding of ions, such as fluoride and calcium ions.

Our results showed that the fluoride uptake in the enamel structure using fluoride-cream and laser was similar to that in the control group, in which there were losses in calcium and phosphorus (Tables 3 and 4, Figs. 2 and 3). A negative change in the inorganic/organic ratio (Table 7, Fig. 6) was also observed in this group after the second cariogenic challenge, indicating solubility of the structure with demineralization.

In the study by Muller et al.,⁶⁰ which used a low power laser, neither the microhardness surface nor the EDS analysis showed any advantageous effects of the laser irradiation on enamel. The authors suggested that the combination of the low power laser and the topical application of the acidulated phosphate fluoride (APF) gel did not have significant effects on the prevention of induced dental caries in rats, although applying the laser before APF gel appeared to diminish caries progression.

Our choice was the concurrent use of the fluoride-cream and laser, as a laser-activated fluoride treatment was also used in a microhardness⁶² and SEM²³ study.

This option did not show satisfactory results, possibly because the interaction between light and fluoridated cream may have prevented the benefits of both fluoride and laser. However, Vlacic et al.⁶² demonstrated statistically significant changes in the enamel surface hardness after lasing with fluoride at the 830 nm wavelength, but the changes were not significant after a demineralizing challenge.

Hydroxyapatite is the principal component of human teeth and its crystallinity is one of the resistant factors against acid agents. The use of μ -EDX permits evaluating the presence of stoichiometric changes in the mineral composition of tooth enamel that has undergone laser treatments, because any change can make us assume hydroxyapatite variation to other types of phosphate and consequently the enamel's degree of crystallization.

Conclusion

Our results suggest that the use of a laser along with a photo-absorbing cream promotes disorganization of the organic content in the tooth enamel with hydroxyapatite crystal reordering and reorganization. In artificial enamel caries lesions, the CL treatment prevented the loss of calcium and phosphorus during new cariogenic challenges, increasing the inorganic/organic ratio of dental enamel. This suggests increased resistance to solubility. However, additional studies

are needed to evaluate the crystallinity of the treated structure (using grazing incident x-ray diffraction, transmission electron microscopy) and the stability of such changes, taking into account the influence of salivary environment.

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Disclosure Statement

No competing financial interests exist.

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