Effect of Nd-YAG laser-irradiation on fluoride uptake by tooth enamel surface (In vitro)

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ABSTRACT

Background: The irradiation of teeth with a laser results in an interaction between the light and the biological constituents of the dental hard substance, which is converted directly into heat.This thermal effect is the cause of the structural and chemical enamel changes.The combined treatment of topical fluoride agent with laser may increase fluoride uptake, and reduce progression of caries-like lesions. The aim of this study was to measure the uptake of the acidulated phosphate fluoride and sodium fluoride to the buccal and lingual caries-like lesion enamel surfaces before and after irradiated by Nd-YAG laser in comparison with matching control group.

Materials and methods: The sample consisted of 30 human healthy upper premolar teeth which were stored in 0.1% thymol solution after extracted. Every tooth divided into: buccal and lingual specimen, each specimen has a rectangular window which was divided to right and left halves (120 specimens). The sample was divided into 2groups (60 specimens) for buccal surface, and the same for lingual surface. The caries-like lesion was formed for all groups except control (1) each group treated with either acidulated phosphate fluoride 1.23% or sodium fluoride 2%, (30 specimens) which contain other subgroups, these are: (10 specimens) one half treated with fluoride agent only and another half as control (first group as control (1) without caries-like lesion, and the second group control (2) with caries-like lesion, then de-ionized water only). (10 specimens) treated with fluoride agent then irradiated by Nd-YAG laser; one half with program (1) (short pulse), andanother with program (2) (long pulse). (10 specimens) irradiated by Nd-YAG laser; one half with program (1) and another with program (2) then treated with fluoride agent. The specimens of enamel were sectioned and the fluoride uptake was determined with using fluoride sensitive electrode.

Results: There was a significant difference between the buccal and lingual enamel surfaces regarding the fluoride uptake in sound tooth, while a non- significant difference was observed after artificial caries-like lesion formation.

Conclusion: Irradiation of Nd-YAG laser program (1) to the buccal and lingual caries-like lesion surfaces of enamel before application of fluoride agents (APF, NaF) was significantly increase fluoride uptake than that of using laser after the application of fluoride agent, as well as from using laser of program (2) after and before the application of fluoride agent, and from using fluoride agent alone in the buccal and lingual surfaces.

Key words: Nd-YAG laser, acidulated phosphate fluoride, sodium fluoride. **(J Bagh Coll Dentistry 2014; 26(1):154-158).**

INTRODUCTION

The decline in dental caries over the last few decades has been attributed to the extensive use of fluoride. Although fluoride is the most powerful treatment to prevent tooth decay, the development of new methods to completely control this disease is still necessary. In this way, lasers, combined or not with fluoride, have been tested on teeth to improve dental enamel properties in order to enhance its resistance to demineralization [1]. Fluoride penetration in the enamel occurs through the replacement of the relatively weak hydroxyl ions in the enamel mineral structure by the much more active fluoride ions, thereby improving the chemical stability of the enamel structure and making it more resistant to acids[2].

The use of laser favors the incorporation of fluoride into enamel, not only on its surface as calcium fluoride (CaF_2) but also within its crystalline structure[3].That the increment of CaF_2-like material formation and retention is a result of the morphological changes promoted by laser irradiation, considering the melting promoted by Nd:YAG [4].

As with all chemical reactions, the degree and speed of penetration of fluoride into the enamel and the formation of fluorapatite are strongly dependent upon concentration and temperature [5].

Low concentrations of fluoride prevented demineralization of sound enamel, and higher concentrations enhanced remineralization of artificial caries-like lesions [6]. Traditional therapy of early childhood caries can be improved with the use of Nd:YAG laser, fluoride gel was applied on the carious surfaces of teeth before irradiation with Nd:YAG laser. Nd:YAG laser tip was used in contact and caries was removed upon irradiation without using local analgesics [7].

MATERIALS AND METHODS

The thirty blocks were divided into 2 groups the buccal surface (A) and the lingual surface (B), The tooth was sliced by a high speed turbine hand piece with flow of de-ionized water to form rectangular slabs (the enamel window technique) [8] on buccal and lingual surfaces of (6mm) in the midpoint of image line running from the highest cusp to the CEJ.The initial cut was made mesio-distally through the tip of the buccal cusp to get buccal and lingual surfaces dissection while

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holding the root and coronal dentine was removed. Enamel sample was then served at approximately one-third of the buccal and lingual surfaces. These specimens of enamel were covered with red hard wax, so that the buccal and lingual enamel surfaces of rectangular slabs (6mm) were exposed to the environment and inner side fixed in wax block. After that therectangular slabs of each surface were separated with a mid-line by the turbine to right and left halves of 3mm and one of this half was covered with varnish nail, so they were subdivided randomly into 12 groups. Each block was soaked for 7 days in continuous stirred artificial saliva 5 ml to develop a pellicle layer in incubator at 37ºc. Sub surface caries like-lesion, was induced in each specimen by immersion for 24 hr at 37ºC in a 14 ml solution of demineralization solution adjusted at pH of (5), after the demineralization solution the specimen rinsed with de-ionized water and analyzed.

These groups of sixty specimens were: 5 specimens as a control (without caries like-lesion formation).5specimens as a control (treated with deionized water only, after caries like-lesion formation).5 specimens treated with acidulated phosphate fluoride (APF) 1.23%. 5 specimens treated with sodium fluoride (NaF) 2%. 5 specimens treated with (APF) then irradiated with Nd-YAG laser of program (1). 5 specimens treated with(NaF) then irradiated with Nd-YAG laser of program (1). 5 specimens treated with (APF) then irradiated with Nd-YAG laserof program(2). 5 specimens treated with (NaF) then irradiated with Nd-YAG laser of program (2). 5 specimens irradiated with Nd-YAG laser of program (1) then treated with (APF). 5 specimens irradiated with Nd-YAG laser of program (1) then treated with (NaF). 5 specimens irradiated with Nd-YAG laser of program (2) then treated with (APF). 5 specimens irradiated with Nd-YAG laser of program (2) then treated with (NaF).

After each enamel specimen treated as mention above, it was separated from enamel block by turbine hand piece (the acid resistant varnish was removed with acetone- soaked cotton applicator from the other specimen of 3mm to return soaked in artificial saliva then demineralization solution to complete the samples number) then dried and powdered using ceramic mortar and pestle.This powder was weighted in electronic balance then immersed in 0.4 mL HCl 1 mol/L was added to the tubes. The tubes were agitated for 30 minutes, then 0.8 mL NaOH 0.5 mol/L was added [9]. An equal volume of TISAB II (1.0 M acetate buffer pH 5.0, 1 MNaCL and 0.4% EDTA) modified with 20 gNaOH/L was added to each solution containing the dissolved enamel layer. Sample was dilution to 10 ml that required to immerging fluoride electrode sufficiently by de-ionized water that was used as the blank were mixed with a magnetic stirrer for 3 minutes.Fluoride measurements were performed using an ion-selective electrode (WTW) GmbH (Germany) fluoride electrode F 500 and fluoride combination electrode F 800, to determine the fluoride ions concentrations in enamel samples by the potential in mV of standard solutions were directly measured by the electrode and calibrated using the concentration scale or by plotting calibration graph or constructed on a standard semi logarithmic paper by plotting the mill volt readings (linear axis) against concentration (long axis).

RESULTS

Fluoride uptake of enamel before and after artificial caries-like lesion formation between buccal and lingual surfaces represented by the percentages of fluoride per 100mg of tooth enamelwas shown in (Table1).It was found that, the a highly significant mean value was reported in buccal surface than that of lingual surface regarding the group control (1) without caries-like lesion. Non significant difference was recorded between two surfaces concerning the group control (2) with caries-like lesion and de-ionized water ($P>0.05$). A highly significant mean values between control (1) and control (2) in buccal and lingual surfaces.By using the LSD test, the comparison (con 2) and other groups in buccal surface was illustrated in (Table 2), not significant differences were observed between group (con 2) and groups (NaF, APF+L_1 and L_2+NaF), while significant differences were recorded with others in the buccal surface. Highly significant differences were observed in (Table 3) between group (con2) and most of the groups except (APF, NaF, APF+L_1 and L_2+NaF), which were not significant differences ($P>0.05$) in the lingual surface.

LSD and P-values were used to compare among all study groups. The data was presented in (Table 4) for buccal surface and in (Table 5) for lingual surface.

Regarding the buccal surface, the results had shown that different mean values of fluoride uptake in group (L_1+APF) and (L_1+NaF) were not significant. While highly significant differences were recorded with the other groups.

DISCUSSION

It is widely known that Nd:YAG laser associated with topical application of fluoride can increase enamel resistance to demineralization; however, this study was conducted to assessed the effect of Nd-YAG laser-irradiation on fluoride uptake by tooth enamel surface (in vitro).In pilot study sound enamel was used without caries like lesion, but the results revealed the same for all groups. Finding of study on smooth surface reported that the administration of 1.23% APF gel one or two times and associated with the daily use of a fluoridated dentifrice was not capable for enhancing surface hardness and fluoride content in bovine enamel, in comparison to blocks submitted to demineralisation alone [10]. As well as, the fluoride acquired after single application with APF was mainly loosely bound and was lost rapidly. While that acquired after three application was mainly permanently retained as fluorapatite[11].The result of present study observed that significant differences between the group control (1) and group control (2) in fluoride uptake, in which enamel demineralization leads to the dissolution of hydroxyapatite and the diffusion of Ca and P ions on the enamel surface. The hyper saturation of these ions causes reprecipitation of hydroxyapatite, forming an intact superficial layer on the enamel surface. Thus, enamel remineralization is achieved through the presence of fluoride in the medium [12].

A likely hypothesis for the results of the present study is the deposition of fluoride ions in the outermost layers of the enamel, impeding the action of fluoride in the mineral deposition in the inner portion of the lesion. The outer layers of human enamel are especially sensitive to fluoride and might be stabilized and condensed due to incorporation of this element [13].Various lasers can reduce the rate of surface demineralization in enamel, when it is submitted to acids (pH 5.5) that cause caries .Several studies emphasized the use of different lasers in association with various forms of fluoride for the reduction of demineralization. The laser is applied to fluoride to increase fluoride diffusibility, which promotes greater ion absorption in the enamel or favors ion linking in the adjacent area [14,15].Nd:YAG laser irradiation provided a reduction in caries incidence compared with the non-treatment control. These findings are in agreement with those reported for the effect of combined fluoride and Nd:YAG laser irradiation treatment on in vitro enamel caries [16].The present study revealed that the irradiation of Nd-YAG laser to the enamel surface before the application of APF or NaF agents was significant higher then after them or were used with unlased enamel. These findings are in agreement with those reported for the effect of combination fluoride and Nd:YAG laser irradiation\ treatment on in vitro enamel caries (reduction of 40% for premolars caries) [16]. A significant synergism has been shown between laser and fluoride in the reduction of enamel solubility. Indeed, topical APF application promotes the dissolution of more soluble apatite crystals, and a large quantity of CaF_2 is formed on the surface [17]. Laser irradiation can retain fluoride ions longer than unlased enamel, and the mechanisms of this fluoride retention are still unknown [18]. It was proposed that laser irradiation can promote the formation of microspaces in enamel, which would facilitate the fluoride incorporation; moreover, laser irradiation can induce the formation of fluorapatite by incorporation of fluoride into the melted layers of the enamel surface, another suggested mechanism is that laser irradiation can increase fluoride diffusion through enamel and generate fluoride reservoirs[19].In the present study, the combination of topical fluoride treatment following laser irradiation provided an even greater degree of caries resistance. Since heat was found to enhance the uptake of fluoride, it was speculated that the thermal effect of the laser was the main factor in promoting fluoride uptake and an increase in enamel resistance to demineralization [20].

The use of program (1) then fluoride application was recorded highly a significant fluoride uptake than program (2), that the higher fluoride incorporation was noted in samples irradiated with the lowest fluencies. These results indicate that the low fluencies generated some structural changes in the enamel surface that propitiated the retention of fluoride. Similar findings were reported when Argon laser was irradiated at low fluency on enamel when it was suggested that laser irradiation could increase the fluoride diffusion through the enamel structure or generated reservoirs for fluoride deposition into enamel [19]. The findings of this study suggest that Nd:YAG, within the parameters tested, followed by topical APF application in both methodologies used, can prevent or retard the demineralization caused by citric acid erosion and lead to preservation of the enamel [21].

Table 1: Concentration of fluoride uptake in enamel before and after artificial caries-like lesion formation between buccal and lingual surfaces.

Group	Buccal		Lingual		P-value
	$^{\Delta}$Mean $\underline{+}$SD		$^{\Delta}$Mean $\underline{+}$SD		
Control 1	0.0047	0.0001	0.0042	0.0001	0.001*
Control 2	0.0194	0.0029	0.0197	0.0018	0.831
P-value	0.001*		0.001*		

*Highly significant; df=8, $^{\Delta}$Percentage of fluoride /100mg enamel.

Table 2: LSD test between control (2) and other groups in buccal surface.

	Mean difference	P-value	Significance
APF	-0.0075	0.036	S
NaF	-0.0066	0.063	NS
APF+L_1	-0.0070	0.050	NS
NaF+L_1	-0.0102	0.005	HS
APF+L_2	-0.0102	0.005	HS
NaF+L_2	-0.0105	0.004	HS
L_1+APF	-0.0217	0.001	HS
L_1+NaF	-0.0251	0.001	HS
L_2+APF	-0.0119	0.001	HS
L_2+NaF	-0.0059	0.098	NS

Table 3: LSD test between control (2) and other groups in lingual surface.

	Mean difference	P-value	Significance
APF	-0.0045	0.110	NS
NaF	-0.0033	0.238	NS
APF+L_1	-0.0049	0.081	NS
NaF+L_1	-0.0080	0.006	HS
APF+L_2	-0.0089	0.002	HS
NaF+L_2	-0.0082	0.005	HS
L_1+APF	-0.0181	0.001	HS
L_1+NaF	-0.0207	0.001	HS
L_2+APF	-0.0077	0.008	HS
L_2+NaF	-0.0034	0.227	NS

Table 4: LSD multiple comparison test in buccal surface.

	APF	NaF	APF+L_1	NaF+L_1	APF+L_2	NaF+L_2	L_1+APF	L_1+NaF	L_2+APF
NaF	0.798								
APF+L_1	0.882	0.914							
NaF+L_1	0.443	0.308	0.361						
APF+L_2	0.443	0.308	0.361	1.000					
NaF+L_2	0.401	0.275	0.324	0.941	0.941				
L_1+APF	0.001*	0.001*	0.001*	0.002*	0.002*	0.002*			
L_1+NaF	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.327		
L_2+APF	0.210	0.133	0.162	0.621	0.621	0.674	0.008*	0.001*	
L_2+NaF	0.641	0.833	0.750	0.220	0.220	0.194	0.001*	0.001*	0.088

* Significant

Table 5: LSD multiple comparison test in Lingual surface.

	APF	NaF	APF+L_1	NaF+L_1	APF+L_2	NaF+L_2	L_1+APF	L_1+NaF	L_2+APF
NaF	0.665								
APF+L_1	0.881	0.560							
NaF+L_1	0.214	0.097	0.273						
APF+L_2	0.121	0.049*	0.159	0.749					
NaF+L_2	0.197	0.087	0.253	0.960	0.787				
L_1+APF	0.001*	0.001*	0.001*	0.001*	0.002*	0.001*			
L_1+NaF	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.368		
L_2+APF	0.267	0.126	0.336	0.893	0.649	0.853	0.001*	0.001*	
L_2+NaF	0.685	0.977	0.580	0.102	0.053	0.093	0.001*	0.001*	0.133

* **Significant**

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