In Vitro Comparison of the Effects of Diode Laser and CO$_2$ Laser on Topical Fluoride Uptake in Primary Teeth

Zahra Bahrololoomi$^1$, Faezeh Fotuhi Ardakani$^{2*}$, Milad Sorouri$^3$

$^1$Associate Professor, Department of Pediatric Dentistry, Member of Social Determinants of Oral Health Research Center, Faculty of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
$^2$Assistant Professor, Department of Pediatric Dentistry, Faculty of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
$^3$Pedodontist, Ahvaz, Iran

Abstract

Objectives: Fluoride therapy is important for control and prevention of dental caries. Laser irradiation can increase fluoride uptake especially when combined with topical fluoride application. The objective of this study was to compare the effects of CO$_2$ and diode lasers on enamel fluoride uptake in primary teeth.

Materials and Methods: Forty human primary molars were randomly assigned to four groups (n=10). The roots were removed and the crowns were sectioned mesiodistally into buccal and lingual halves as the experimental and control groups. All samples were treated with 5% sodium fluoride (NaF) varnish. The experimental samples in the four groups were irradiated with 5 or 7 W diode or 1 or 2 W CO$_2$ laser for 15 seconds and were compared with the controls in terms of fluoride uptake, which was determined using an ion selective electrode after acid dissolution of the specimens. Data were analyzed by SPSS version 16 using ANOVA treating the control measurements as covariates.

Results: The estimated amount of fluoride uptake was 59.5± 16.31 ppm, 66.5± 14.9 ppm, 78.6± 12.43 ppm and 90.4± 11.51 ppm for 5W and 7 W diode and 1W and 2 W CO$_2$ lasers, respectively, which were significantly greater than the values in the conventional topical fluoridation group (P<0.005). There were no significant differences between 7W diode laser and 1W CO$_2$ laser, 5W and 7 W diode laser, or 1W and 2 W CO$_2$ laser in this regard.

Conclusion: The results showed that enamel surface irradiation by CO$_2$ and diode lasers increases the fluoride uptake.

Key words: Lasers; Gas; Semiconductor; Dental Enamel; Tooth, Deciduous; Fluorides

Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran (2015; Vol. 12, No. 8)

INTRODUCTION

Despite the decreasing incidence of dental caries, it remains the most common disease of the childhood and adulthood [1]. Topical application of fluorides, use of fluoride toothpastes and fluoridation of drinking water are methods to prevent dental caries by decreasing the demineralization and increasing the mineral content of the saliva, which may inhibit enamel demineralization [2,3]. Laser application along with the application of fluoride is a new technique to improve fluoride
binding to the enamel [4]. Heat generated by the laser light not only prevents the formation of caries on enamel surfaces [5,6], but also leads to decomposition of the organic matrix, loss of water and carbon, and formation of the refractory phases of hydroxyapatite, such as calcium phosphate or calcium pyrophosphate. In addition, it helps the incorporation of fluoride into the hydroxyapatite structure when combined with topical fluoridation and results in the formation of fluoroapatite and calcium fluoride (CaF$_2$) on the enamel surface serving as a reservoir of fluoride against dental caries in the process of demineralization [6]. Diode laser is a laser containing active solid ingredients made of semiconductor crystals of indium, gallium and arsenide with a wavelength between 800 and 980 nm, near the non-ionizing invisible infrared spectrum [7]. Continuous wavelength of diode laser makes it excellent for soft tissue surgery. A major advantage of diode laser is its small size and portability [8]. CO$_2$ laser with the carbon dioxide gas as its active substance and a wavelength of 10600 nm, which lies at the end of the non-ionizing, non-visible infrared spectrum is transmitted through continuous pulse with the highest absorption rate in water after erbium laser. Among all dental lasers, CO$_2$ laser wavelength has the maximum absorption rate in hydroxyapatite, which is 1,000 times the absorption rate of diode laser [9]. Some authors believe that laser therapy may have adverse effects on the enamel causing melting zones, pitting and fracture lines and thermal damage to dentin or pulp [10,11]. However, it has been shown that the application of diode laser, combined with topical fluoridation inhibits damage caused by acid attack in vitro [4].

Villalba-Moreno et al. [4] assessed the effect of diode laser on fluoride uptake. In their study, 25 third molars were sectioned mesiodistally and divided into five groups of 10 specimens. The first group received no treatment. The second group was treated with fluoride varnish (NaF: 1.0 mg) for 15 seconds. The third group was treated with fluoride varnish and diode laser (wavelength of 809 nm, power of 5W). The fourth group was treated with fluoride varnish and 7W diode laser. The fifth group was treated with fluoride varnish and 10W diode laser. The results showed that laser treatment significantly increased fluoride binding to the enamel surface [4].

González-Rodríguez et al. [12] compared the effects of CO$_2$ laser and diode laser on 90 specimens obtained from 45 third molars, which were divided into six groups. Diode laser (wavelength of 809 nm, power of 5 and 7W, for 15 seconds) and CO$_2$ laser (wavelength of 10.6 μm, power of 1 and 2W, for 15 seconds) with the interval of 30 ms, along with 1.0 mg amine fluoride were used. There was a significant difference in the amount of fluoride absorption between the CO$_2$ laser and 5W diode laser [12]. Sayed et al. [13] in 2012 evaluated four common laser wavelengths including neodymium-doped yttrium aluminum garnet (Nd:YAG), potassium-titanyl-phosphate (KTP), diode, and CO$_2$ in combination with fluoride therapy in acidic challenges. Forty sound human premolars were divided into buccal and lingual halves and 1.23% NaF gel was applied on each sample. The samples were divided into five groups of control, KTP, Nd: YAG, diode and CO$_2$ laser. The minimum reduction in enamel hardness following acidic challenge was seen in KTP, Nd: YAG, CO$_2$ and diode lasers, respectively [13]. The aim of this study was to compare the effects of CO$_2$ and diode lasers on topical fluoride uptake by primary teeth surfaces after NaF varnish treatment.

**MATERIALS AND METHODS**

In this parallel-group in vitro study, 40 primary molars with no visible defects on their enamel surfaces were selected and stored in 5% chloramine T solution at room temperature for one week. Then, they were randomly assigned to four groups of 10 specimens. The roots were sectioned and the crowns were divided into
buccal and lingual halves using a diamond disc and a handpiece. One section was used as the control and the other as the experimental specimen. A total of 80 samples were assigned to four groups each containing 20 specimens. In each specimen, a 4 × 4 mm window was considered, then the periphery of the window was coated with nail varnish and the specimen was stored in distilled water.

All specimens (experimental and control) were then dried and treated with 5% NaF varnish (Sultan Topex®, USA) for 15 seconds, and were then laser irradiated (control specimens did not receive laser treatment). In the first group, diode laser (A.R.C. Laser GmbH., Nürnberg, Germany) was used (wavelength of 980 nm, frequency of 16 Hz, power of 5 W, beam diameter of 600 μm and pulse duration of 30 ms) in pulse mode with exposure time of 15 seconds. The same diode laser was used in the second group with a power of 7 W. In the third group, CO₂ laser (Daeshin Enterprise Corp., Seoul, South Korea) was used (wavelength of 10.6 μm, frequency of 30 Hz, power of 1 W, beam diameter of 0.6 mm and pulse duration of 15 ms) in pulse mode with exposure time of 15 seconds; the same CO₂ laser with a power of 2 W was used in the fourth group. The power density of the four groups was 53, 74, 5 and 10.5 J/cm², respectively. Dental samples were irradiated by an operator using the same method described above from a distance of 5 mm. Putty molds were used to ensure equal distance between the tip of the laser handpiece and the enamel surface. After laser irradiation, the specimens were stored in distilled water for 24 hours; then excess fluoride varnish was removed from the surface of all specimens by an electric toothbrush (Oral-B Laboratories Inc., Iowa City, IA, USA) for 5 seconds followed by rinsing with distilled water for 30 seconds.

Fluoride uptake was measured by the enamel biopsy technique using a fluoride ion selective electrode (Fluoride ISE Metrohm 2000, Metrohm AG, Herisau, Switzerland) and a potentiometer. Each specimen was placed in a test tube containing 1 cc of perchloric acid (0.5 M HClO₄) and removed after 30 seconds. Then, the enamel surfaces were washed with 2 cc of 0.2 M KOH. All these procedures were carried out in a test tube with 2 mL of the above-mentioned solution to obtain 3 mL of biopsy solution. For fluoride measurement, EDTA was added to the solution until the pH reached 5.2. Standard electrode of the potentiometer and fluoride electrode were inserted into the solution to find the electric potential difference. This value was placed in the linear equation obtained from the graph of the electric potential difference versus concentration in order to determine the concentration of fluoride in the specimens.

ANOVA and post hoc tests were used for data analysis treating the control measurements as covariates.

RESULTS

Table 1 compares enamel fluoride concentration in different study groups. The results showed that the difference among the laser irradiated groups was significant (P<0.001). Post hoc test was used to compare the mean enamel fluoride concentrations between each laser-irradiated group and other irradiated groups. A significant difference was observed between 5 W diode group and both 1 W and 2 W CO₂ laser groups (P<0.05). The mean fluoride concentration in 7 W diode laser group was significantly different from that in 2 W CO₂ group (Table 2).

A significant difference was found in the mean fluoride concentration of each laser-irradiated group and its respective control (P<0.001). The lowest and the highest difference was observed in 5 W diode and 2 W CO₂ laser groups, respectively.

DISCUSSION

This study assessed the efficacy of CO₂ and diode lasers in increasing fluoride uptake by the enamel.
CO₂ laser irradiation effectively reduces the enamel solubility in acidic conditions and increases fluoride uptake, when used in combination with fluoride [14]. However, despite the widespread use of diode laser in dentistry, its beneficial effects as an adjunct to topical fluoridation have yet to be completely understood [15]. The method used to measure fluoride uptake in the current study was based on studies that evaluated fluoride uptake following the application of several topical fluoridated products in vitro [16-18].

It should be noted that in the above-mentioned studies, there were variables that were difficult to control, such as differences in tooth structure and composition. Confounding factors such as place of birth, place of residence, amount of fluoride in drinking water and proper nutritional habits can affect the results. In the current study it was impossible to control for all these variables. Thus, the teeth in each group were divided into two buccal and lingual halves. Studies have shown that buccal and lingual tooth halves are similar with regard to the pretreatment fluoride concentration [17]. This method decreases the error due to variable pre-treatment fluoride concentrations in different human teeth [19].

### Table 1. Comparison of enamel fluoride concentration in the laser-treated (experimental) groups and the control group

| Groups   | Enamel sections | Fluoride concentration (ppm) | P value |
|----------|-----------------|------------------------------|---------|
|          |                 | Mean | SD   | Min. | Max. |         |
| Experiment |                 |      |      |      |      |         |
| 5W diode | 10              | 59.50 | 16.31 | 37  | 79  |         |
| 7W diode | 10              | 60.50 | 14.90 | 44  | 87  | <0.001  |
| 1W CO₂  | 10              | 78.60 | 12.43 | 62  | 104 |         |
| 2W CO₂  | 10              | 90.40 | 11.51 | 76  | 108 |         |
| Total    | 40              | 73.75 | 17.93 | 37  | 108 |         |
| Control  |                 |      |      |      |      |         |
| 5W diode | 10              | 52.20 | 16.81 | 31  | 76  |         |
| 7W diode | 10              | 49.90 | 13.67 | 30  | 70  |         |
| 1W CO₂  | 10              | 50.90 | 13.98 | 35  | 81  | 0.007   |
| 2W CO₂  | 10              | 52.50 | 11.96 | 35  | 71  |         |
| Total    | 40              | 51.37 | 13.69 | 30  | 81  |         |

### Table 2. The mean differences of fluoride concentration among the laser-irradiated groups

| Group a       | Group b   | Mean difference (a-b) | P value |
|---------------|-----------|-----------------------|---------|
| 5W Diode      |           |                       |         |
| 7W Diode      | 7W Diode  | -7.00                 | 0.622   |
|               | 1W CO₂    | -19.10                | 0.065   |
|               | 2W CO₂    | -30.90                | 0.002   |
| 7W Diode      |           |                       |         |
| 1W CO₂        | 7W Diode  | -12.10                | 0.172   |
| 2W CO₂        | 2W CO₂    | -23.90                | 0.007   |
| 2W CO₂        | 1W CO₂    | -11.80                | 0.162   |
In the treatment protocol that includes a combination of laser irradiation and fluoride therapy, the exact mechanism of fluoride absorption by the enamel is not clear. Two mechanisms have been suggested: 1. Increase in penetration and uptake of fluoride by the enamel due to the heat generated by laser treatment [20] and 2. Retention of fluoride in the enamel surface porosities and micro-cracks caused by laser irradiation [21]. Of the two mechanisms, the thermal effect is more important in increasing fluoride uptake by the enamel surface. High temperature of the laser converts acid phosphate to carbonate pyrophosphate, which leads to enamel water loss. As the enamel crystals lose carbonate they become more stable and more resistant substances such as tetra-calcium diphosphate monoxide, alpha-tricalcium phosphate and beta-tricalcium phosphate may form, which lead to obstruction of interprismatic spaces and low permeability [14,22].

Some studies have found elevated concentrations of fluoride and hardness of enamel after laser treatment [12,22,23] attributed to an increase in the amount of fluoride loosely bonded to the enamel surface (calcium fluoride) and strong coupling of fluoride with the crystalline structure of enamel (fluorapatite). Both types of fluoride can be released during the acidic attack to tooth structure and will facilitate remineralization and inhibit demineralization [24].

According to Chin-Ying et al, laser treatment can increase fluoride uptake by loose binding rather than strong coupling; however, the difference was not statistically significant [25]. Therefore, the authors concluded that the beneficial effects of combined use of laser and fluoride in vivo were less than those in vitro, due to the reduction in fluoride topically applied on the enamel surface because of release and ion exchange with the surrounding tissue fluid, saliva or dental plaque [26].

In this study, treatment with 5W and 7W diode laser in combination with NaF varnish increased fluoride uptake by the enamel of primary teeth compared to the control group. Although the mean fluoride concentration in the 7W laser group was more than in 5W laser group, this difference was not statistically significant. These results are similar to those of Villalba-Moreno et al, [4] and González-Rodríguez et al [12].

The lower mean fluoride concentration in 5W and 7W laser treatment groups in the study by Villalba-Moreno et al. [4] compared to our study can be related to lower initial concentration of fluoride in the specimens, because their dental specimens were prepared from impacted third molars that were not exposed to the oral environment and therefore did not have the ability to absorb fluoride topically over time. Our study specimens were primary molar teeth, which had the opportunity to absorb fluoride topically from various sources. Moreover, the concentration of the fluoride varnish applied by Villalba-Moreno et al. [4] (1% NaF) was lower than that used in our study (5% NaF). Also, we used a stronger acid, i.e. perchloric acid versus chloridric acid in the study by Villalba-Moreno et al, [4] which causes greater solubility of calcium fluoride in the enamel surface. Scanning electron microscopic (SEM) observations in the study by Villalba-Moreno et al. [4] showed changes in the enamel surface due to 5W and 7W diode laser irradiation, which strongly confirms the efficacy of diode laser combined with topical fluoride treatment. In their study, enamel surface after laser application was free of cracks and grooves [4], while changes are observed in the enamel surface following treatment with other types of lasers [27]. However, further studies are recommended. In our study, the group treated with CO₂ laser and fluoride had a significantly higher fluoride uptake by the enamel than the control group. Although the mean fluoride concentration in 2W laser group was greater than in 1W laser group, this difference was not statistically significant.
These findings were also demonstrated by Tepper et al. [28] and Chin-Ying et al. [25]; although they reported higher amounts of fluoride uptake by their specimens. This difference can be justified by the use of CO₂ laser in pulse mode with lower power in our study compared to the continuous mode in their study, which results in lower heat generation and less adverse effects on the enamel [29]. The increase in fluoride uptake by both groups of 1W and 2W CO₂ laser was significantly higher than that in 5W diode laser; but a significant difference was seen only between the 2W CO₂ laser and 7W diode laser. Thus, given the absence of a significant difference between the 1W CO₂ laser and 7W diode laser, it can be stated that the effect of both 1W CO₂ laser and 7W diode laser on fluoride uptake was the same. González-Rodríguez et al. reported similar results [12]. Microscopic examination in their study showed that increase in fluoride uptake with 2W CO₂ laser was not possible without morphological damage to the enamel structure [12].

Specimens treated with 1W CO₂ laser showed changes in almost all laser-irradiated surfaces but without any cracks. No change in enamel surface was seen following application of 5W diode laser; however, morphological changes and detachment of small areas with 7W laser occurred. CO₂ laser wavelength has higher absorption coefficient by the enamel surfaces compared to diode laser; thus, it may have more significant effects on the surface even in lower powers. Therefore, they concluded that the fracture and detachment zones are seen more frequently following the application of 2W CO₂ laser compared to 7W diode laser. These defects are caused by the steam released from the enamel organic matrix due to heat generation following laser treatment. It should be noted that the use of diode laser has advantages such as low cost, portability and easy use and that it is a fiber laser (compared to CO₂ laser), which facilitates its use.

CONCLUSION
1. The simultaneous use of fluoride and laser in all four groups significantly increased fluoride uptake by the primary enamel compared to fluoride treatment alone.
2. Treatments with 1W CO₂ laser and 7W diode laser were equally effective with regard to fluoride uptake.
3. Given that the safety of 7W diode laser is confirmed in future studies, it may be suitable for use as an adjunct to fluoride therapy.

REFERENCES
1- Marinelli CB, Donly KJ, Wefel JS, Jakobsen JR, Denhey GE. An in vitro comparison of three fluoride regimens on enamel remineralization. Caries Res. 1997;31(6):418-22.
2- International collaborative research on fluoride. J Dent Res. 2000 Apr;79(4):893-904.
3- Esteves-Oliveira M1, Zezell DM, Meister J, Franzen R, Stanzel S, Lampert F, et al. CO₂ Laser (10.6 microm) parameters for caries prevention in dental enamel. Caries Res. 2009;43(4):261-8.
4- Villalba-Moreno J, González-Rodríguez A, López-González Jde D, Bolaños-Carmona MV, Pedraza-Muriel V. Increased fluoride uptake in human dental specimens treated with diode laser. Lasers Med Sci. 2007 Sep;22(3):137-42.
5- Apel C, Meister J, Schmitt N, Gräber HG, Gutknecht N. Calcium solubility of dental enamel following sub-ablative Er:YAG and Er:YSGG laser irradiation in vitro. Lasers Surg Med. 2002;30(5):337-41.
6- Hsu CY, Jordan TH, Dederich DN, Wefel JS. Effects of low-energy CO₂ laser irradiation and the organic matrix on inhibition of enamel demineralization. J Dent Res. 2000 Sep;79 (9):1725-30.
7- Aoki A, Mizutani K, Takasaki AA, Sasaki KM, Nagai S, Schwarz F, et al. Current status of clinical laser applications in periodontal therapy. Gen Dent. 2008 Nov-Dec;56(7):674-87.
8- Schoop U, Kluger W, Moritz A, Nedjelik N, Georgopoulos A, Sperr W. Bactericidal effect of different laser systems in the deep layers of dentin. Lasers Surg Med. 2004;35(2):111-6.
9- Konishi N, Fried D, Staninec M, Featherstone JD. Artificial caries removal and inhibition of artificial secondary caries by pulsed CO₂ laser irradiation. Am J Dent. 1999 Oct;12(5):213-6.
10- McCormack SM, Fried D, Featherstone JD, Glena RE, Seka W. Scanning electron microscope observations of CO₂ effects on dental enamel. J Dent Res. 1995 Oct;74(10):1702-8.
11- Malmström HS, McCormack SM, Fried D, Featherstone JD. Effects of CO₂ laser on pulpal temperature and surface morphology: an in vitro study. J Dent. 2001 Nov;29(8):521-9.
12- González-Rodríguez A, de Dios López-González J, del Castillo Jde D, Villalba-Moreno J. Comparison of effects of diode laser and CO₂ laser on human teeth and their usefulness in topical fluoridation. Lasers Med Sci. 2011 May;26(3):317-24.
13- Sayed A, Hegde V, Thukral N. Prevention of enamel from erosion by laser activated fluoride treatment. J Dent Lasers 2012;6:7-10.
14- Rodrigues LK, Nobre dos Santos M, Pereira D, Assaf AV, PardiV. Carbon dioxide laser in dental caries prevention. J Dent. 2004 Sep;32(7):531-40.
15- Klim JD, Fox DB, Coluzzi DJ, Neckel CP, Swick MD. The diode laser in dentistry. Rev Wavelengths 2000;8(4):13–6.
16- Tveit AB. Fluoride uptake by enamel surfaces, root surfaces and cavity walls following application of a fluoride varnish in vitro. Caries Res. 1980;14(5):315-23.
17- Chan S, Hill FJ, Newman HV. Uptake of fluoride by sound and artificially carious enamel in vitro following application of topical sodium and amine fluorides. J Dent. 1991 Apr;19(2):110-5.
18- Carll JJ, Silverstone LM, Clarkson BH, Wefel JS, Wei SH. Fluoride uptake and in vitro caries-like lesion formation in enamel after two-step topical fluoride applications. Caries Res. 1982;16(2):162-9.
19- Mc Donald R, Avery D. Dentistry for the child and Adolescent.7th ed. St. Louis: Mosby;2011, chap:10.:243.
20- Putt MS, Beltz JF, Muhler JC. Effect of temperature of SnF₂ solution on tin and fluoride uptake by bovine enamel. J Dent Res. 1978 Jul-Aug;57(7-8):772-6.
21- Oho T, Morioko T. A possible mechanism of acquired resistance of human dental enamel by laser irradiation. Caries Res. 1990;24:86–92.
22- Bahrololoomi Z, Lotfian M. Effect of diode laser irradiation combined with topical fluoride on enamel microhardness of primary teeth. J Dent (Tehran). 2015 Feb;12(2):85-9.
23- Vitale MC, Zaffe D, Botticell AR, Caprioglio C. Diode laser irradiation and fluoride uptake in human teeth. Eur Arch Paediatr Dent. 2011 Apr;12(2):90-2.
24- Hsu CY, Jordan TH, Dederich DN, Wefel JS. Laser-matrix-fluoride effects on enamel demineralization. J Dent Res. 2001 Sep;80(9):1797-801.
25- Chin-Ying SH, Xiaoli G, Jisheng P, Wefel JS. Effects of CO₂ laser on fluoride uptake in enamel. J Dent. 2004 Feb;32(2):161-7.
26- Smith GE. The action of fluoride in teeth and bone. Med Hypotheses. 1986 Feb;19(2):139-54.
27- Tsai CL, Lin YT, Huang ST. In vitro acid resistance of CO₂ and Nd–YAG laser-treated human tooth enamel. Caries Res. 2002 Nov-Dec;36(6):423-9.
28- Tepper SA, Zehnder M, Pajarola GF, Schmidlin PR. Increased fluoride uptake and acid resistance by CO₂ laser – irradiation through topically applied fluoride on human enamel in vitro. J Dent. 2004 Nov;32(8):635-41.
29- Klocke A, Mihailova B, Zhang S, Gasharova B, Stosch R, Güttler B, et al. CO₂ laser-induced zonation in dental enamel: a Raman and IR microspectroscopic study. J Biomed Mater Res B Appl Biomater. 2007 May;81(2):499-507.