Inhibition of enamel remineralization with blue LED: an *in vitro* study

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ABSTRACT

Blue light, especially from LED devices, is a tool very frequently used in dental procedures. However, the investigations of its effects on dental enamel are focused primarily on enamel demineralization and fluoride retention. Despite the fact that this spectral region can inhibit enamel demineralization, the consequences of the irradiation on demineralized enamel are not known. For this reason, we evaluated the effects of blue LED on enamel remineralization. Artificial lesions formed in bovine dental enamel samples by immersion in undersaturated acetate buffer were divided into three groups. In the first group (DE), the lesions were not submitted to any treatment. In the second (RE), the lesions were submitted to remineralization. The lesions from the third group (LED+RE) were irradiated with blue LED (455nm, 1.38W/cm^2, 13.75J/cm^2 and 10s) before the remineralization. Cross-sectional microhardness was used to assess mineral changes induced by remineralization under pH-cycling. The mineral deposition occurred preferably in the middle portion of the lesions. Specimens from group RE showed higher hardness value than the DE ones. On the other hand, the mean hardness value of the LED+RE samples was not statistically different from the DE samples. Results obtained in the present study show that the blue light is not innocuous for the dental enamel and inhibition of its remineralization can occur.

Keywords: light emitting diode, light-tissue interaction, caries, microhardness

1. INTRODUCTION

Light-tissue interaction is the major topic for understanding the effects promoted by the irradiation of the biological tissue. Dental enamel is a strongly light-scattering composite material of the outer layer of the tooth crown whose optical properties are still very little understood when compared to other biological tissue such as the eye. Although the effects of infrared light on dental enamel were extensively studied, the consequences of blue light irradiation on this tissue were less investigated. Also, the absorption properties in the blue spectral region of its different components, composed of 87% inorganic material, 11.5% water and 1.5% organic matrix [1], are not totally known. Despite of this fact, dental enamel is frequently irradiated by blue light during typical dental office procedures such as curing resin, tooth bleaching and diagnosis [2, 3].

There are several ways to investigate light-tissue interaction. Asides from the investigation of the tissues primary optical response to irradiation, the interactions between light and tissue can also be indirectly studied by evaluating the effects caused when the irradiated biological material faces challenges. Such a challenge is the exposure of dental enamel to its daily, continuous and dynamic exchange of minerals inside the oral cavity [4]. If the rate of loss (demineralization) outstrips the rate of deposition (remineralization) in repeated episodes, caries lesion would be formed [5]. On the opposite, natural reversal of this process can be induced changing the balance for remineralization [1]. The diffusion pathway for these minerals consists of the spaces between the crystals and prisms that are filled with water and organic components [6]. Previously reports showed that alteration in this organic matrix leads to changes in the mineral loss [6, 7] and deposition [8] .

Several *in vitro* and *in vivo* studies showed that interaction between light from argon laser and dental enamel results in alteration of enamel demineralization [9, 10]. Relative low fluences (11.5-13.5 J/cm^2) and short time of irradiation (10 s) were investigated and promoted inhibition of mineral loss in samples submitted to different periods of acid challenge[9, 10].

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Furthermore, the association with fluoride can alter the interaction between this topical agent and the enamel causing an increase of fluoride retention time [11]. The wavelength emitted by the argon laser devices used in these studies may vary between blue (λ = 488 nm) and blue/green light (λ = 488 nm and 514.5 nm), whereas it seems that the effects are primarily due to emission at 488 nm [9, 11].

So far, research has concentrated its efforts on the effect of blue light on enamel demineralization. The hypothesis tested in this study is that the blue light interacts with enamel tissue altering the process of remineralization.

2. MATERIALS AND METHODS

2.1 Experimental design

Enamel blocks obtained from bovine incisors were used as experimental units to perform this *in vitro* study. These samples were immersed in undersaturated acetate buffer to induce caries like-lesion formation and were arbitrarily divided into three groups: (DE) samples not submitted to any additional treatment, (RE) control, specimens only submitted to remineralization; (LED+RE) samples irradiated with blue light and submitted to remineralization. The remineralization of the RE and LED+RE samples was induced by pH-clycling. Then, the cross-sectional microhardness analysis was performed in all specimens and the integral area of hardness profile (ΔS) and the percentage of hardness recovery were calculated.

2.2 Samples preparation

Thirty enamel blocks of 4 mm x 2 mm were cut (Isomet Low Speed Saw, Buehler, USA) from the buccal surface of recently extracted bovine incisors teeth and selected after analysis under optical microscope to ensure the absence of cracks or any other defects. The samples were brushed with pumice and washed with distilled de-ionized water. In order to evade alterations of the organic components of the enamel, none chemical reagent or heating technique was used for cleaning the teeth. The blocks were then covered with acid resistant nail varnish and dental wax, except for the enamel surface. During all the experiment, the samples were individually kept in eppendorf with a small portion of sterile cotton slightly dried with distilled de-ionized water.

2.3 Lesion formation

Enamel lesions were produced by immersing each enamel block in 50 ml of acetate buffer 50% saturated with respect to enamel mineral[12], contained ina coveres plastic bottle. The composition of the demineralizing solution was 0.05M acetate buffer, 1.28 mM Ca, 0.74 mM P, 0.03 µg F/mL, pH 5.0[13] and and 4.3 mmol/l thymol as a bacteriostat[14]. The low fluoride concentration was used in the buffer to avoid loss of enamel surface. The bottles were maintained at 37^{o}C for 32 hours in a constant-temperature incubator, without agitation. After this period, the samples were removed from the demineralizing solutions, rinsed with distilled de-ionized water, and gently dried with absorbent paper.

2.4 Irradiation

After lesion formation, the blocks from group LED+RE were irradiated with LED (Blue Star 1, Microdont, Brazil) emitting 455 nm with width of 20 nm. The output power was measured in the central area of the light beam using a power meter with precision of ±1 mW (Sensor head PS19 and Analyzer Field Master, Coherent, USA). A mask with a window of 4x2 mm was fixed in front of the power meter at the working distance in order to allow the measurement of the energy delivered to the sample. The parameters used were: 110 mW, 1.38 W/cm^2, 10 s, 13.75 J/cm^2.

2.5 pH cycling

The enamel blocks were submitted to the pH cycling regimen for 8 days at 37^{o}C. The samples were individually kept in 25 ml of remineralizing solution containing 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 µg F/ml, 0.1 M Tris buffer, pH 7.0 (modified from ten Cate *et al.*)[15] and and 4.3 mmol/l thymol as a bacteriostat[14] for 22 h; rinsed with distilled de-ionized water and immersed for 2 h in 50 ml of the buffer acetate described above. On the fourth day, both solutions were changed.[13]

2.6 Microhardness

After pH cycling, the enamel blocks were longitudinally cut at the center of the surface. One half of each block was embedded in epoxy resin and polished on a polishing machine (PLF, Fortel, Brazil) using 800, 2500 and 4000 grade silicon carbide paper (Buehler, USA), followed by 1 μm diamond abrasive suspension (Metadi, Buehler, USA) on polishing cloth (G-Cloth, Buehler, USA).

Hardness indentations were made crosswise the cut face of the sample using a microhardness tester (HMV-2000, Shimadzu, Japan) and a Knoop diamond under 25 g load for 5 s[13]. Three rows of indentations were made all over the extension of the demineralized region, beginning at 10 μ from the enamel surface in 10 μm steps. Other four indentations were made in sequence at 20 μm intervals to determine the mean hardness of sound enamel. In each sample, the three values measured at the same depth were averaged and these data were normalized by the mean value of its sound enamel. Normalized Knoop hardness number, expressed as KHN, was plotted in function of the depth. The numerically integrated area above the curve of hardness profile in the outer (10-30 μm), middle (40-90 μm) and inner (100 μm to the end of the lesions) portion of the lesions from the DE (ΔS_{DE}), RE and LED+RE ($\Delta S_{pH\text{-}cycled}$) groups was made. Also, the percentage of hardness recovery ($\%\Delta S_R$) was calculated for the samples from groups RE and LED+RE as follows:

$$\%\Delta SR = \left(\Delta S_{DE} - \Delta S_{pH-cycled}\right) \times 100 / \Delta S_{DE}$$ [13]

2.7 Statistical analysis

The ΔS and the $\%\Delta S_R$ data were analyzed by the Shapiro-Wilk W test to verify the assumption of normality and by Brown-Forsythe test for the equality of group variances. Next, analysis of variance was performed followed by Bonferroni test.

3. RESULTS AND DISCUSSION

The Shapiro-Wilk W test showed that the distribution of ΔS and $\%\Delta S_R$ data can be assumed as normal for all groups since p-values were superior to 0.05. The group variances were considered homogeneous for ΔS and $\%\Delta S_R$ ($p>0.05$) data according to Brown-Forsythe test. Results of both tests justified the comparison of the mean ΔS and $\%\Delta S_R$ values using ANOVA followed by Bonferroni test.

Hardness number presents a high correlation (0.91) with mineral content measured by microradiography in enamel caries lesion[16] and it has been extensively employed to evaluate mineral changes[16-18]. The integrated area above the curve of hardness profile (ΔS) is related to the lesion size[19] and %ΔSR represents the percentage of remineralization. Based on theses concepts, the analysis of cross-sectional microhardness data showed that irradiation with blue light altered the remineralization of enamel lesion in view of the fact that the percentage of remineralization of the irradiated enamel blocks was statistically lower ($p<0.01$) than the non-irradiated ones (fig. 1). This result provides evidence that the blue light irradiation, with the parameters used in this study, can inhibit enamel remineralization.

The analysis of the integrated area above the curve in the outer, middle and inner portions shows that preferential remineralization in the middle portion of RE samples was achieved; however, it was not observed in the samples from LED+RE group (fi. 2). In this region, only Re samples presented a mean ΔS value significantly different from DE group ($p<0.05$). In addition, the ΔS of the outer and inner portion of the lesions was similar in all groups ($p>0.05$), which indicates the absence of mineral deposition in these regions. This pattern of remineralization could be caused by the low fluoride concentration (0.05 μg/ml) added to the remineralizing solution which did not induce preferably mineral deposition close to the surface[20]. The surface porosity, characteristic of demineralized lesion, was maintained during pH cycling and it allowed mineral diffusion and deposition in the deeper regions of enamel lesion[21]. Further exposure of the samples to the remineralizing solution would probably promote an increase of mineral deposition in the superficial layers[22].

Besides the different remineralization behavior of irradiated samples observed in the present study, the inhibition of material diffusion out of the tooth was reported in samples irradiated with argon laser with similar parameters (1.38 W/cm^2, 270 mW, 13.5 J/cm^2, 10 s; 250 mW, 12 J/cm^2, 10 s).[9, 10] From these observations it can be assumed that the blue

light may alter the mineral exchange into and out of dental enamel. Thus, this treatment could inhibit progression of enamel lesions after new caries challenges. Nevertheless, further studies should be carried out to investigate this issue.

Since an effect could be observed, it is reasonable to expect that interaction between light and enamel occurred. Spitzer & Bosch[23] showed that this spectral region of emission is poorly absorbed by inorganic components of human and bovine dental enamel whereas the organic matrix absorbs below 300nm. However, the organic material was extracted from the enamel by an acid dissolution (0,01M HCl), which can cause alteration or fragmentation of the molecules, changing the absorption properties of the organic matrix.

Regardless the absorption properties, morphological changes in enamel after argon laser irradiation with similar parameters used in this study (231 mW, 11.5 J/cm^2, 10 s, 1.2 W/cm^2) were also reported.[24] The authors suggested that the irradiation could have altered or eliminated the organic material causing the morphological alterations observed. However, if this spectral region were able to eliminate enamel organic content, it should be expected that the LED irradiation would have increased the remineralization in the present study.[8] This fact suggests that the alteration of organic material is the most probable reason for the effects observed.

In this context, the mechanism supporting the results obtained in the present study could be the denaturation of organic components resulting from photochemical effect, since the temperature increase of enamel surface reached after irradiation with a similar LED device ($\lambda = \pm 460$ nm)[25] was much lower than the temperature reported for the denaturation of protein[26]. The energy absorbed by the organic molecule would cause changes in its structure leading to alterations in the organic-inorganic interaction.

4. CONCLUSION

In conclusion, the irradiation with blue light, under the conditions used in this study, inhibited enamel remineralization. The alterations in mineral uptake by irradiated enamel may be a result of photochemical interaction between blue light and organic matrix. Additionally to the results obtained in this study, the evidences of organic material influence on de-remineralization process seem to encourage further studies.

5. ACKNOWLEDGMENTS

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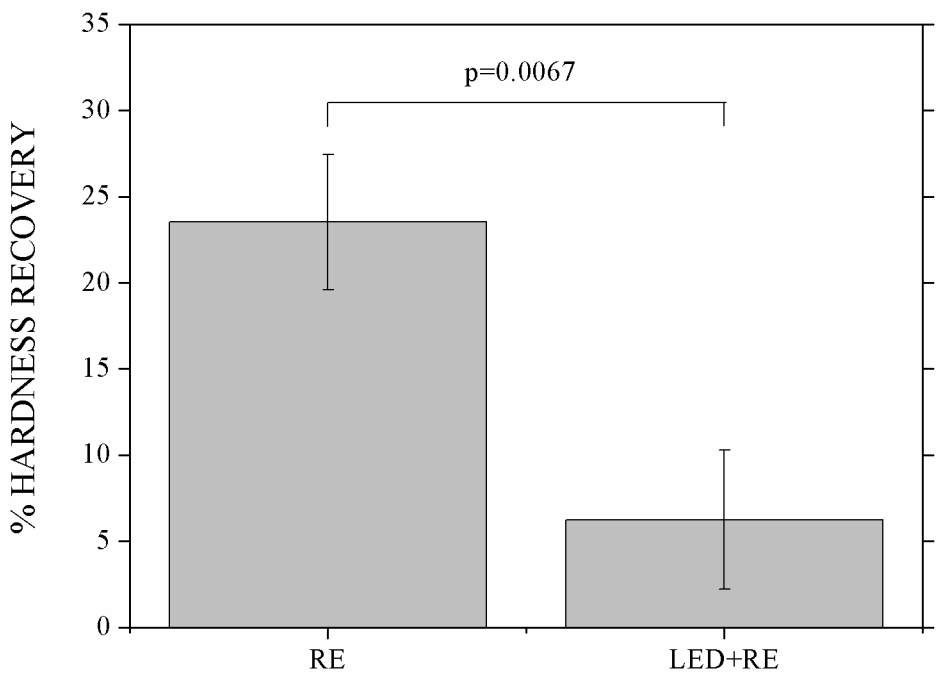

Fig. 1 - Percentage of hardness recovery (%ΔSR) of the samples from groups RE and LED+RE. Columns represent the mean %ΔSR values and bars are standard errors. The p-value was calculated by Bonferroni test.

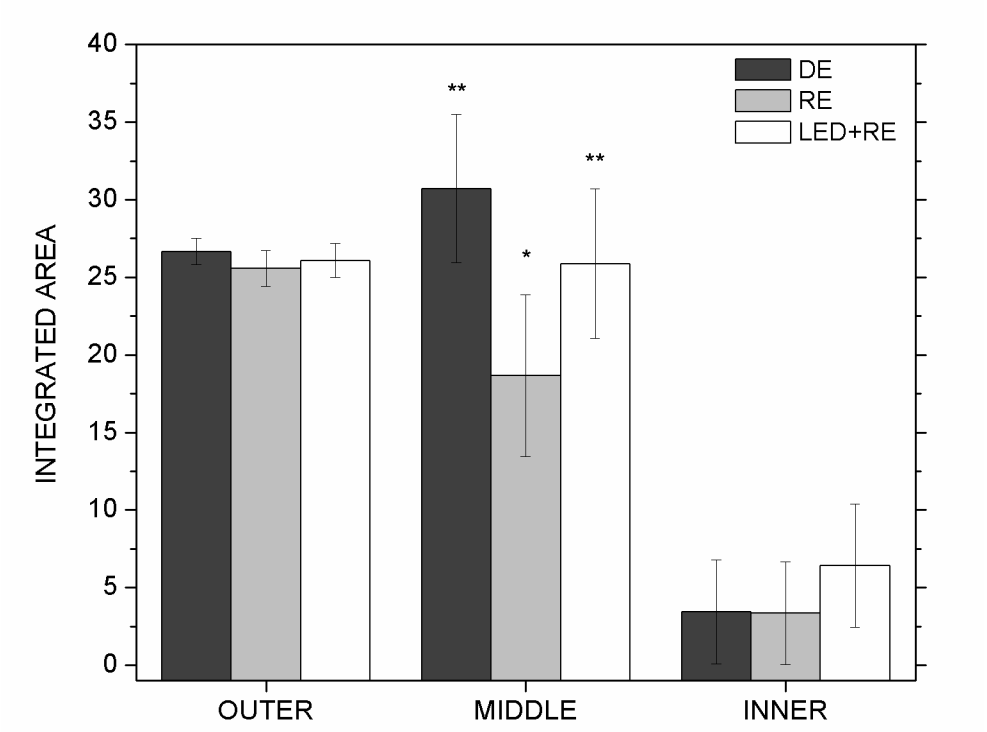

Figure 2 – Numerically integrated area above the curve of hardness profile (ΔS) in the outer (10-30 μm), middle (40-90 μm) and inner (100 μm to the end of the lesions) portions of lesions from the DE, RE and LED+RE groups. Columns represent the mean ΔS values and error bars represent standard deviation. Significant differences ($p<0.05$) between groups were observed only in the middle region (in the figure is represented by columns marked with different symbols).