Photobiomodulation, Photomedicine, and Laser Surgery
Volume 39, Number 6, 2021
© Mary Ann Liebert, Inc.
Pp. 381–385
DOI: 10.1089/photob.2020.4974

Effect of Er:YAG Laser and Association of Protocols on the Demineralized Enamel Microhardness

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Abstract

Objective: The aim of this study was to analyze the microhardness of demineralized enamel following different treatments (fluoride varnish, Er:YAG laser, and Er:YAG laser associated with fluoride varnish).
Methods: Forty-eight enamel blocks ($4 \times 4 \times 7$ mm) were divided into six groups ($n = 8$): (S) Sound; (DE) Demineralized; (DED) DE + Duraphat® 5% (fluoride varnish); (DEL20) DE + Er:YAG laser (20 mJ pulse mode; 0.20 W; 10 Hz; 60 sec; 1.18 J/cm^2; 11.83 W/cm^2); (DEL50) DE + Er:YAG laser (50 mJ pulse mode; 0.50 W; 10 Hz; 60 sec; 2.95 J/cm^2; 29.58 W/cm^2); (DEL20D) DE + Er:YAG laser (20 mJ) + Duraphat 5%. The irradiation was performed at 1 mm distance from the surface using a tip (AS7066X, L-14 mm, D-1.3 mm in diameter) in water/air spray refrigeration (level 6). The enamel blocks were submitted to pH cycling (4 h into DES solution +20 h into RE solution for 8 days and the solutions were changed every day). Knoop microhardness was measured (50 g/15 sec, six readings per sample) and data were analyzed by Kruskal–Wallis test at 5% significance.
Results: After treatments, DF group showed higher microhardness values than all the groups. Also, DEL20D group showed similar results with H group according to the microhardness analysis ($p < 0.05$).
Conclusions: It could be concluded that Duraphat 5% treatment showed better results when compared with all tested groups, however, the association of Er:YAG Laser 20 with Duraphat 5% also showed promising results.

Keywords: dental enamel, microhardness, phototherapy

Introduction

Dental caries is observed initially as a white spot lesion in enamel, which shows the initial demineralization process and loss of minerals from the enamel below the surface. This results in increase of subsurface porosities of ~25 vol.%.[1] When this volume exceeds 50%, cavitations occur in enamel and the reversion of the lesion is not possible anymore.[2] The treatments used to be a challenge due to the multifactorial severity and etiology[3] of the disease.

Some laser protocols were used in previous studies[4,5] for the prevention and treatment of enamel lesions, such as the Nd:YAG laser, which increased enamel resistance to demineralization.[4] Also, after irradiation with Nd:YAG laser (60 mJ pulse mode, 10 Hz, 84.9 J/cm^2) and topical fluoride application for 4 min, Nd:YAG laser irradiation changed the chemical composition of enamel regardless of fluoride concentration, and inhibited demineralization of enamel on primary teeth after 1 year.[4,5] High concentration of phosphates and carbonates that increased surface hardness and roughness were observed in the irradiated specimens,[6] however, the generated heating would be a problem when using this laser device.

In this way, other studies[2,7,8] were carried out to optimize surface preparation using another type of laser: the Er:YAG laser. These previous studies were performed on enamel surface and showed precise and uniform cuts, which could maintain its prismatic structure.[7] There was ablation of enamel and dentin with a minimum thermal effect when the frequencies of 2 and 5 Hz were used.[8] For energy between 25 and 365 mJ pulsed in enamel and dentin specimens (0.5–0.75 mm), there was minimal thermal effect on the enamel with energy density below 80 J/cm^2 and minimal thermal effect on dentin was noticed with density below 74 J/cm^2.

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Zhegova et al.[9] observed that the interaction of Er:YAG laser with enamel and dentin is influenced by tissue composition and that photoablation should be performed with low energy on the primary teeth, which has higher amount of water and less mineralization than the permanent teeth. However, using low-energy Er:YAG laser for remineralization of enamel white spot lesions is poor in the literature up to now.

Thus, the aim of this study was to evaluate the microhardness of the demineralized enamel according to different surface treatments (fluoride varnish, Er:YAG laser, and Er:YAG laser associated with fluoride varnish). The hypothesis of the study was that there would be no difference among the treatments on the enamel microhardness.

Materials and Methods

After the approval of the Human Research Ethics Committee of the Dental School, Nove de Julho University (under the Protocol number 2.514.150), twenty-four caries-free third molars were selected by the Biobank of Human Teeth of Dental School of Nove de Julho University. The teeth were stored in 0.1% thymol solution at 4°C during 7 days. The proximal surfaces (mesial and distal) were abraded with 400, 600, and 1200 silicon carbide papers (Buehler, São Paulo, Brazil), washed between each sandpaper exchange, polished with felt disc and diamond paste of 3, 1, and 0.5 μm (FOX Foco Tools, São Paulo, Brazil). The samples were also rinsed between each diamond paste exchange to remove residues from the previous abrasive. Enamel blocks (4×4×7 mm) were obtained by cutting the proximal surfaces of the teeth with double-sided diamond disc (7020; KG Sorensen, São Paulo, Brazil) and low-speed handpiece by water cooling. The thickness of the specimens was measured with a digital caliper (Mitutoyo, São Paulo, Brazil). The blocks were randomly distributed into six groups ($n = 8$): (1) S—sound (positive control); (2) D—Demineralized (negative control); (3) DF—Demineralized + Duraphat® 5% (Colgate-Palmolive Ind e Com LTDA, São Paulo, Brazil); (4) DL20—Demineralized +20 mJ Er:YAG laser (20 mJ pulse mode; 0.20 W; 10 Hz; 60 sec; 1.18 J/cm^2; 11.83 W/cm^2); (5) DL50—Demineralized +50 mJ Er:YAG laser (50 mJ pulse mode; 0.50 W; 10 Hz; 60 sec; 2.95 J/cm^2; 29.58 W/cm^2); and (6) DL20D—Demineralized + Laser 20 mJ + Duraphat 5% (Colgate-Palmolive Ind e Com LTDA). The enamel microhardness was evaluated on the sound and demineralized enamel surface and after the different proposed treatments for the enamel demineralization. In this manner, for comparison purposes, the sound and the demineralized enamel were considered as positive and negative control groups, respectively, as described in Table 1.

Enamel demineralization was artificially produced by pH cycling challenge for 8 days.[10] Except for the S group, the enamel blocks of the other groups were immersed in 5 mL demineralization solution (DE) during 4 h [DS; H_2O 2400 mL, $Ca(OH)_2$ 462 mg, CH_3COOH 8.94 mL, $C_2H_3NaO_2$ 9.30 g, H_3PO_4 0.39 mL; pH 3.5–4.0; pH = 3.5–4]; (Fórmula & Ação, São Paulo, Brazil), rinsed under current deionized water (30 sec), and immersed in 5 mL of remineralizing solution (RE) for 20 h [H_2O, HCL, KOH, $CaCl_2$, $(HOCH_2)_3CNH_2$; pH 7]; (Fórmula & Ação). The solutions were exchanged every day. During pH cycling, the samples were stored at 37°C. For the Duraphat 5% group, after the pH cycling, the varnish was applied on the whole surface of the enamel blocks (4 applications of 60 sec each, with intervals of 7 days among them).[10,11] The samples were stored in deionized water at 37°C and the varnish was totally removed from the enamel blocks surface to measure the microhardness. Two Er:YAG laser protocols were used: (A) 20 mJ pulse mode, 0.20 W, 10 Hz, 60 sec, 1.49 J/cm^2, 0.02 W/cm^2; and (B) 50 mJ pulse mode, 0.50 W, 10 Hz, 20 mJ, 60 sec 2.95 J/cm^2, 29.58 W/cm^2. The protocols were adjusted on the LiteTouch™ (Light Instruments Ltd., Yokneam, Israel) device by selecting the ''Gentle Treatment'' mode.[9] The samples were irradiated at 1 mm distance from their surfaces (2×30 sec each in perpendicular directions) using a cylindrical tip (AS7066X, L-14 mm, D-1.3 mm in diameter) in continuous cooling with water spray at level 6. To evaluate the association of Er:YAG laser with fluoride varnish, protocol A (20 mJ) was used followed by application of Duraphat 5% (Colgate-Palmolive Ind e Com LTDA) as described in Table 1.

The Knoop microhardness was measured (50 g for 15 sec) using a microhardness testing machine (HMV, Shimadzu, Japan). The indentations were initially performed in the center of each enamel block, then 5 additional equidistant readings were taken (in 100 μm each), totalizing 6 readings per specimen. Data were analyzed by Kruskal–Wallis with an overall significance level of 5%.

Results

According to the results obtained and as shown in Table 2, the demineralized enamel followed by fluoride application (DF; Duraphat 5%; Colgate-Palmolive Ind e Com LTDA) showed the highest microhardness value ($p < 0.05$; power of test = 1), whereas the lowest hardness value was observed to demineralized enamel (D) alone ($p < 0.0001$; power of test = 1). Also, the sound enamel (S) showed higher microhardness value when compared with the other groups ($p < 0.0001$; power of test = 1).

Following the pH cycling (DES-RE), the DF group showed higher microhardness value than DL20 and DL50 groups ($p < 0.0001$; power of test = 1). The DF group microhardness value was higher than the DL20F ($p < 0.01$; power of test = 1).

When comparing the groups irradiated with Er:YAG laser, a higher microhardness value to DL20 group was observed when compared with DL50 group ($p < 0.0001$; power of test = 1) and also, a higher microhardness value of DL20F group when compared with DL20 ($p < 0.0001$; power of test = 1) and DL50 groups ($p < 0.0001$; power of test = 1).

Regarding the treatments after the pH cycling (DES-RE), it was observed that the association between Er:YAG laser followed by Duraphat 5% application (DL20F) showed similar microhardness values to the sound group (S), which showed a promising treatment for white spot lesion condition as well.

Discussion

This study aimed at evaluating whether different treatments would influence the microhardness of the demineralized

Table 1. Experimental Groups and Procedures

Group	*Procedures*
(S) Sound	Prophylaxis with slurry of pumice and water Rinsing in distilled water (30 sec)
(D) Demineralized (DE-RE cycle)[a]	4 h DE solution, Rinsing in distilled water (30 sec) 20 h RE solution Rinsing in distilled water (30 sec) Total: 8 days. Change solutions every day
(DF) Duraphat™ (5% NaF, 2.26%F, pH 5.0)	DE-RE[a] cycling 60 sec application (1st application) Storage in distilled water at 37°C 2nd, 3rd, and 4th applications (1 min each) Total: 4 applications of 60 sec each, with 7-day interval among them
(DL20) Er:YAG laser (LiteTouch™)	Parameters: 2940 nm; 20 mJ pulse mode; 0.20 W; 10 Hz, 60 sec (2 × 30 sec each); 1.18 J/cm^2; 11.83 W/cm^2) 1 mm distance from the surface Spot size 1.3 mm in diameter (tip AS7066X, L-14 mm, D-1.3 mm in diameter Water/air spray refrigeration (level 6)
(DL50) Er:YAG laser (LiteTouch)	Parameters: 2940 nm; 50 mJ pulse mode; 0.50 W; 10 Hz, 60 sec (2 × 30 sec each); 2.95 J/cm^2; 29.58 W/cm^2) 1 mm distance from the surface Spot size 1.3 mm in diameter (tip AS7066X, L-14 mm, D-1.3 mm in diameter Water/air spray refrigeration (level 6)
(DL20F) Er:YAG laser (LiteTouch) + Duraphat 5%	Parameters: 2940 nm; 20 mJ pulse mode; 0.20 W; 10 Hz, 60 sec (2 × 30 sec each); 1.18 J/cm^2; 11.83 W/cm^2) 1 mm distance from the surface Spot size 1.3 mm in diameter Water/air spray refrigeration (level 6) 60 sec Duraphat application, Storage in distilled water at 37°C 2nd, 3rd, and 4th applications (1 min each) Total: 4 applications of 60 sec each, with 7-day interval among them

[a] DE-RE cycling according to Queiroz et al.[17]

enamel after pH cycling challenge. A high microhardness value was observed for sound enamel (positive control) and a low microhardness value was shown to the demineralized enamel (negative control). These results were expected for control groups as the best (positive control) and worst (negative control), and then comparisons were possible to the treated groups (Duraphat varnish, Er:YAG laser in two different protocols and association Er:YAG laser with Duraphat varnish). As the hypothesis of the study was that there would be no difference of the different treatments on the enamel microhardness, it could be partially rejected.

The demineralized enamel treated with Duraphat 5% alone showed higher microhardness value than the sound enamel. Considering that fluoride varnish prolongs the contact between fluoride and enamel, we suppose that the fluoride was released on the enamel surface for a long time and then remineralized the enamel lesions. Systematic review studies have recommended the application of Duraphat 5% varnish twice a year to prevent demineralization of enamel in high-risk caries patients, but the authors emphasize the use of complementary approaches (dietary and oral habits changes) to prevent enamel demineralization[11] as well. In the present study, Duraphat 5% was applied more than twice (one application a week during 4 weeks, in a total of four applications), which improved the enamel microhardness.

Given the multifactorial etiology of the caries disease, it is important to study different treatment proposals for white spot lesions, reversing the condition prior cavitation and thus, to work with minimal intervention as often as possible. In this way, our study investigated the aforementioned different protocols. Thus, other treatments were used after the pH cycling and the association between Er:YAG laser under the DL20 protocol followed by Duraphat 5%, remineralized

Table 2. Mean (SD) of Knoop Microhardness of the Sound and Demineralized Enamel as Well as the Treatments Carried Out After Enamel Demineralization

Groups	*Means (SD)*
Sound (S)	333 (38.3)[a]
Demineralized (D)	174.5 (17.9)[b]
Demineralized + Duraphat (DF)	367.3 (35.9)[c]
Demineralized + Er:YAG Laser 20 (DL20)	240.9 (24.5)[d]
Demineralized + Er:YAG Laser 50 (DL50)	151.8 (29.1)[b]
DL20+Duraphat (DL20F)	321.1 (28.6)[a]

Superscript lower-case letters indicate the statistically significant differences. ($p < 0.0001$)

SD, standard deviation.

the enamel previously demineralized showing microhardness values similar to the sound enamel. It could be speculated that the DL20 erbium laser prepared the enamel surface by removing the demineralized tissue and then, the remaining enamel would have been remineralized by the fluoride varnish.

A previous study[12] showed that enamel resistance to acid dissolution was observed after irradiation with Er:YAG laser in 100 mJ (12.7 J/cm^2), 200 mJ (25.5 J/cm^2), and 300 mJ (38.2 J/cm^2) because there was a decrease in the percentage of carbon and an increase in the percentages of oxygen, phosphorus, and calcium. The increase in the Ca/P ratio after irradiation was associated with the use of high energy density.[12] In our work, we could observe the increase of microhardness of the enamel using only 20 mJ of Er:YAG followed by the application of Duraphat 5%, which demonstrates a promising result.

Liu et al.,[13] observed few demineralization in enamel after cariogenic challenge and irradiation with Er:YAG laser (2.94 μm; 100 μsec; 2 Hz) at low energy (subablative effect: 5.1 J/cm^2 reduction of 45% and 2.0 J/cm^2 reduction of 25%). The mineral composition of enamel was quantified using a microcomputed tomography. Thus, the authors concluded that subablative low-energy Er:YAG laser irradiation following fluoride treatment may instantaneously transform enamel hydroxyapatite into fluoridated hydroxyapatite to reduce enamel solubility as a preventive treatment for enamel demineralization. The results of our work corroborate with the aforementioned study as the use of fluoride varnish increased the enamel microhardness after pH cycling (DES-RE) probably due to the longer contact time of fluoride with enamel.

Zhegova et al.[9] described the removal of enamel and dentin caries in primary teeth by the Er:YAG laser (300 mJ in enamel, 200 mJ in dentin, 20 Hz, 1.3 mm diameter of the sapphire tip, spray air/water ratio 8 (39 mL/min), 50 μsec pulse, 15.08 J/cm^2 for dentin and 22.61 J/cm^2 for enamel). The authors concluded that the interaction of Er:YAG laser with enamel and dentin was influenced by tissue composition and photoablation should be performed with low energy in the primary teeth because of its higher amount of water and less mineralization than the permanent teeth.

Our study used two protocols: DL20 and DL50 (Table 1). In both protocols, the energy was <100 mJ and the other parameters were all lower than the parameters used by Zhegova et al.[9] However, in pulsed mode, they used microseconds against 60 sec (2 × 30 sec) that was used in ours. It could be suggested that the early enamel lesions simulated by the pH cycling using fresh extracted third molars might be likened to the demineralized enamel lesions of a primary tooth and then justify the higher microhardness value of DL20 group when compared with the DL50 group.

The use of the Er:YAG laser equipment and the parameters described in our study were decisive to simulate different minimally invasive irradiation protocols, since the equipment offers many possible adjustments. Two protocols were tested in the most subtle mode, called ‘‘Gentle treatment,’’ which operates irradiations of 100 mJ or less, not found in the literature up to now. Considering the enamel fragility in enamel white spot lesions, our purpose was to deliver the lower energy as possible to treat the structure.

The Er:YAG LiteTouch equipment (LiteTouch; Light Instruments) used was designed for minimally invasive interventions and applicability in the treatment of caries lesions with minimal tissue ablation. It is classified as type IV laser and operates with Er:YAG laser at 2940 nm, pulsed energy up to 700 mJ, adjustable power up to 8.4 W, frequency between 10 and 50 Hz, and tip size between 0.2 and 1.3 mm. The equipment offers versatility in the adjustment of the parameters and optimizes the applicability for the treatment in different clinical situations. In the present study, it allowed to simulate different treatments for enamel demineralized lesions.

Within the limitations of this study, it could be concluded that Duraphat 5% treatment (*gold standard*) showed the better results, however, Er:YAG Laser 20 associated with Duraphat 5%, respectively, also showed promising results to improve the enamel microhardness. Further studies should be continued to assess Er:YAG laser protocols associated with fluoride varnish or infiltrating materials in clinical scenarios of white spot lesions.

Author Contributions

All authors have contributed significantly, and they are all in agreement with the article.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee (Human Research Ethics Committee of the Dental School, Nove de Julho University, protocol number 2.514.150) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.’’

Author Disclosure Statement

No competing financial interests exist.

Funding Information

The authors declare that there is no funding.

References

1. Crabb HS, Mortimer KV. Dental caries and enamel structure. Nature 1966;209:611–612.
2. ten Cate JM, Featherstone JD. Mechanistic aspects of the interactions between fluoride and dental enamel. Crit Rev Oral Biol Med 1991;2:283–296.
3. Restrepo M, Jeremias F, Santos-Pinto L, Cordeiro RCL, Zuanon ACC. Effect of Fluoride varnish on enamel remineralization in anterior teeth with molar incisor hypomineralization. J Clin Pediatr Dent 2016;40:207–210.
4. Raucci-Neto W, Spinola LM, Lepri CP, Faraoni-Romano JJ, Silva JMG, Palma-Dibb RG. Nd:YAG laser in occlusal caries prevention of primary teeth: a randomized clinical trial. Lasers Med Sci 2015;30:761–768.
5. Neto WR, Lepri CP, Romano JJF, et al. Chemical and morphological changes of primary teeth irradiated with Nd:YAG laser: an ex vivo long-term analysis. Photomed Laser Surg 2015;33:266–273.

6. Zezell DM, Boari HGD, Ana PA, Eduardo CP, Powell GL. Nd:YAG laser in caries prevention: a clinical trial. Lasers Surg Med 2009;41:31–35.
7. Belal MH, Yassin A. A comparative evaluation of CO_2 and erbium-doped yttrium aluminium garnet laser therapy in the management of dentin hypersensitivity and assessment of mineral content. J. Periodontal Implant Sci 2014;44: 227–234.
8. Zhao-zhang L, Code JE, Van de Merwe WP. Er:YAG laser ablation of enamel and dentin of human teeth: determination of ablation rates at various fluences and pulse repetition rates. Lasers Surg Med 1992;12:625–630.
9. Zhegova G, Rashkova M, Rocca JP. Minimally invasive treatment of dental caries in primary teeth using an Er:YAG laser. Laser Ther 2015;23:249–254.
10. Queiroz CS, Hara AT, Paes Leme AF, Cury AJ. pH-Cycling models to evaluate the effect of low fluoride dentifrice on enamel De and Remineralization. Braz Dent J 2008;19:21–27.
11. Azarpazhooh A, Main PA. Fluoride varnish in prevention of dental caries in children and adolescents: a systematic review. JCDA 2008;74:73–79.
12. Díaz-Monroy JM, Contreras-Bulnes R, Olea-Mejía OF, et al. Chemical changes associated with increased acid resistance of Er:YAG laser irradiated enamel. Sci World J 2014;2014:6.
13. Liu Y, Hsu CYS, Teo CMJ, Teoh SH. Potential mechanism for the laser-fluoride effect on enamel demineralization. J Dent Res 2013;92:71–75.

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Received: October 28, 2020.
Accepted after revision: February 12, 2021.
Published online: May 21, 2021.