EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 70, 889:897, JANUARY, 2024
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 21-11-2023 • Accept Date : 31-12-2023 • Available online: 10-01-2024 • DOI : 10.21608/EDJ.2023.249483.2786

REMINERALIZATION OF ENAMEL WHITE SPOT LESION USING ERBIUM CHROMIUM LASER, FLUORIDE VARNISH, OR THE COMBINATION OF BOTH VERSUS SOUND ENAMEL: IN VITRO STUDY

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ABSTRACT

Objectives: This study aimed to evaluate the effect of Er, Cr: YSGG LASER, fluoride varnish, and their combination on treating white spot lesions before and after pH cycling.

Methods: A total number of fifteen freshly extracted intact human molars were used. The teeth crowns were sectioned buccolingual and mesiodistal to form four quadrants resulting in 60 specimens. They were divided into six groups of ten. I1: positive control (sound enamel), I2: negative control (demineralized enamel), I3: Er, Cr: YSGG LASER, I4: fluoride varnish, I5: Er, Cr: YSGG LASER followed by fluoride varnish, I6: fluoride varnish and then Er, Cr: YSGG LASER. Enamel microhardness (SMH) was measured for all these six groups. All groups were subjected to a pH cycling model for three days except the I1 group. Enamel SMH was measured again after pH cycling.

Results: The mean SMH values before pH cycling showed no statistically significant difference between all the tested groups. After pH cycling, there was a statistically significant difference in the mean SMH values p=0.001, where I2 showed the lowest statistically significantly mean SMH values and I5 showed the highest. When comparing each tested group separately before and after pH cycling the mean SMH values statistically significantly increased only in I3 and I5 groups where p= 0.027 and 0.047 respectively.

Conclusion: The use of Er, Cr: YSGG LASER with the tested specifications followed by fluoride application is an effective method for enhancing remineralization. pH cycling is necessary to examine the effect of the different remineralization protocols.

KEYWORDS: Er Cr: YSGG LASER, surface microhardness, fluoride, remineralization, pH cycling

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INTRODUCTION

The earliest sign of dental caries is demineralization or the development of white spot lesions. [1] These lesions appear chalky white as a result of mineral loss and the microporosities that occured in the enamel. Several studies have shown that these white spot lesions can be arrested, rehardened, and remineralized with fluoride, casein phosphopeptide-amorphous calcium phosphate [CPP-ACP], sodium calcium phosphosilicate, nano hydroxyapatite, arginine bicarbonate calcium carbonate complex, or trimetacalcium phosphate. [1, 2, 3]

In the last few years, LASER irradiation seemed to be very promising through its positive effects on increasing enamel acid resistance. [4] Water and hydroxyapatite in the tooth tissues notably absorb high-power LASERs like CO_2 and Erbium LASER. These LASERs change the enamel microcrystalline structure, thus increasing its resistance to demineralization, decreasing its permeability, and acid solubility.[1] The impact of fluoride on reducing enamel demineralization or enhancing remineralization can be demonstrated clearly in vitro using pH cycling models that mimic clinical situations.[5]

That's why this study was carried out to evaluate the effect of fluoride varnish, Er: chromium LASER, and their combination on the remineralization of enamel white spot lesions before and after pH cycling.

MATERIALS AND METHODS

The material used:

i- Sodium fluoride clear varnish 5%, Dura Shield SULTAN® health care (USA).

ii- Erbium, Chromium: Yttrium-Scandium-Gallium-Garnet Waterlase® (Er, Cr: YSGG waterlase) Millennium™ Biolase Technologies, USA.

iii- Demineralizing solution:[6] NaH_2PO_4 (2.2 mM), Lactic acid (0.05 M), $CaCl_2$ (2.2 mM), Fluoride (0. 2 ppm) and 50% NaOH was used as an acidic buffer to lower the solution's pH to 4.5.

iv- Remineralizing solution (Artificial saliva): [6] KCl (17.98 mM), $NaCl_2$ (4.29 mM), Na_3PO_4 (3.90 mM), $NaHCO_3$ (3.27 mM), $CaCl_2$ (1.10 mM), H_2SO_4 (0.50 mM), $MgCl_2$ (0.08 mM), distilled water and the pH was adjusted to be 7.2.

Selection of Teeth:

Fifteen freshly extracted intact human third mandibular molars were collected from patients of 20 to 40 years old. The selected teeth were free from caries, defects, or restorations. They were cleaned using an ultrasonic scaler (Coxo, China) to remove any debris of calculus, plaque, or any attached periodontal tissue. They were kept in a 10% formalin solution for one week.[7] They were then stored in a physiological saline solution to be used within one month after extraction.

Specimen preparation:

Each tooth was immersed vertically such that its roots were embedded inside a plastic mold filled with a recently mixed chemically activated acrylic resin (Acrostone, Egypt). The crown of each tooth was then sectioned mesiodistally and buccolingual to form four equal quadrants. These sectioned quadrants were then separated from the roots at the CEJ. Then each quadrant was immersed into another acrylic resin dough leaving the buccal or lingual surface exposed. After curing the acrylic resin, a vertical and a horizontal line were drawn on the cured acrylic block bisecting the exposed tooth surface into four equal quadrants. The point of intersection between these two lines was considered a reference point. **Figure (1)** shows the vertical and horizontal lines that are drawn to bisect each acrylic block. The width of each block was measured at this reference point using a caliper (Healthco, Germany).

Each block was given a number, and written on the acrylic block. A diamond disc (Edenta Golden, Switzerland) mounted in a straight handpiece was used to remove 0.5 mm from each tooth specimen in order to obtain a flat enamel surface. Then the width of each block was remeasured at the same reference point to make sure that only 0.5 mm was reduced from the specimen surface.

Grouping of the Specimens:

Sixty (15 X4) specimens were obtained and divided into six groups of ten. I1=Sound enamel (positive control), I2= demineralized enamel (negative control), I3= Er, Cr: YSGG LASER, I4= fluoride varnish, I5= Er, Cr: YSGG LASER then fluoride varnish, I6= fluoride varnish then Er, Cr: YSGG LASER. After measuring the SMH of all specimens, a pH cycling model was employed to all groups except the positive control group (I1) .

Artificial caries formation:

A freshly prepared demineralizing solution (50 ml) previously mentioned was used to create artificial caries lesion. Before the application of any material all specimens except the positive control group (I1) were immersed in this solution and left in an incubator for 72 hours at 37°C.

Fluoride varnish application:

A thin layer of fluoridated varnish Dura Shield 5% sodium fluoride clear varnish (Dura Shield, Sultan H C, USA) was applied with a micro brush to the dried enamel surface and left for 4 minutes. This fluoride varnish was applied alone in (I4) group, after LASER application in (I5) group, and before LASER application in (I6) group.

LASER application:

Er, Cr: YSGG LASER of photon wavelength 2.78 µm and pulse set at 140 micro seconds was used, The parameters were adjusted to emit a power output of 0.75Watt, a frequency of 20 Hz, and 8.5 J/ cm^2 of energy for one minute. The handpiece's beam diameter at the focal point was 800 µm and positioned perpendicular to the enamel surface at one mm away from it. A custom-made plastic assembly was used to fix the handpiece in its position while being used as shown in Figure (2). LASER was applied alone in group I3, before fluoride varnish in group I5, and after fluoride varnish in group I6.

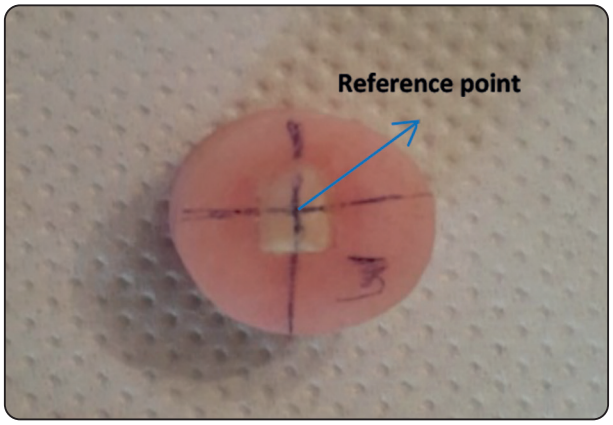

Fig. (1) Vertical and horizontal lines were drawn bisecting each acrylic block

Fig. (2) Custom made plastic assembly used to fix the LASER hand piece

Micro-hardness Testing:

Each specimen was leveled on a stage such that the prepared tooth specimen would be placed exactly parallel to the stage plane. Vicker's micro-hardness testing machine (Wilson digital microhardness tester, China) was used. The SMH of the positive control group was measured before any demineralization was carried out. A digital optical microscope was used to adjust the SMH indent on the flattest area of the prepared enamel. A 50-gram load was applied for 5 seconds. The length and depth of the rhomboid indent were digitally measured and the micro-hardness value was automatically calculated. Three indentations were made and measured for each specimen and their average values were considered the SMH value of each specimen. SMH test was carried out on all specimens before and after pH cycling except the positive control group (I1).

Demineralization/remineralization cycle regimen

After measuring the SMH of all specimens, they were exposed to a pH cycling model for five days.[7] Specimens were placed in 50 ml of demineralized solution for three hours. The specimens were then washed for 10 seconds with deionized water. This was followed by the placement of the specimens in a remineralizing solution for 21 hours.

Statistical Analysis:

Data were calculated as mean and standard deviation (SD). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine whether the data were normal or not. Mean SMH values showed a parametric distribution so One Way ANOVA was used to study to compare between the different tested groups. Tukey's post hoc test was used for pairwise comparison when ANOVA was significant. The independent t-test was used to detect the effect of pH cycling for the different treatment models. The level of significance has been set at $P \leq 0.05$. Statistical analysis was carried out using IBM SPSS Statistics Version 24 for Windows (SPSS Inc., IBM Corporation, NY, USA).

RESULTS

Statistical analysis of the mean SMH values of the different treatment models before and after pH cycling were shown in Table (1) and Figure (3). The mean SMH values before pH cycling showed no statistically significant difference between all the tested groups. After pH cycling, there was a statistically significant difference in the mean SMH values p=0.001, where I2 showed the lowest statistically significantly mean SMH values and I5 showed the highest. When comparing each tested group separately before and after pH cycling

TABLE (1) Mean SMH value of the different treatment models before and after pH cycling:

Treatments	Group name	Mean SMH values (VHN) and SD before pH cycling	Mean SMH values (VHN) and SD after pH cycling	p-value
Control	I1	108±24	117± 16 ^{ab}	0.494 NS
Demineralized	I2	91±6	97±10 ^{b}	0.362 NS
LASER	I3	90±12	108±7 ^{ab}	0.027*
Fluoride	I4	109±13	101±9 ^{ab}	0.332 NS
LASER + fluoride	I5	113±11	144±23 ^{a}	0.043*
Fluoride +LASER	I6	113±15	115.7±4 ^{ab}	0.837 NS
p-value		0.077	0.001*	

the mean SMH values statistically significantly increased only in I3 and I5 groups where p= 0.027 and 0.047 respectively.

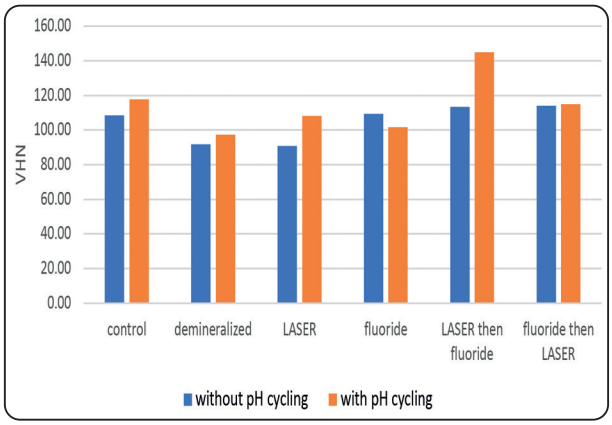

Fig. (3): Bar chart representing the mean Micro-hardness values (VHN) of the different treatment models before and after pH cycling

DISCUSSION

Surface microhardness (SMH) offers a simple, non-destructive, and a quick mean of assessing demineralization and remineralization.[8] It is also suitable for structures like enamel that are non-homogenous, with fine microstructure and brittle.[7] It is well known that when the pH decreases to the critical pH 5.5, hydroxy apatite dissolves while fluorapatite starts to dissolve at pH 4.5. That's why when fluorapatite is precipitated at the enamel surface it causes a decrease in demineralization.[9] The mineral redeposited differs from the lost one because hydroxyapatite dissolves from the enamel's subsurface, whereas fluorapatite is deposited on the surface layer. This mineral gain as fluorapatite has been interpreted as a decrease in demineralization rather than as remineralization process. Researchers consider that the most important action of fluoride is reducing enamel demineralization,[10] whilst others believe that fluoride accelerates the remineralization process, which is the primary mechanism of caries control.[11]

Several studies found that the optimal NaF concentration to saturate the binding sites is 2.5% to provide maximal protection against demineralization.[12] That explains why 5% NaF concentration was used in the present study. This concentration of 50,000 ppm NaF (22,600 ppm fluoride) in a resin varnish is required because saliva washes the varnish and dissolves the fluoride salts. These fluoride salts become deposited in the fluoride reservoirs found in the dental plaque, and soft tissues of the mouth. Subsequently, when pH decreases these reservoirs re-release these fluoride ions again to reach the teeth demineralized sites.[12]

The influence of fluoride on enamel demineralization or remineralization can be clearly demonstrated in vitro utilizing pH cycling models that reflect clinical situations of recurrent demineralization or remineralization.[2],[13] pH cycling models entail subjecting the dental surfaces to demineralization and remineralization cycles in order to simulate the dynamics of mineral loss and gain that occur during caries formation. This information aided in the comprehension of the caries process and the potential processes via which fluoride prevents caries.[14]

In the present study, there was no statistically significant difference in the mean SMH values between all groups before pH cycling, but after pH cycling I5 group showed the statistically significant highest mean SMH values when compared to the other groups and I2 group which showed the statistically significant lowest mean SMH values. These results are in agreement with several studies.[2, 15, 16]

This agrees with Alsharief et al., 2023[17] who examined untreated enamel after pH cycling using polarized microscope and found loss of typical subsurface enamel structure as well as a high level of positive birefringence. Several studies confirmed these results by scanning electron microscope analysis which showed uneven enamel surface

pitting, selective removal of the enamel rod core, and selective removal of the rod perimeter while the rod core remained mostly intact.[18, 19] All these lead to destruction of enamel prismatic pattern with the development of initial enamel caries (white spot lesion).[20],[21]

There was no statistically significant difference between I1, I3, I4, I6 groups after pH cycling. This agrees with many studies who showed that enamel treated with fluoride only; showed globular precipitates all over the surface of the enamel, but also few porosities and defects were observed by polarized microscope images. [22]

They also found that the surface treated with LASER alone exhibited uneven enamel crystals with numerous porosities and irregular topography, as well as minimal amorphous surface precipitation. [22] Apel et al., 2005[22] stated that fine enamel cracks that occurs after LASER treatment act as a starting point for acid attachment, which may cause deep demineralization and reduce the positive effect of enamel caries prevention.

That is why some studies[22], [23] explained that LASER absorbs water from the hydroxyapatite of dental tissues and decrease acid solubility, but are not recommended to be used alone for caries prevention. Karandish in 2014, found that when the lased enamel was challenged with acid, the following features were found; crater-like holes 1–20 μm in diameter that exhibit positive birefringence and reverse birefringence.[23] These features increased the roughness of the lased enamel facilitating fluoride precipitation and adhesion.[24]

The selected LASER type must be sufficiently absorbed and transformed into heat without endangering the soft or hard tissues of the mouth. As a result, certain wavelengths were chosen to be highly absorbed by hydroxyapatite and water in the dental hard tissues. Ana et al., 2012[2] found that Er, Cr: YSGG LASER of 8.5 J/cm^2 fluency was able to reduce the loss of hardness when compared to the demineralized untreated group, this confirms that higher temperatures are necessary to change the chemical properties of enamel. This agrees with the present study results where I2 group showed a higher statistically significant mean SMH values after pH cycling. This fluency was selected in the present study which agrees with many other studies [25], [26], [27] who recommended this LASER fluency. On the other hand, Santos et al., 2014[28] found that Er, Cr: YSGG LASER of 8.9 J/cm^2 fluency promoted morphological alterations on the demineralized enamel surface, which precluded the preventive action of fluoride varnish and allowed the penetration of acid through the enamel subsurface.

Several studies agree that LASER therapy combined with topical fluorides were found to be more effective than LASER therapy alone.[1], [29],[30], [31] In group I6 fluoride was applied followed by the application of LASER. Bahrololoomi et al., 2015 [29] found microstructural changes in irradiated dental hard tissue when LASER was applied after the application of remineralizing agents. These microstructural changes include re-crystallization and melting of areas exposed to the emitted LASER light. They suggested that these changes would facilitate absorption of the remineralizing agent into the demineralized sites in the tooth structure. Nair et al., 2016 [32] found that pre-treatment with topical fluoride before ER:YAG LASER had higher remineralization effect than without LASER. De Sant'Anna et al., 2009[33] proved that the application of a photo-absorbing material to teeth prior to LASER therapy increased remineralization outcomes. Several studies used LASER irradiation after fluoride application.[34, 35, 36] In the present study, I6 group showed a non-statistically significant difference in the mean SMH values after pH cycling when compared with the other groups which is in agreement with Moslemi et al., 2009. [26] Ana et al., 2012[2] found that when the enamel surface was pretreated by LASER irradiation at 8.5 J/cm2 followed by fluoride application it resulted in the

formation of an adherent CaF_2 like material which is more resistant to acid attack. The literature presents conflicting results on whether LASER should be applied before or after the fluoride varnish. Most studies who applied the remineralizing agent before LASER application did not compare it by the opposite application.

According to several studies,[37, 38, 39] application of LASER before fluoride application is done to benefit from the thermal action of LASER which change the enamel microstructure to better retain fluoride and form a structure that is more resistant to acid attack. Nammour et al., 2003[37] showed that LASER irradiation created enamel micro spaces increasing its microroughness and improving fluoride diffusion through the enamel, leading to fluoride reservoirs. Zamataro et al., 2013 and many other studies [2], [16], [15] proved that the morphology of irradiated enamel is altered by Er, Cr: YSGG LASER radiation, leading to an increase in the hydroxyapatite rods' exposition and roughness. This agrees with our study where I5 group showed the highest statistically significant mean SMH values after pH cycling. This indicates that LASER created a very retentive niches for fluoride that resisted dissolution during cariogenic challenge.

CONCLUSION

The following conclusions were made within the scope of this study:

1. NaF varnish (5%) is one of the remineralizing agents that can arrest non cavitated enamel white spot lesions in the presence of cariogenic challenge.
2. The use of Er, Cr: YSGG LASER with the tested specifications followed by fluoride application is an effective method for enhancing remineralization.
3. pH cycling is mandatory to examine the effect of the different remineralization protocols.

RECOMMENDATION

1. Further studies should evaluate the effect of combining the different remineralizing agents and LASER irradiation.
2. A clinical investigation is still required to demonstrate the long-term effect of LASER on demineralization.

REFERENCES

1. Heravi F, Ahrari F, Mahdavi M, Basafa S (2014) Comparative evaluation of the effect of Er:YAG laser and low level laser irradiation combined with CPP-ACPF cream on treatment of enamel caries. Journal of Clinical and Experimental Dentistry 6:e121. https://doi.org/10.4317/JCED.51309
2. Ana PA, Tabchoury CPM, Cury JA, Zezell DM (2012) Effect of Er,Cr:YSGG laser and professional fluoride application on enamel demineralization and on fluoride retention. Caries research 46:441–451. https://doi.org/10.1159/000333603
3. Kalra D, Kalra R, Kini P, Allama Prabhu C (2014) Non-fluoride remineralization: An evidence-based review of contemporary technologies. Journal of Dental and Allied Sciences 3:24. https://doi.org/10.4103/2277-4696.156525
4. Liu Y, Hsu CYS, Teo CMJ, Teoh SH (2013) Potential mechanism for the laser-fluoride effect on enamel demineralization. Journal of dental research 92:71–75. https://doi.org/10.1177/0022034512466412
5. Roberto AR, Jassé FF, Boaventura JMC, et al (2011) Evaluation of tooth color after bleachingwith and without light-activation. Revista Odonto Ciencia 26:247–252. https://doi.org/10.1590/S1980-65232011000300010
6. Lata S, Varghese N, Varughese J (2010) Remineralization potential of fluoride and amorphous calcium phosphate-casein phospho peptide on enamel lesions: An in vitro comparative evaluation. Journal of conservative dentistry : JCD 13:42. https://doi.org/10.4103/0972-0707.62634
7. Lee JJ, Mettey-Marbell A, Cook A, et al (2007) Using extracted teeth for research: the effect of storage medium and sterilization on dentin bond strengths. Journal of the American Dental Association (1939) 138:1599–1603. https://doi.org/10.14219/JADA.ARCHIVE.2007.0110
8. Karlinsey RL, Mackey AC, Walker TJ, et al (2011) In vitro remineralization of human and bovine white-spot enamel lesions by NaF dentifrices: A pilot study. Journal of dentistry and oral hygiene 3:22–29

9. Cury JA, Tenuta LMA (2009) Enamel remineralization: controlling the caries disease or treating early caries lesions? Brazilian oral research 23 Suppl 1:23–30. https://doi.org/10.1590/S1806-83242009000500005
10. Ten Cate JM (1999) Current concepts on the theories of the mechanism of action of fluoride. Acta odontologica Scandinavica 57:325–329. https://doi.org/10.1080/000163599428562
11. Featherstone JDB (2008) Dental caries: a dynamic disease process. Australian dental journal 53:286–291. https://doi.org/10.1111/J.1834-7819.2008.00064.X
12. Carey CM (2014) Focus on fluorides: update on the use of fluoride for the prevention of dental caries. The journal of evidence-based dental practice 14 Suppl:95–102. https://doi.org/10.1016/J.JEBDP.2014.02.004
13. Delbem ACB, Bergamaschi M, Sassaki KT, Cunha RF (2006) Effect of fluoridated varnish and silver diamine fluoride solution on enamel demineralization: pH-cycling study. Journal of applied oral science : revista FOB 14:88–92. https://doi.org/10.1590/S1678-77572006000200005
14. Queiroz CS, Hara AT, Paes Leme AF, Cury JA (2008) pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization. Brazilian dental journal 19:21–27. https://doi.org/10.1590/S0103-64402008000100004
15. Malik A, Parmar G, Bansal P, et al (2015) Effect of laser and fluoride application for prevention of dental caries: A polarized microscope analysis. Journal of Dental Lasers 9:11. https://doi.org/10.4103/0976-2868.157590
16. Zamataro CB, Ana PA, Benetti C, Zezell DM (2013) Influence of Er,Cr:YSGG laser on CaF_2 -like products formation because of professional acidulated fluoride or to domestic dentifrice application. Microscopy research and technique 76:704–713. https://doi.org/10.1002/JEMT.22221
17. Alsherif AA, Farag MA, Helal MB (2023) Efficacy of Nano Silver Fluoride and/or Diode Laser In Enhancing Enamel Anticariogenicity around orthodontic brackets. BDJ open 9:. https://doi.org/10.1038/S41405-023-00151-X
18. Salvati E, Besnard C, Harper RA, et al (2020) Finite Element Modelling and Experimental Validation of the Enamel Demineralisation Process at the Rod Level. Journal of advanced research 29:167–177. https://doi.org/10.1016/J.JARE.2020.08.018
19. Silversrone LM, Saxton CA, Dogon IL, Fejerskov O (1975) Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries research 9:373–387. https://doi.org/10.1159/000260179
20. Melrose CA, Appleton J, Lovius BB (1996) A scanning electron microscopic study of early enamel caries formed in vivo beneath orthodontic bands. British journal of orthodontics 23:43–47. https://doi.org/10.1179/BJO.23.1.43
21. Younis SH, Obeid RF, Ammar MM (2020) Subsurface enamel remineralization by Lyophilized Moringa leaf extract loaded varnish. Heliyon 6:. https://doi.org/10.1016/J.HELIYON.2020.E05054
22. Apel C, Meister J, Götz H, et al (2005) Structural changes in human dental enamel after subablative erbium laser irradiation and its potential use for caries prevention. Caries research 39:65–70. https://doi.org/10.1159/000081659
23. Karandish M (2014) The efficiency of laser application on the enamel surface: a systematic review. Journal of lasers in medical sciences 5:108–14
24. Chin-Ying SH, Xiaoli G, Jisheng P, Wefel JS (2004) Effects of CO2 laser on fluoride uptake in enamel. Journal of Dentistry 32:161–167. https://doi.org/10.1016/j.jdent.2003.10.004
25. Moslemi M, Fekrazad R, Tadayon N, et al (2009) Effects of ER,Cr:YSGG laser irradiation and fluoride treatment on acid resistance of the enamel - PubMed. Journal of Paediatric Dentistry 31:409–413
26. Anaraki SN, Serajzadeh M, Fekrazad R (2012) Effects of laser-assisted fluoride therapy with a CO2 laser and Er,Cr:YSGG Laser on enamel demineralization. Pediatric Dentistry 34:e92-96
27. Geraldo-Martins VR, Lepri CP, Palma-Dibb RG (2013) Influence of Er,Cr:YSGG laser irradiation on enamel caries prevention. Lasers in Medical Science 28:33–39. https://doi.org/10.1007/s10103-012-1056-9
28. Santos DM, Nogueira RD, Lepri CP, et al (2014) In vitro assessment of the acid resistance of demineralized enamel irradiated with Er, Cr:YSGG and Nd:YAG lasers. Pediatric dentistry 36:137–142
29. Bahrololoomi Z, Fotuhi Ardakani F, Sorouri M, Fotuhi Ardakani F (2015) In Vitro Comparison of the Effects of Diode Laser and CO2 Laser on Topical Fluoride Uptake in Primary Teeth. Journal of Dentistry (Tehran, Iran) 12:585
30. Poosti M, Ahrari F, Moosavi H, Najjaran H (2014) The effect of fractional CO2 laser irradiation on remineralization of enamel white spot lesions. Lasers in medical science 29:1349–1355. https://doi.org/10.1007/S10103-013-1290-9

31. Moslemi M, Erfanparast L, Fekrazad R, et al (2010) The effect of Er,Cr:YSGG laser and air abrasion on shear bond strength of a fissure sealant to enamel. Journal of the American Dental Association (1939) 141:157–61. https://doi.org/10.14219/jada.archive.2010.0133

32. Nair AS, Krishnakumar R, Philip ST, et al (2016) A Comparative Analysis of Caries Inhibitory Effect of Remineralizing Agents on Human Enamel Treated With Er:YAG Laser: An In-vitro Atomic Emission Spectrometry Analysis. Journal of Clinical and Diagnostic Research : JCDR 10:ZC10. https://doi.org/10.7860/JCDR/2016/21058.9007

33. De Sant'Anna GR, Dos Santos EAP, Soares LES, et al (2009) Dental Enamel Irradiated with Infrared Diode Laser and Photo-Absorbing Cream: Part 2—EDX Study. Photomedicine and Laser Surgery 27:771. https://doi.org/10.1089/PHO.2008.2401

34. Vlacic J, Meyers IA, Kim J, Walsh LJ (2007) Laser-activated fluoride treatment of enamel against an artificial caries challenge: comparison of five wavelengths. Australian dental journal 52:101–105. https://doi.org/10.1111/J.1834-7819.2007.TB00472.X

35. Noureldin A, Jupta H, Hunyh T, et al (2015) Re-hardening effect of two-applications of combined fluoride-laser on incipient-enamel lesions. Journal of Dental Health, Oral Disorders & Therapy Volume 3: https://doi.org/10.15406/JDHODT.2015.03.00088

36. Moshonov J, Stabholz A, Zyskind D, et al (2005) Acid-etched and erbium:yttrium aluminium garnet laser-treated enamel for fissure sealants: a comparison of microleakage. International journal of paediatric dentistry 15:205–209. https://doi.org/10.1111/J.1365-263X.2005.00624.X

37. Nammour S, Demortier G, Florio P, et al (2003) Increase of enamel fluoride retention by low fluence argon laser in vivo. Lasers in surgery and medicine 33:260–263. https://doi.org/10.1002/LSM.10219

38. Patrick R Schmidlin, Iris Dörig, Adrian Lussi, et al (2007) CO2 laser-irradiation through topically applied fluoride increases acid resistance of demineralised human enamel in vitro - PubMed. Oral Health Prev Dent 2007;5(3):201-8 5:201–208

39. Demortier G, Nammour S (2008) In vivo PIXE–PIGE study of enhanced retention of fluorine in tooth enamel after laser irradiation. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 266:2408–2411. https://doi.org/10.1016/J.NIMB.2008.03.053