Scientific Article

Effects of Laser-assisted Fluoride Therapy With a CO_2 Laser and Er,Cr:YSGG Laser on Enamel Demineralization

Saeid Nemati Anaraki, DDS, MSc[1] • Maryam Serajzadeh, DDS[2] • Reza Fekrazad, DDS, MSc[3]

Abstract: ***Purpose:*** *The purpose of this in vitro study was to evaluate the irradiation efficacy of the CO_2 laser and the Er,Cr:YSGG laser—either unassisted or assisted by acidulated phosphate fluoride (APF) treatment—on enamel's acid resistance.* ***Methods:*** *One hundred twenty enamel samples, obtained from 20 extracted human molars, were randomly assigned to 6 groups as follows: (1) control (C); (2) exposed to acidulated phosphate fluoride (APF) gel (F); (3) Er,Cr:YSGG laser (EL); (4) irradiated with Er,Cr:YSGG laser through APF gel (EL/F); (5) CO_2 laser (CL); and (6) irradiated with CO_2 laser through APF gel (CL/F). The specimens were individually demineralized in an acidified hydroxyethylcellulose system, and the acid resistance was evaluated by determining the calcium ion using atomic absorption spectrometry.* ***Results:*** *The average concentration of the calcium ion determined in groups C, F, EL, EL/F, CL, and CL/F was, respectively, 3.36, 2.63, 2.26, 2.32, 2.24, and 1.51 ppm. The results showed that demineralization in the: CL/F group was significantly less than the other groups; and the control group was significantly more than the other groups (P<.001).* ***Conclusion:*** *The effect of CO_2 laser irradiation, used with acidulated phosphate fluoride, in decreasing the enamel demineralization was more than all the other groups.*
(Pediatr Dent 2012;34:e92-e96) Received June 17, 2011 | Last Revision October 2, 2011 | Accepted October 25, 2011

KEYWORDS: CO_2 LASER, YSGG LASER, APF, ACID RESISTANCE

Enamel demineralization is a significant clinical problem.[1] Demineralization can lead to caries,[1] which can result in other problems such as tooth loss.[2] Although the incidence of dental caries has declined significantly over time, it is still the most prevalent disease in the world.[3]

Different methods have been proposed to prevent caries, such as using casein phosphopeptide-amorphous calcium phosphate,[4] fluoride-containing products, and laser irradiation. However, one of the most important means is to encourage patients to consider their oral hygiene and include recommendations for increased fluoride exposures.[5] Fluoride-containing products are well known to be effective for the prevention of dental caries.[5,6] Fluoride works via 3 different mechanisms to induce its cariostatic effect. It: (1) inhibits bacterial metabolism; (2) inhibits demineralization[2,7]; and (3) enhances remineralization.[7]

There are different systems for topical application of fluoride, such as sodium fluoride, stannous fluoride, and acidulated phosphate fluoride (**APF**).[8] APF at a 1.23% concentration is available in the forms of gel, solution, and foam and is ready to use. The pH should be between 3 and 5. All of these systems should be in contact with teeth for at least 4 minutes to achieve the maximum cariostatic effect.[5,8] Comparing these systems, APF has proved to be better than the others, since it produces more fluorohydroxyapatite during its chemical reaction with hydroxyapatite.[8]

Since 1964, when Stern and his coworkers first used a ruby laser to assess its effect on preventing caries, many investigations have demonstrated that exposure of enamel to laser irradiation increases its resistance to demineralization.[9-11] Eventually, laser therapy was introduced as an alternative for the prevention of dental caries.

There are several theories regarding the mechanism in which laser irradiation could increase enamel's resistance to demineralization. Hsu et al.,[12] proposed the "organic blocking" theory, which explains partial denaturation of an organic matrix that may block the diffusion pathway in enamel.[13] Other theories include melting, fusion, recrystallization of hydroxyapatite crystals, creation of microspaces, and the reduction in organic, carbonate, and water content of enamel.[14]

The efficacy of fluoride treatment either after or before CO_2 laser treatment for caries prevention has been demonstrated by some authors.[13,15,16-21] There are also some other investigations regarding the efficacy of the Er,Cr:YSGG laser before or after fluoride treatment in prevention of demineralization.[22,23] None of these investigations, however, have attempted to compare the effect of the CO_2 laser or Er,Cr:YSGG laser alone or assisted by laser fluoride therapy (**LAFT**).

Thus, the purpose of this study was to compare the ability of the CO_2 laser and Er,Cr:YSGG laser, assisted by acidulated phosphate fluoride therapy, to inhibit demineralization in human enamel.

Methods

Preparation of the enamel samples. Twenty nonerupted human third molars were collected. After ensuring they were

[1]Dr. Nemati is an assistant professor, Operative Department, School of Dentistry, Tehran branch, Islamic Azad University; [2]Dr. Serajzadeh is a dentist in private practice; and [3]Dr. Fekrazad is an assistant professor, Laser Research Center in Medical Sciences (LRCMS), AJA University of Medical Sciences, Laser Research Center in Dentistry (LRCD), Tehran University of Medical Sciences, all in Tehran, Iran.

Correspond with Dr. Fekrazad at dr_rfekrazad@yahoo.com

free from cracks, erosion, caries, or any structural defect via radiography, the teeth were stored in 0.1% thymol solution.[22] The crowns were removed approximately to the cementoenamel junction. The crowns were cut mesiodistally in the middle, and 2 cuts were made buccolingually, making 6 specimens from each crown. All specimen surfaces were covered with nail varnish, except for a 2 × 2 mm window on the enamel surface, which was measured by a cullis. An enamel sample from each tooth was randomly divided into the 6 groups (total=120, with 20 in each group). This approach provided a sample from each tooth in each group, so that all materials were tested in each tooth. This is the equivalent of a within subject control design.

Experimental design. The surface treatment was performed under the following conditions: no treatment or control (**C**); APF gel alone (**F**); Er,Cr:YSGG laser alone (**EL**); Er,Cr:YSGG laser irradiated through APF treatment (**EL/F**); CO_2 laser alone (**CL**); CO_2 laser irradiated through APF gel treatment (**CL/F**). This study used 120 human enamel samples (20 samples for each group) obtained from 20 molars. We evaluated the acid resistance by determining the calcium ion released from demineralization using atomic absorption spectrometry.

Fluoride treatment and laser irradiation. There was no treatment done in group C. The samples in group F were treated with the APF gel (Topex APF fluoride gel, Sultan Healthcare Inc, Englewood, Calif, USA) for 4 minutes, according to the manufacturer's instructions, and then the gel was washed off.

The samples in group EL were irradiated for 10 seconds with an Er,Cr:YSGG laser (Waterlase, Biolase Technologies Inc, San Clemente, Calif, USA) via the following settings: WaveLength=2,780 nm; Power=0.25 Watt; pulse energy= 12.5 mJ; repetition rate=20 Hz; pulse duration=140 micro second; 11% air; and 0% water. A G6 tip, 600 µm in diameter, was used. The irradiation was done at a distance of 1 to 2 mm from the tooth surface.

The samples in group EL/F were first treated with APF gel for 2 minutes and then irradiated with the Er,Cr:YSGG laser for 10 seconds without removing the gel. The output power in this group was 0.5 W. The APF gel was left on for another 2 minutes and then washed off.

The samples in group CL were irradiated with a CO_2 laser (Ultra Dream Pulse V, DS_40U [all in one], Daeshin Enterprise, Seoul, South Korea), emitting energy on a 10.6 µm wavelength for 10 seconds. The parameter settings used were based on the manufacturer's recommendations: peak power=291 W; pulse duration=100 µs; and interval time=20 ms. The beam spot size was 0.2 mm. The irradiation was done at a distance of 1 cm in the focal point of the laser beam.

The enamel samples in group CL/F were first treated with APF gel for 2 minutes and then irradiated with the CO_2 laser for 10 seconds without removing the gel. The output power in this group, according to the manufacturer's recommendations, was 252 W and the pulse duration was 200 µs. The gel was kept on for another 2 minutes before being washed off.

All irradiations were completed by a standardized expert dentist in a scanning style that allowed the entire surface to be covered.

Demineralization. Each enamel sample was placed in 50 ml of an acidified hydroxyethyl cellulose (**HEC**) system (pH=4.5) for 3.5 days.[24] The samples were removed and prepared to be examined by atomic absorption spectrometry. To ensure accurate results, a pilot study was performed to prove the solution was effective for demineralization.

Atomic absorption spectrometry. The acid resistance of each enamel sample was evaluated by determining the parts per million of calcium ions of each solution using atomic absorption spectrometry (Thermo Elemental Company, Solar M5, Cambridge, England).

The data was imported into SPSS 14 software (SPSS Inc, Chicago, Ill, USA) for statistical analysis. An analysis of variance model was first constructed. This was followed by a Tukey Honestly Significant Difference (**HSD**) test for multiple pairwise comparisons of mean values, since the variances were similar (Leven's test; *P*<.09). Alpha was set at 0.05.

Results

The mean value of the calcium ions in each group and the standard deviation of each group are shown in Table 1. The results of the study showed there was a statistically significant difference (*P*<.001) between group C and all the other groups and between groups CL and CL/F and all the other groups. The Control group had the most decalcification, and group CL/F had the least. There was no statistically significant difference between the other groups. However, the group exposed to APF gel had more decalcification than the CL group, the EL group or the EL/F group. Enamel demineralization was more in the EL/F group than the CL and the EL group. The EL group had more decalcification than the CL group. The multiple comparisons between groups are shown in Table 2.

Discussion

The current study showed that fluoride treatment, CO_2 and Er,Cr:YSGG laser irradiation by itself or assisted by fluoride therapy could significantly decrease enamel demineralization. One of the specifications of this study was using LAFT. In most studies, the use of fluoride was either before the laser irradiation[13,20,23] or after it.[17,23] The other specification was using 2 different kinds of lasers. The reason for choosing these 2 lasers (CO_2 and Er,Cr:YSGG) was due to the recommendations of the previous investigations and their high surface absorbance.[11,15]

It is believed that using laser irradiation combined with fluoride treatment can control caries in high-risk areas, such as around brackets during orthodontic treatment[9] or rest areas during prosthodontic treatments. Using CO_2 laser irradiation has also resulted in caries inhibition around composite restorations.[25] The CO_2 laser has abundant usage in dental applications,

Table 1. MEAN VALUE OF THE CALCIUM ION AND STANDARD DEVIATION OF EACH GROUP

Groups*	Mean±(SD)	95% confidence interval for mean		Minimum	Maximum	*P*-value
		Lower	Upper			
C	3.36±0.48	3.13	3.58	2.70	4.30	.00
F	2.63± 0.73	2.29	2.97	1.50	4.10	
EL	2.26± 0.46	2.04	2.48	1.50	3.20	
EL/F	2.32± 0.86	1.91	2.73	0.80	4.30	
CL	2.24± 0.92	1.81	2.67	1.00	5.50	
CL/F	1.51± 0.39	1.33	1.69	0.80	2.50	

Table 2. SIGNIFICANT DIFFERENCE BETWEEN GROUPS (*P*-VALUE) AND MULTIPLE COMPARISONS BASED ON TUKEY HSD*

Groups*	*P*-value					
	C	F	EL	EL/F	CL	CL/F
C		>.01	.00	.00	.00	.00
F			<.52	.70	<.46	.00
EL				1.00	1.00	.009
EL/F					>.99	.003
CL						>.01
CL/F						

* HSD = Honestly Significant Difference.

** Control group=C; group exposed to acidulated phosphate fluoride gel=F; group irradiated with Er,Cr:YSGG laser=EL; group irradiated with Er,Cr:YSGG laser through APF gel=EL/F; group irradiated with CO_2 laser=CL; group irradiated with CO_2 laser through APF gel=CL/F.

especially in soft tissue surgery. The Er,Cr:YSGG laser is used for enamel ablation and soft and hard tissue surgeries. Therefore, the aim was to find another usage for the Er,Cr:YSGG laser, which is used widely in dental offices.

The reason to use HEC for our demineralization solution was its composition, since it didn't contain any calcium. In this study, the amount of calcium ions from demineralization of the samples was determined to be very little. Therefore, this solution was the best choice for reducing the incidence of inaccuracy.

In several studies CO_2 laser irradiation either alone or with APF treatment could inhibit demineralization in enamel caries.[16,26,27] We reached the same conclusions as some of the other studies in that the combination of laser irradiation and fluoride treatment was more effective than using each one alone.[15,19,20,25,28] In several investigations, Er,Cr:YSGG laser irradiation would increase the enamel's acid resistance.[22,23,29,30]

According to the investigation by Moslemi et al.[23] in 2009, there was a significant difference between Er,Cr:YSGG irradiation alone and when combined with fluoride treatment. In another study, there was no significant difference when using a CO_2 laser alone or in combination with fluoride treatment. These 2 studies were both in contrast with the results of the present study. This is possibly due to different parameters of irradiation, laser settings, and demineralization solutions.

There are several theories regarding the mechanism by which laser irradiation could increase enamel's acid resistance. Some believe by decreasing the carbonate that is in hydroxyapatite (**HA**), enamel's acid resistance would increase. This is acquired through laser irradiation and changing the carbonated HA in the enamel to an HA which is less soluble by altering its crystal structure.[15,17,31] Another theory posits that laser irradiation causes the HA crystals to melt and fuse again. Therefore, the surface of the enamel would be sealed and more resistant to demineralization.[23,25,32]

The increase of enamel's acid resistance after laser irradiation on the threshold of subablative parameters is attributed to photothermal effects rather than photomechanical effects.[22] According to some investigations, to achieve these photothermal effects a temperature ranging from 100°C to 600°C is necessary.[32,33] The energy density required for enamel's acid resistance and this change of temperature when using an Er,Cr:YSGG laser is 8 to 13 J/cm^2.[32,34] Therefore, although using lasers with high energy would melt and fuse enamel's HA crystals and decrease enamel's demineralization, the high temperature (>1,000°C) that they produce may increase the temperature in the pulp or underlying dentin, which can be harmful.[35]

There are different concepts about the mechanisms in which laser-fluoride treatments decrease demineralization. One of the concepts explains that laser irradiation creates microscopic spaces in the enamel.[36,37] These small spaces are important in preventing enamel dissolution, since they can trap free ions that are in the solution after the demineralization phase.[14,27] On the other hand, in the presence of fluoride, HA changes to fluoroapatite (**FAP**). During fluoride treatment, the fluoride ions spread between enamel prisms and create a layer of fluoride coating. After irradiating with CO_2 laser, this fluoride coating, together with several microns on the enamel's outer surface, will melt and crystallize again—thus making a new structure, which is the FAP.[38] Results from a study by Chin-Ying and his coworkers showed that, after CO_2 irradiation, the fluoride concentrations in enamel would increase in both forms of loosely bound (on enamel surfaces) and firmly bound apatitic fluoride. Both forms can facilitate remineralization and inhibit demineralization during an acid attack by being released from the tooth structure.[20,39]

Since the laser irradiation and fluoride treatment were used simultaneously (LAFT) in the present study, it seems that the possible mechanism is the thermal effect of the laser; hence, there shouldn't be much alteration in the surface structure, because the laser was not in contact with the surface of the enamel. Actually, the laser worked as an accelerator, and it is believed that the probable mechanism in these groups was simply fluoride's effect in preventing demineralization.

To prevent ablation of enamel, subablative parameters were used and laser irradiation was done without water cooling to increase surface temperature and not harm the pulp.[32,38]

It is recommended that future studies conduct SEM examinations or other investigations to evaluate surface changes and chemical alterations. Different demineralization solutions and various methods for the evaluation of this demineralization should also be tested to evaluate and establish optimal prophylactic effects. It is also recommended to compare LAFT with laser irradiation either before or after fluoride treatment.

Conclusions

Based on this study's results, it can be concluded that:

1. APF treatment could decrease decalcification of enamel.
2. Er,Cr:YSGG laser irradiation either by itself or assisted by fluoride treatment could decrease decalcification of enamel.
3. CO_2 laser irradiation or Er,Cr:YSGG laser irradiation can prevent demineralization of enamel more than Er,Cr:YSGG laser irradiation assisted by fluoride treatment in the conditions of this study.
4. Using a CO_2 laser assisted by fluoride therapy can prevent enamel demineralization.

Acknowledgment

The authors wish to thank Zohreh Torabian MSc in Chemistry, Researcher in Iranian Research Organization for science and technology, Tehran, Iran for preparing the HEC solution and assisting throughout this study.

References

1. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: A contemporary review. Aust Dent J 2006;51:284-9.
2. Roberson TM, eyman H, Swift, Edward M, Sturdevant CM. Sturdevant's Art and Science of Operative Dentistry. 5th ed. Maryland Heights, Mo: Mosby Co; 2006:67-114.
3. Bowen WH. Dental caries: Is it an extinct disease. J Am Dent Assoc 1991;22:19-52.
4. Azarpazhooh A, Limebach H. Clinical efficacy of casein derivatives: A systemic review of literature. J Am Dent Assoc 2008;139:915-24.
5. Mount G.J, Hume W-R. Preservation and Restoration of Tooth Structure. 2nd ed. Maryland Heights, Mo: Mosby Co; 2005:30-78.
6. McDonald R, Avery D, Dean J. Dentistry for the Child and Adolescent. 8th ed. Maryland Heights, Mo: Mosby Co; 2004:223-31.
7. Featherstone JDB. The science and practice of caries prevention. J Am Dent Assoc 2000;131:887-99.
8. Harris NO, Garcia-Goday F, Nathe CN. Primary Preventive Dentistry. 7th ed. New Jersey: Pearson Higher Ed; 2008:6-9, 246-51.
9. Harazaki M, Hayakawa K, Fukui T, Isshiki Y, Powell L, The Nd-YAG laser is useful in prevention of dental caries during orthodontic treatment. Bull Tokyo Dent Coll 2001;42:79-86.
10. Simunovic Z. Lasers in Medicine and Dentistry. Rijeca Croatia: Vitgraf DOO; 2000:19-40.
11. Featherstone JDB, Fried D. Fundamental interactions of lasers with dental hard tissues. Med Laser Appl 2001;16: 181-94.
12. Hsu C-YS, Jordan TH, Dederich DN, Wefel JS. Effects of low-energy CO_2 laser irradiation and the organic matrix on inhibition of enamel demineralization. J Dent Res 2000;79:1725-30.
13. Meurman JH, Hemmerle J, Voegel J-C, Rauhamaa-Makinen R, Loumanen M. Transformation of hydroxyapatite to fluorapatite by irradiation with high-energy CO_2 laser. Caries Res 1997;31:397-400.
14. Haider SM, White GE, Rich A. Combined effects of argon laser irradiation and fluoride treatments in prevention of caries-like lesion formation in enamel: An in vitro study. J Clin Pediatr Dent 1999;23:247-57.
15. Rodrigues LKA, Nobre dos Santos M, Pereira D, Assaf AV, Pardi V. Carbon dioxide laser in dental caries prevention. J Dent 2004;32:531-40.
16. Featherstone JDB, Zhang SH, Shariati M, McCormack SM. Carbon Dioxide Laser Effects on Caries-like Lesions of Dental Enamel: Lasers in Orthopedic, Dental, and Veterinary Medicine. Bellingham, Wash: SPIE; 1991:145-9.
17. Fox JL, Yu D, Otsuka M, Higuchi WI, Wong J, Powell G. Combined effects of laser irradiation and chemical inhibitors on the dissolution of dental enamel. Caries Res 1992; 26:333-9.
18. Souza-Gabriel AE, Colucci V, Turssi CP, Serra MC, Corona SAM. Microhardness and SEM after CO_2 laser irradiation or fluoride treatment in human and bovine enamel. Microsc Res Tech 2010;73:1030-5.
19. Nobre dos Santos M, Featherstone JDB, Fried D. Effect of a new carbon dioxide laser and fluoride on sound and demineralized enamel. In: Lasers in Dentistry VII. Bellingham, Wash: SPIE; 2001:169-74.
20. Hsu Chin-Ying S, Xiaoli G, Jisheng P, Wefel J. Effects of CO_2 laser on fluoride uptake in enamel. J Dent 2004;32: 161-7.
21. Steiner-Oliveira C, Nobre dos Santos M, Zero DT, Eckert G, Hara AT. Effect of a pulsed CO_2 laser and fluoride on the prevention of enamel and dentine erosion. Arch Oral Biol 2010;55:127-33.
22. de Freitas P, Rapozo-Hilo M, Eduardo C, Featherstone JDB. In vitro evaluation of erbium, chromium:yttrium-scandium-gallium-garnet laser-treated enamel demineralization. Lasers Med Sci 2010;25:165-70.
23. Moslemi M, Fekrazad R, Tadayon N, Ghorbani M, Torabzadeh H, Shadkar MM. Effects of Er,Cr:YSGG laser irradiation and fluoride treatment on acid resistance of the enamel. Pediatr Dent 2009;31:409-13.
24. Amaechi BT, Higham SM, Edgar WM. Factors affecting the development of carious lesions in bovine teeth in vitro. Arch Oral Biol 1998;43:619-28.
25. Klein ALL, Rodrigues LKA, Eduardo CP, Nobre dos Santos M, Cury JA. Caries inhibition around composite restorations by pulsed carbon dioxide laser application. Eur J Oral Sci 2005;113:239-44.
26. Tagliaferro EPS, Rodrigues LKA, Nobre dos Santos M, Soares LES, Martin AA. Combined effects of carbon dioxide laser and fluoride on demineralized primary enamel: An in vitro study. Caries Res 2007;41:74-6.
27. Rodrigues LKA, Nobre dos Santos M, Featherstone JDB. In situ mineral loss inhibition by CO_2 laser and fluoride. J Dent Res 2006;85:617-21.
28. Hossain MM, Hossain M, Kimura Y, Kinoshita J, Yamada Y, Matsumoto K. Acquired acid resistance of enamel and dentin by CO_2 laser irradiation with sodium fluoride solution. J Clin Laser Med Surg 2002;20:77-82.
29. Qiao LY, Yu JT, Jia XY. A study on acquired acid resistance of enamel and dentin irradiated by Er,Cr:YSGG laser in vitro. Zhonghua Kou Qiang Yi Xue Za Zhi 2005;40: 34-7.
30. Hossain M, Kimura Y, Nakamura Y, Yamada Y, Kinoshita JI, Matsumoto K. A study on acquired acid resistance of enamel and dentin irradiated by Er,Cr:YSGG laser. J Clin Laser Med Surg 2001;19:159-63.
31. Fried D, Glena RE, Featherstone JDB, Seka W. Permanent and transient changes in the reflectance of CO_2 laser-irradiated dental hard tissues at λ=9.3, 9.6, 10.3, and 10.6 μm and at fluences of 1-20 J/cm^2. Laser Surg Med 1997;20:22-31.
32. Freitas PM, Soares-Geraldo D, Biella-Silva AC, Silva AV, Silveira BL, Eduardo CDP. Intrapulpal temperature variation during Er,Cr:YSGG enamel irradiation on caries prevention. J Appl Oral Sci 2008;16:95-9.
33. Fowler BO, Kuroda S. Changes in heated and in laser-irradiated human tooth enamel and their probable effects on solubility. Calcif Tissue Int 1986;38:197-208.
34. Fried D, Featherstone JDB, Visuri SR, Seka W, Walsh JT. The caries inhibition potential of Er:YAG and Er:YSGG laser irradiation. In: Lasers in Dentistry. Proceedings of the SPIE Meeting. Bellingham, Wash: SPIE; 1996:73-8.

35. Ying D, Chuah GK, Hsu Chin-Ying S. Effect of Er:YAG laser and organic matrix on porosity changes in human enamel. J Dent 2004;32:41-6.
36. Nelson DGA, Shariati M, Glena R, Shields CP, Featherstone JDB. Effect of pulsed low energy infrared laser irradiation on artificial caries-like lesion formation. Caries Res 1986;20:289-99.
37. Nelson DGA, Wefel JS, Jongebloed WL, Featherstone JDB. Morphology, histology, and crystallography of human dental enamel treated with pulsed low-energy infrared laser radiation. Caries Res 1987;21:411-26.
38. Apel C, Schafer C, Gutknecht N. Demineralization of Er:YAG and Er,Cr:YSGG laser-prepared enamel cavities in vitro. Caries Res 2003;37:34-7.
39. Hsu C-YS, Jordan TH, Dederich DN, Wefel JS. Laser-matrix-fluoride effects on enamel demineralization. J Dent Res 2001;80:1797-801.