Analysis of photodynamic cream effect in dental caries using Optical Coherence Tomography.

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ABSTRACT

The aim of this study was to assess the effect in the enamel demineralization of low-intensity infrared laser (λ=810 nm, 100 mW/cm^2, 90 sec, 4.47 J/cm^2, 9 J) with or without photodynamic cream fluorinated or not fluorinated, using Optical Coherence Tomography (OCT). ***Background data:*** Lasers can be used as tools for the prevention of tooth enamel demineralization.

All enamel specimens (n= 105) were analyzed using OCT at baseline, and randomly assigned into seven groups (*n*=15): C (+), laser application; C(-), no treatment; (F), acid fluoride gel; cream (IV); cream and neutral fluoride (IVF); cream and laser (IVL); and cream with neutral fluoride+ laser (IVFL). The specimens were submitted to all kind of treatments before demineralizing pH cycling challenge and were reanalyzed.

ANOVA and Tukey's multiple comparative analysis ($p < 0.01$) demonstrated a greater delta attenuation between baseline and post challenge for C + (0.034 ± 0.011) compared to IVF (0.016 ± 0.007) F (0.018 ± 0.010) IVFL (0.019 ± 0.008), and IVL (0.014 ± 0.010). The cream laser group (IVL) also showed lower delta (0.014 ± 0.010) compared to C - (0.025 ± 0.008).

The OCT technique demonstrated that cream associated with laser showed the lowest quantitative enamel mineral looses after cariogenic challenge.

`Keywords:` Tooth; Dental Caries; Lasers; Dental Enamel; Fluorine Compounds; Tomography; Demineralization; Prevention, Control; Tomography, Optical.

1. INTRODUCTION

Despite the decline in its prevalence, tooth decay remains a disease with alarming epidemiological data and 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].

Research on the pathogenesis of dental caries have been recognized over the years and have greatly contributed to more conservative professional actions[3].

Light as a preventive and therapeutic approach to dental caries, is already a technological reality.[4], however the literature offers, in this regard, data on high-intensity lasers, which still economically unfeasible for routine use. Thus, the possibility of low-intensity lasers uses due to the economic viability is an issue to consider.

Biophotonics South America, edited by Cristina Kurachi, Katarina Svanberg, Bruce J. Tromberg,
Vanderlei Salvador Bagnato, Proc. of SPIE Vol. 9531, 95313A · © 2015 SPIE
CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2180962

In order for a laser acts biologically, it has to interact with the tissue. The tooth enamel has little absorption in the visible (400–700 nm) and in the near-infrared (780-2500 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].

Near infrared and red low intensity lasers appear an alternative approach, since literature suggest that its association with topical fluorine or not, cause an increase in the resistance facing the dental caries [6-12]. Some authors affirm that for caries prevention, in order to alter the composition or the solubility of hard dental tissues, laser should be strongly absorbed and transformed in heat without damaging the tissues around or underlying[13].

Researchers suggest that the use of low intensity laser, and fluorinated or not photoabsorbing creams may alter morphological, structural, and biochemically dental enamel[9-12,14]. Some authors irradiated surface of deciduous tooth enamel with low intensity diode laser using a fluorinated and not fluorinated photoabsorbing cream; and observed the morphological and structural aspects pointing the presence of a surface coverage that could possibly act as reservoirs for mineral phases during cariogenic challenge and provide some degree of protection against dental caries[14].

Following the same principle, research[10] with X Ray dispersive energy spectroscopy demonstrated preservation and maintenance of elemental weight percentages of calcium and phosphorus in irradiated enamel with infrared laser and photoabsorbing cream fluorinated or not. In the same study, the authors demonstrated that the samples treated with non-fluorinated indocyanine cream and laser irradiation showed increases in elemental weight percentage of calcium and phosphorus and increase in inorganic/ organic ratio under cariogenic challenge. However, for irradiated fluorinated indocyanine green cream, inorganic / organic ratio decreased[10].

De Sant'Anna et al.[9] with FT-Raman spectroscopy study demonstrated using the same approach concept of enamel treatment that there was a significant laser-induced reduction and possible modification of the organic matrix content in enamel treated with the low-level diode laser and photoabsorbing fluorinated or non-fluorinated creams.

In the same philosophy of preventative approach, research evaluating surface microhardness after cariogenic challenge showed that photoabsorbing indocyanine cream fluorinated or not under laser irradiation demonstrated lower microhardness decreases[11].

An vitro study investigated the effect of low-power near infrared laser on inhibition of enamel demineralization around orthodontic brackets using as treatment, laser and photoabsorbing cream using indocyanine gree as cromophore, the authors observed the effectiveness of such association and verifyed the inhibition of caries development through quantitative light induced fluorescence (QLF)[12] .

The pigments of the dye act selectively absorbing parts of the light spectrum and reflecting others; the indocyanine green dye is widely used in biomedicine with infrared absorbing properties[15]; absorption peak at about 800nm and little or no absorption in the visible spectrum[16].

In photodynamic action as the proposals mentioned in the above studies, there is the intention of the free radicals and reactive oxygen species formation, which in turn act on biological tissue generating photochemical actions.

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 aiming to change the enamel structure making it more resistant is in line with the pursuit of better health.

New tools are needed to non-destructively assess carious lesion depth and severity, efficacy of chemical intervention, and testing of anti-caries agents to serve as a likely surrogate end point in dental clinical trials [17].

Several studies have demonstrated that optical coherence tomography (OCT) can be used to nondestructively measure[18-25].

The optical coherence tomography (OCT) has been developed for the past years as a non-invasive method used in the diagnosis of different biomedical events[26].

The OCT is an emerging technology of bio-imaging, which promises to have a broad and significant impact on the diagnosis and clinical picture in relation to tooth decay and can be used to monitor the progression of carious lesions, or the effect of treatments aiming prevention [20].

The present in vitro study evaluated the effect of photoabsorbing creams irradiated with low-intensity near-infrared laser in human deciduous enamel under demineralization (DE) challenge using OCT.

2. METHODOLOGY

2.1 Ethical Aspects and Samples Preparation

This study was approved by Ethics and Research Committee, Cruzeiro do Sul University/# 185/2013. Caries-free human deciduous molars (n = 53) analyzed with a stereoscopic magnifying glass (Nikon, instrumental Group) were sectioned mesiodistally with a micromotor (LB100, Beltec; Brazil) and a carborundum disk (Dremel, USA) with water cooling, obtaining 105 human enamel samples. They were cleaned with ultrasound for 10min (Cristofoli, PR, Brazil) and Robinson brush (KG Sorensen, SP, Brazil) in micro motor (Dabi Atlante, SP, Brazil) with pumice (SS-White, SP, Brazil) and water.

The allocation of the specimens in groups was random taking care that each enamel fragment was in different treatment groups. Seven treatment groups were established (*n*=15): C (+), laser application; C(-), no treatment; (F), acid fluoride gel; (IV), cream; (IVF), cream and neutral fluoride; (IVL), cream and laser; and (IVFL), cream with neutral fluoride+ laser, all samples stored individually, and kept in deionized water.

For treatment and demineralization assay areas standardization in each sample, each block had an area of 6,25mm^2 marked in the center of the face, which were carried out OCT analysis. The area delimitation was obtained by gluing square labels (Pimaco-Bic, RJ, Brazil), with 2.5mm side, on the dental enamel, which were removed after the remaining enamel was covered by acid resistant varnish (Revlon, NY, USA).

2.2 OCT System and Analyses

The samples were examined using OCT technique at the beginning of the experiments, establishing a baseline and submitted to demineralization challenge after the treatments and once more examined by OCT. Before testing, each sample was slightly dried with absorbent paper merely for the removal of the superficial liquid film, assuring same hydration conditions for the OCT imaging.

The OCT system used employs a light source with a central wavelength of 930 nm, with power of 2 mW (OCP930RS, Thorlabs Inc, available at Optics Tomography Laboratory of Lasers and Applications Center IPEN-CNEN, SP, Brazil).

In OCT scans were obtained images of the whole area extent, which was initially defined during the samples preparation. The axial resolution was 4.0 μm (considering the enamel refractive index of approximately 1.6) [27], since the equipment has a nominal resolution of 6.0 μm in the air and side resolution of 6.0 μm.

The images of the central portions of the bounded areas were evaluated using a program developed in programming environment LabView 8.

In calculating the optical attenuation coefficient was used simple model of exponential intensity decay of the detected light (backscattered), according to the equation: $f(z)=\alpha.e^{-2bz} + C$; $f(z)$ represents the detected intensity as a function of depth z, the parameter α is the incident intensity, b is the optical attenuation coefficient, and C is a constant (from signal background).

The software allows selecting a region of interest (ROI) as the use of delimiters, important in the exclusion of edges and irregularities in this type of sample. After determining the ROI and chosen the refractive index for samples (1.6), the program automatically performed the analysis of all the images of all samples and groups.

2.3 Treatments and Cariogenic Challenge Simulation

The seven groups received specific surface treatments according to the coding described (Table 1). The near-infrared diode laser parameters (UltraBlue IV Plus II, DMC equipment; São Carlos, Brazil) were as follows: λ = 810 nm, 30mW, 90 sec, 4.47 J/cm^2, 9 J, and continuous wave (CW) laser source. The laser beam touched the sample over 1mm of cream when used, and maintained the same distance in the groups without cream. The pre irradiation used time of all products was 60 seconds.

Table 1. Groups and Surface Treatments

Groups	**n**	**Treatment**
C (-)	15	No treatment
C (+)	15	Irradiated with infrared diode laser Samples were washed in deionized Water
IVL	15	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	15	Indocyanine green gel cream (Acros; NJ; lot: A0232896) (0.05 g, Buenos Aires Lab, Brazil) (60 sec) Samples were washed in deionized Water
IVFL	15	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	15	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
F	15	Sodium fluoride, pH 1,23% (lot: 12040574)(DFL Industry and Trade S.A, RJ, Brazil)

After these treatments, the samples were submitted to *in vitro* induced artificial dental caries simulation using acidic gel composed by 8% of metilcelulosis proposed by Linch and Ten Cate[28].

The samples were placed individually in plastic pots and fixed using cosmetic nail lacquer. The samples were covered with a layer of metilcelulosis gel (0.5 cm), which remained on the specimen for 12 hours in a cold chamber at 4 °C. In sequence was added the same volume of 0.1M lactic acid (Sigma-Aldrich, St. Louis, MO, USA), pH 4.6 adjusted with 10 M KOH (0.5 cm = 1.5 ml) remaining on the specimens for 14 days at 37 °C [28,29].

They were then rinsed in deionized water and stored individually immersed in double-distilled and deionized water until the time of use for the final analysis using OCT.

2.4 Data Processing

The total optical attenuation coefficient was determined from the samples signal analysis of OCT, based on the exponential decay of this signal to the depth from 25 to 500 μm within the enamel.

This depth range was considered for the analysis because, for most of the samples prior to a depth of 25 μm was observed the presence of a very high intensity peak intensity caused by the change of light reflectivity of the medium (the air into tooth), while from 500 μm observed signal extinction.

The delta attenuation values of each sample were compared, taking into account the mean of difference before and after simulated demineralization. The OCT data as mentioned was analyzed using a computer program we developed in LabView 8 (National Instruments), which allows the user to define a region of interest in order to exclude border irregularities. From this region, the software calculates the average Ascan signal, the area under this curve and the optical attenuation coefficient using the following model of exponential decay: $f(z)=a.e^{-2bz}+C$.

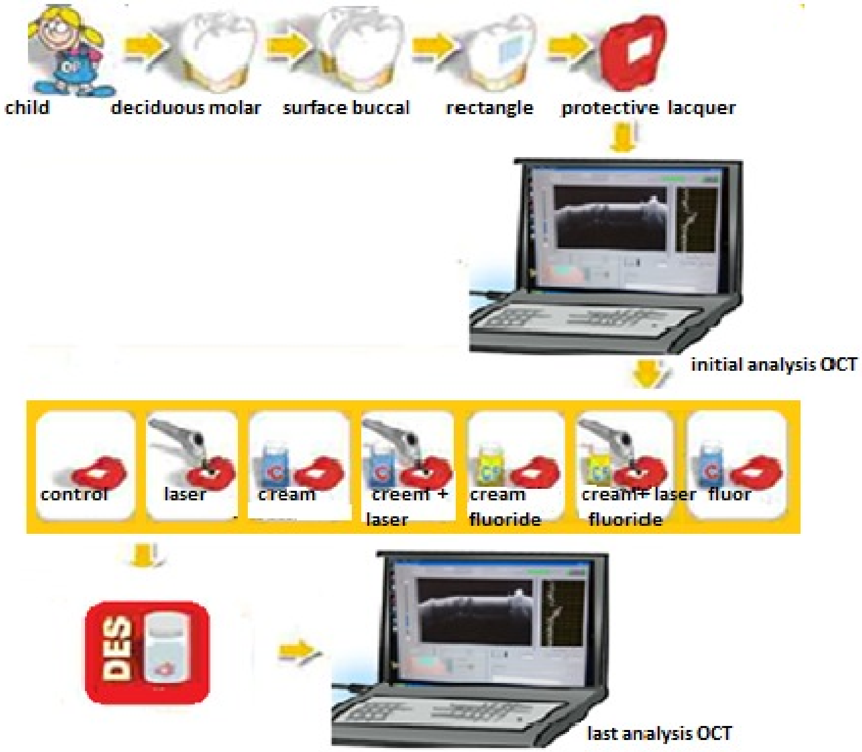

Figure 1- Study design

2.5 Statistycal analyses

The means and standard deviations assessed in the OCT were calculated, and one way analysis of variance (ANOVA) was performed (*R for Windows,* statistical software 3.1.0) 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.

3 DATA AND RESULTS

All samples demonstrated greater optical attenuation coefficient after demineralization compared to sound enamel (figure2). These results are due to a demineralization process that creates voids in the enamel structure, increasing the number of interfaces and hence the light scattering.

Statistically significant difference was observed ($p < 0.005$) for all groups evaluated before and after (figure3).

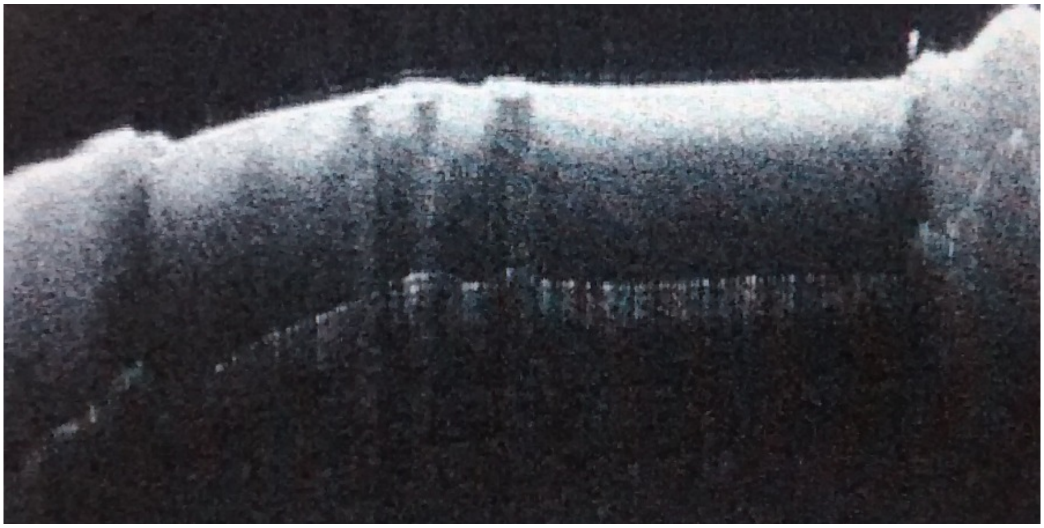

Figure 2 OCT image for demineralized sample after cariogenic challenge

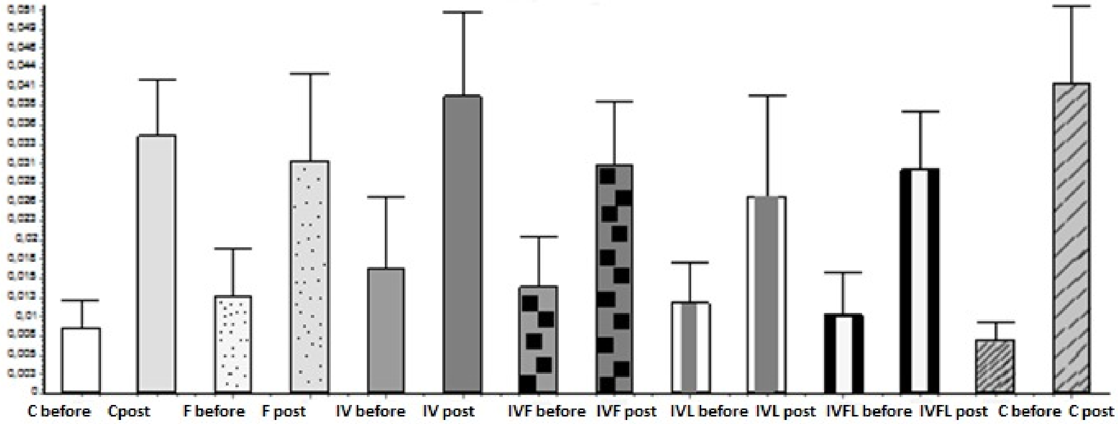

Figure 3. Media±SD for all groups before and after treatment.

The intergroup comparison of attenuation coefficient for the tested treatments are shown in Figure4.

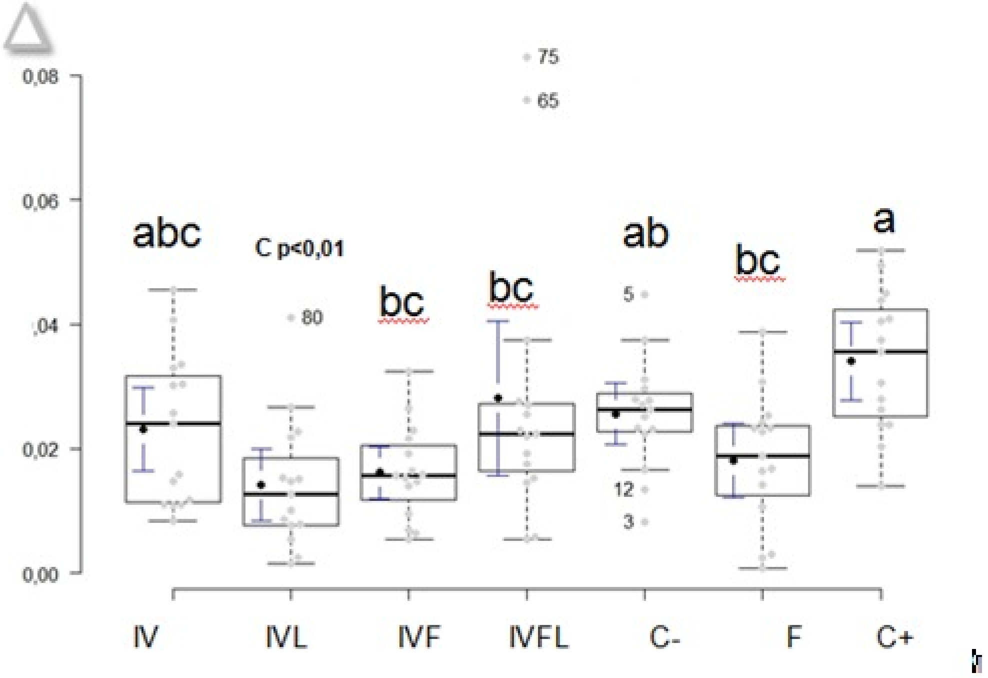

Figure 4: Difference (Δ) between the attenuation coefficient obtained before and after the simulation of the cariogenic challenge, for each proposed surface treatments.

The comparison between groups was performed using the difference between the optical attenuation coefficients obtained before and after demineralization, i.e., the delta (figure 4).

Analysis of variance and multiple comparative statistical analysis of Tukey ($p < 0.01$) showed a greater delta attenuation between pre and post challenge for the positive control group treated only with laser irradiation (C +) (0.034 ± 0.011) compared fluorinated cream (IVF) (0.016 ± 0.007), fluor gel (F) (0.018 ± 0.010) , irradiated fluorinated cream (IVFL) (0.019 ± 0.008), and Cream + laser (IVL) (0.014 ± 0.010). The cream laser group (IVL) also demonstrated lower delta (0.014 ± 0.010) compared to the negative control (C -) (0.025 ± 0.008) and had the same behavior of fluoride gel, the gold pattern.

4 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[10, 30]. It may even enhance the action of conventional therapies[31].

In tooth decay prevention, high-intensity lasers work primarily through a thermal effect, promoting physical [32, 33] and chemical changes in the target tissue[34]. 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[35-37].

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.[38] 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-absorbing 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 photoabsorbent in irradiations with Nd:YAG lasers[39]; and also alter the protein conformation causing denaturation[11, 40].

Photoabsorbing 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[41], in this study the option was low-level laser conjugated with photoabsorbing substances, once is a routine tool in clinical practice.

Indocyanine green, which is a biocompatible dye, is used in the medical sciences with an absorbance at 800 nm. This indocyanine green cream was used to maximize the absorption of near infrared laser energy on the enamel surface[9-14].

The combined use of these creams and low-level laser improve the light interaction with tissue from the optical point of view as well as has a photodynamic action resulting from the light interaction with the chromophore. It is known that in consequence of this reaction there is the production of reactive oxygen species and free radicals with several biological effects[42].

The OCT technique has been successfully applied to the diagnosis of dental caries due to changes in the optical properties of enamel after undergoing demineralization[19; 43, 44,45]. Images obtained by OCT can be evaluated qualitatively by identification of structures, dimensions, and proportions [46, 47], and quantitatively, for example by analyzing the reflectivity [19, 23;48] what drives us to use this analysis tool.

A research demonstrated that the quantitative technique using the total optical attenuation coefficient [49] obtained using OCT is capable to identify sound and demineralized tissue with accuracy of 0.95, our results also demonstrated that enamel under demineralization simulation presented in all groups differences in attenuation coefficient post challenge (figures 2and 3).

The highest total attenuation coefficient found in all groups after demineralization when compared to healthy enamel is in agreement with results found in other studies.[27, 49]. One possible reason is that the demineralization process creates voids in tooth structure and thereby there is increased interface, which increase the light scattering . Indeed, when demineralised tooth enamel undergoes prism changes being a disorganized structure contributing to light backscattering

Other studies[44;45] found lowest attenuation coefficient in carious samples, which can be justified due to a more intense demineralization once both studies used natural white spot lesions as their samples. With the intense increase in mineral loss, larger pores are formed and there is a higher probability of light goes through the pores do not interacting with the scattering centers.[49]

As regards laser therapy study demonstrated that OCT was a tool well-suited for in vivo measurements of caries inhibition after laser CO_2 treatments[35].

The presentstudy demonstrated that in the positive control group using only low-power laser (C +) (0.034 ± 0.011), the attenuation coefficient before and after challenge was greater than for the other groups with no statistical difference from the control (C-) and cream (IV) groups. This result may be related to increased susceptibility to mineral loss of such treatments, since no photodynamic action can be correlated with these types of treatment and no chemical product was added to these protocols (i.e., fluoride).

In this study the only difference from C- with statistical significance is the group (IVL), similar results were found by Barbosa *et al.*[11] using the same protocol and analyzing the surface microhardness and pulp temperature observed the conjugate use of laser and chromophore resulted in decrease mineral loss post challenge as Lacerda[12] and de Sant'Anna *et al.*[10].

A possible explanation for this finding may be found in results of the surface temperature analysis obtained in the same study[11] for the groups receiving the cream and laser treatment, in that the elevated temperature may cause protein 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[40].

At temperatures lower than those necessary to change the mineral phases of dental enamel may result in partial decomposition of the organic matrix, with obstruction of the interprismatics spaces in the enamel and impairment of acid ions diffusion and thus a reduction of enamel demineralization. [35].

Some authors[10] used the same treatment protocol of this study. Using X-ray energy dispersive spectroscopy, analyzed the variable organic balance (oxygen and carbon), which provides information about the organic components of the enamel and their links with water. The authors 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. The same authors in other study[9] with FT-Raman spectroscopy also demonstrated changes in organics of enamel under the same treatment , i.e., cream and laser.

It should be noted that this photodynamic association with its attendant by- products, namely reactive oxygen species and free radicals may result depending upon the quantum efficiency in damage to the matrix proteins by oxidative stress, blocking access acid and therefore demineralization, and the studies[9,10] has shown. Radicals and two-electron oxidants can undergo a variety of reactions with aminoacids, peptides and proteins, including hydrogen abstraction (removal of hydrogen by a radical), eléctron transfer (oxidation or reduction of the substrate), addition, fragmentation and rearrangement, dimerisation, disproportionation, and substitution (concerted addition and elimination) reactions. Oxidative damage can occur to both the protein backbone as well as the 20 common amino acid side chains, most of which have multiple possible sites of attack[50] and this could be a possible mechanism of decrease tooth demineralization under challenge observed in OCT analysis.

5 CONCLUSION

The results of cream associated with laser treatments and the use of OCT as an analytical tool were promising; however more research is importante in order to adapt them to treatment procedures protocols, both *in situ* and *in vivo.* Further, studies comparing this analysis tool with other traditional ones are also important in order to establish correlations.

Acknowledgements

The analyses in the present study were performed at the Institute of Energy and Nuclear Research (IPEN), São Paulo, SP, Brazil and the treatment protocols used a laser unit of DMC group Ltd. (São Carlos, SP, Brazil).

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