ORIGINAL RESEARCH

Effect of CO_2 Laser and 1.23% Acidulated Phosphate Fluoride on Acid Resistance and Fluoride Uptake of Human Tooth Enamel: An *In Vitro* Assessment

Adesh Kakade[1], Parag Kasar[2], Dimple Padawe[3], Vilas Takate[4], Akansha Juneja[5], Anil Patil[6]

Abstract

Aim: To evaluate the effectiveness of CO_2 laser treatment before applying 1.23% acidulated phosphate fluoride (APF), through topically applied 1.23% APF solution, and after applying 1.23% APF on acid resistance and fluoride uptake of the enamel.

Materials and methods: Sixty non-carious human premolars were extracted due to the orthodontic reason and stored in distilled water solution under refrigeration. Using a water-cooled diamond disc, enamel slabs of 4 mm × 4 mm × 1.5 mm were cut from the buccal surface of each tooth. Sixty samples were randomly divided into one control group and five test groups of 10 premolars each. Solution was prepared for wet chemical analysis followed by fluoride analysis that was carried out using a fluoride ion selective electrode (Thermo Scientific Orion 4-Star Plus ISE Meter). The weight of enamel (WE) was determined from the amount of calcium (Ca) etched away considering the fact that the Ca content of the human enamel is 37.4 wt%. The subgroups were statistically analyzed using ANOVA for fluoride determination and evaluation of acid resistance.

Results: There was a significant increase in acid resistance of enamel slabs when treated individually or in combination of a low-power CO_2 laser and 1.23% APF solution. Application of 1.23% APF solution after low-power CO_2 laser treatment showed maximum increase in acid resistance.

Conclusion: Application of a low-power pulsed CO_2 laser through topically applied 1.23% APF solution resulted in a detrimental effect of the human tooth enamel with resultant decrease in acid resistance. High fluoride uptake does not necessarily indicate increased acid resistance.

Clinical significance: The present study provides evidence that a low-power CO_2 laser can be used effectively in combination with topically applied 1.23% APF solution in order to make the enamel more resistant to acid attack, thereby helping in controlling dental caries.

Keywords: 1.23% Acidulated phosphate fluoride, Acid resistance, CO_2 laser, Fluoride uptake, Human tooth enamel.

The Journal of Contemporary Dental Practice (2019): 10.5005/jp-journals-10024-2684

Introduction

Acidulated phosphate fluoride (APF) is most effective among all topical fluorides.[1–3] But it has been reported that APF when applied for 4 minutes is insufficient for the optimum effect.[4] Prevention through increased acid resistance as a result of the laser occurs due to decreased enamel permeability,[5] alterations in chemical composition,[6] or both.[7] CO_2 laser irradiation of the human dental enamel at 10.6 microns and the continuous[8–11] as well as the pulsed[12–15] wave mode led to significantly reduced dissolution rates. Similar results have been found when investigating the effect of additional fluoride application before or after laser treatment, leading to an increased fluoride uptake and a decreased rate of dissolution in acidic solution.[11,16–18]

Spontaneous conversion of hydroxyapatite to fluorapatite by irradiation with a high-energy CO_2 laser in the presence of sodium fluoride has been reported.[19] Also, 1.23% APF application followed by laser shows high fluoride uptake and acid resistance in comparison to NaF.[16] Several advantages have been reported when laser is performed in combination with fluoride application.[20]

Hence, the present study aimed at evaluating the effect of a CO_2 laser before applying 1.23% APF, through topically applied 1.23% APF solution, and after applying 1.23% APF on acid resistance and fluoride uptake of the enamel.

Materials and Methods

The present *in vitro* study was carried out in the Department of Pediatric and Preventive Dentistry, Nair Hospital Dental College, Mumbai, in collaboration with Department of Earth Sciences and Department of Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Powai, Mumbai, to assess the effect of a CO_2 laser and 1.23% APF solution on the human tooth enamel. All subjects enrolled in this research responded to an informed-consent protocol, which had been approved by the Ethics Committee, Nair Hospital Dental College, Mumbai.

Preparation of Enamel Slabs

Sixty non-carious human premolars were extracted due to the orthodontic reason and stored in distilled water solution under

[1,2]Department of Pediatric and Preventive Dentistry, Nair Hospital and Dental College, Mumbai, Maharashtra, India

[3,4]Department of Pediatric and Preventive Dentistry, Government Dental College and Hospital, Mumbai, Maharashtra, India

[5]Department of Pediatric Dentistry, Jamia Millia Islamia, New Delhi, India

[6]Department of Pediatric and Preventive Dentistry, Bharati Vidyapeeth Dental College and Hospital, Sangli, Maharashtra, India

Corresponding Author: Parag Kasar, Department of Pediatric and Preventive Dentistry, Nair Hospital and Dental College, Mumbai, Maharashtra, India, Phone: +91 9619345878, e-mail: parag.pedopg@gmail.com

How to cite this article: Kakade A, Kasar P, *et al.* Effect of CO_2 Laser and 1.23% Acidulated Phosphate Fluoride on Acid Resistance and Fluoride Uptake of Human Tooth Enamel: An *In Vitro* Assessment. J Contemp Dent Pract 2019;20(9):1082–1089.

Source of support: Nil

Conflict of interest: None

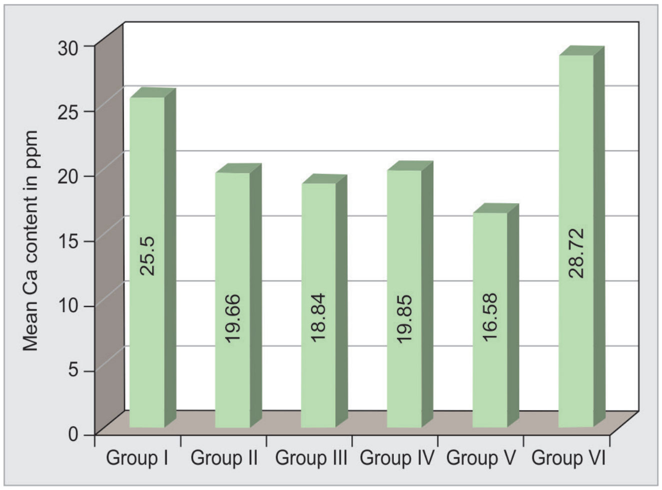

Fig. 1: Comparison of the mean Ca content of solutions from different groups

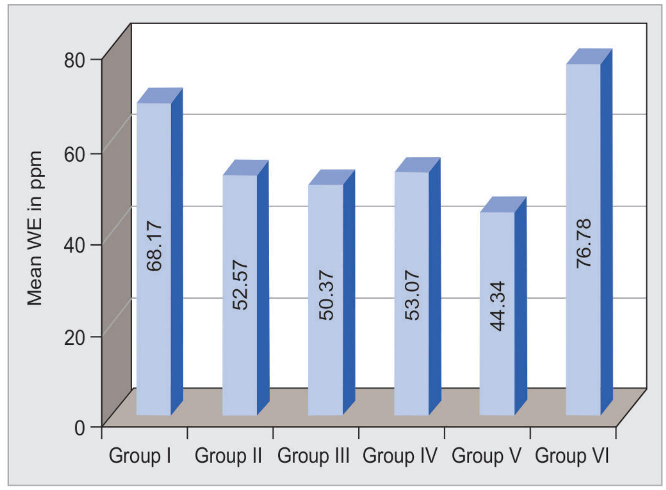

Fig. 2: Comparison of mean WE loss from each group

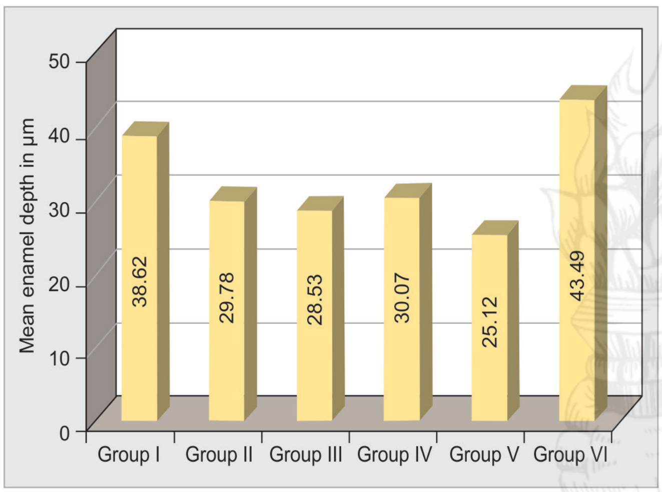

Fig. 3: Comparison of mean enamel depth from different groups

refrigeration. The teeth were then cleaned and polished with nonfluoridated polishing paste and rubber cup. The selected teeth were checked for surface cracks, white spots, or mottling using contrast light (Fig. 1). Enamel slabs of 4 mm × 4 mm × 1.5 mm were cut using a water-cooled diamond disc on the buccal surface of each teeth (Fig. 2). A vernier caliper was used to standardized the dimensions. The enamel slabs were embedded in acrylic blocks (Fig. 3).

Preparation of 1.23% APF Solution

Four grams of sodium fluoride were added in 200 mL of 0.1 M phosphoric acid in a plastic beaker. The 48% hydrofluoric acid was added to adjust the pH between 3 and 3.5 using a Eutech pH meter and fluoride concentration at 1.23%. Constant stirring using a magnetic stirrer and fischer was done to prepare the solution.

Carbon Dioxide Laser

Commercially available dream pulse III surgical laser system (Daeshin Enterprise, Korea) was used at super pulse setting and mode A (having pulse duration of 800 µs), each pulse separated by 100 ms with energy output of 5 W per pulse. The laser system operated at a wavelength of 10.6 µm.

Lasing the Samples

The entire enamel surface of the samples was irradiated for 15 seconds by moving the laser probe tip continuously at a distance less than 1 mm from the sample surface by a single operator. Sixty samples were randomly divided into one control group and five test groups of 10 premolars each as follows:

- Group I: control samples, sound tooth enamel slabs receiving no treatment.
- Group II: enamel slabs treated with 1.23% APF solution for 4 minutes.
- Group III: enamel slabs lased for 15 seconds using super pulse setting and mode A (having pulse duration of 800 µs), each pulse separated by 100 ms and energy output of 5 W per pulse.
- Group IV: enamel slabs that were treated with 1.23% APF solution for 4 minutes before lasing for 15 seconds.
- Group V: enamel slabs that were treated with 1.23% APF solution for 4 minutes after lasing for 15 seconds.
- Group VI: enamel slabs that were lased for 15 seconds immediately through the topically applied 1.23% APF solution at the end of 4-minutes application.

All the 60 samples underwent the following procedures.

Method of Preparation of Solution for Wet Chemical Analysis (Fig. 4)

One layer of the enamel in samples was etched away by exposure to 1 mL of 0.5 M perchloric acid contained in plastic beakers for 30 seconds. The 0.5 M perchloric acid was prepared by adding 4.4 mL of 70% pure perchloric acid to 100 mL of double distilled water. After removing the sample subsequent to etching with 0.5 M perchloric acid, the enamel slab was washed carefully with 4 mL of total ionic strength adjustment buffer (TISAB) solution dispensed through a plastic syringe, in such a way that all the solution was collected in a beaker containing perchloric acid. The solution in a beaker was then prepared to 10 mL by adding distilled water. This analysis was done to evaluate calcium levels of all the groups.

Fluoride Analysis (Fig. 4)

The fluoride analysis was carried out using a fluoride ion selective electrode (Thermo Scientific Orion 4-Star Plus ISE Meter). The same solution (mixture of 0.5 M perchloric acid and 4 mL TISAB was

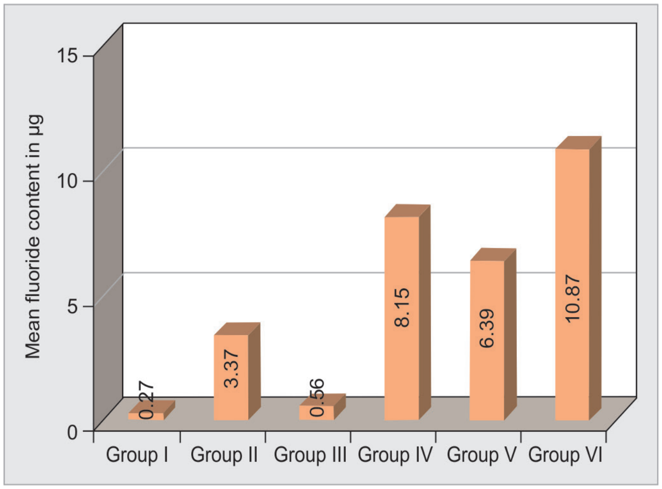

Fig. 4: Comparison of the mean fluoride content of solutions from different groups

mixed and prepared to 10 mL by adding distilled water) was used to evaluate the fluoride level in all groups.

Estimation of Calcium Content

The weight of enamel (WE) was determined from the amount of calcium (Ca) etched away considering the fact that the Ca content of the human enamel is 37.4 wt%. The thickness of the enamel layer removed was also determined using the following formula:[21]

$$\text{Depth in } \mu\text{m} = \frac{\mu\text{gCa} \times 10^2}{37.4 \times 16 \times 2.95}$$

where, 37.4 wt% is the calcium content in the enamel, 2.95 g/cm^3 is the density of the enamel, and 16 mm^2 is the surface area of the enamel slab.

The Ca content in the solution was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The same solution (mixture of 0.5 M perchloric acid and 4 mL TISAB was mixed and prepared to 10 mL by adding distilled water) was used to evaluate the Ca content level in all groups.

Statistical Analysis

The subgroups were statistically analyzed using ANOVA for fluoride determination and evaluation of acid resistance. Individual *post hoc* comparisons were performed using a unpaired *t* test. Significance was set at 95% ($p \leq 0.05$).

Results

Among all the groups, group V showed lowest values of mean Ca content in prepared solution (16.58 ± 2.03 ppm), mean WE (44.34 ± 5.44 ppm), and mean enamel depth (25.12 ± 3.08 µm) suggestive of maximum acid resistance (Tables 1 to 7, Figs 5 to 7). The fluoride content of prepared solutions was highest in group VI (10.87 ± 0.79 µg) (Tables 1 to 7 and Fig. 8). Fluoride uptake was highest in group IV (15372.10 ± 4277.46 ppm) with comparable fluoride uptake in group V (14509.00 ± 1625.53 ppm) and group VI (14226.90 ± 1543.75 ppm) (Table 7 and Fig. 9).

Discussion

Fluoride prevents dental decay by inhibition of demineralization and promoting subsurface remineralization.[22–25] The human enamel is considered the substrate of choice for preparing the enamel slabs, because it is identical, or nearly so, to the substrate it is intended to represent. Use of premolars intended for extraction also has the advantage that they are in the functional and natural state.[26] So, enamel slabs for the present study were prepared from sound human premolars extracted for the orthodontic reason. Synthetic hydroxyapatite shows high-energy CO_2 laser treatment converting hydroxyapatite into fluorapatite in the presence of fluoride.[19] Thus, APF solution was used in the present study.

Oral hard tissues absorb light in the infrared spectrum.[13] The radiation produced by a CO_2 laser in the infrared spectrum coincides with the apatite absorption bands.[26] For this reason, a CO_2 laser was used in the present study. At a continuous wave[11] mode or a pulsed wave[15] mode, a CO_2 laser shows high resistance to acids. Energy is dissipated if the pulse duration is short, whereas if it is longer, some amount of heat is deposited in upper layers. So, a pulsed wave CO_2 laser was used in the present study. A low-power surgical laser system was used in this study. The test groups were lased with a CO_2 laser at 10.6 µm wavelength, energy level of 5 J/cm^2 for 15 seconds with pulse duration of 800 µs and each pulse separated by 100 ms.[27–30]

Among the various methods of fluoride estimation, acid etch biopsy along with the use of a fluoride ion selective electrode is comparatively easier[31] and has been used previously by various authors like Mellberg,[32] Mellberg and Loertscher,[33] Margill,[34] Hattab and Frostell,[21] Hattab,[35] Crall and Bjerga,[36] and Tepper et al.[11]

While determination of fluoride using acid etch biopsy, sharp etch is of interest because an irregularly removed layer directly influences the fluoride profiles in the enamel. The 0.5 M perchloric acid was used for acid etch biopsy in this study because of its ability to give a sharper etch pattern.[37] Fluoride ion concentration in biopsied solutions from test and control groups was determined using a fluoride ion selective electrode. Same solutions were then used for the estimation of the Ca content, which was then used for the determination of the dissolved enamel, i.e., WE etched away. Weight of the dissolved enamel was calculated considering the Ca content of the human tooth enamel to be 37.4%. The resultant fluoride content of test and control solutions and calculated enamel weight etched away were then used for the calculation of the fluoride content in human tooth enamel slabs using the formula given by Hattab and Frostell:[21]

$$F \text{ content of tooth in ppm} = 10^6 \, \frac{\text{Weight of dissolved fluoride } (\mu\text{g})}{\text{Weight of dissolved enamel } (\mu\text{g})}$$

The test and control samples were evaluated by ICP-AES for relative acid resistance. The calcium levels were used to calculate the WE etched away and enamel depth penetration. Calcium analysis was performed using an atomic emission spectrometer. Use of an atomic emission spectrometer for analysis of the Ca content has also been done by Jetpurwala and Damle.[38]

Group II samples showed reduced enamel solubility when compared to the control group. On comparing with the WE of the control group, i.e., 68.17 ± 5.19 ppm, the difference was statistically significant similar to findings of authors such as Bibby,[1] Mellberg,[32] Garcia-Godoy et al.,[39] and Delbem and Cury.[40] Significant increased resistance to acid attack and changes in enamel permeability have been obtained after lasing the tooth samples with a high-energy CO_2 laser.[5,7,9,15,41] The findings of these authors cannot be correlated with the present study due to the difference in the methodology and the type of CO_2 laser used. High acid resistance after exposure

Table 1: Calcium content, WE etched away, enamel depth, fluoride content in solution, and fluoride content in tooth enamel slabs of group I

Group I						
Sample no.	*Ca in solution (ppm)*	*WE in solution (ppm)*	*Calculated depth (μm)*	*Fluoride in solution (ppm)*	*Total fluoride content in solution (μg)*	*Calculated fluoride in tooth (ppm)*
1	25.14	67.22	38.08	0.03	0.27	406
2	25.87	69.17	39.18	0.02	0.25	359
3	23.25	62.17	35.22	0.03	0.34	552
4	26.02	69.57	39.41	0.03	0.31	450
5	25.01	66.87	37.88	0.03	0.29	438
6	22.13	59.17	33.52	0.03	0.34	581

Table 2: Calcium content, WE etched away, enamel depth, fluoride content in solution, and fluoride content in tooth enamel slabs of group II

Group II						
Sample no.	*Ca in solution (ppm)*	*WE in solution (ppm)*	*Calculated depth (μm)*	*Fluoride in solution (ppm)*	*Total fluoride content in solution (μg)*	*Calculated fluoride in tooth (ppm)*
1	18.22	48.72	27.60	0.31	3.10	6361
2	23.73	63.45	35.94	0.26	2.63	4146
3	22.19	59.33	33.61	0.27	2.68	4518
4	19.98	53.42	30.26	0.30	3.03	5675
5	20.18	53.96	30.57	0.44	4.43	8218
6	17.22	46.04	26.08	0.46	4.58	9957
7	17.59	47.03	26.64	0.30	3.03	6446
8	20.62	55.13	31.23	0.38	3.83	6952
9	19.6	52.41	29.69	0.50	4.99	9513
10	17.29	46.23	26.19	0.42	4.23	9148
Mean	19.66 ± 2.17	52.57 ± 5.79	29.78 ± 3.28	0.37 ± 0.09	3.37 ± 1.25	6546.82 ± 2659.15

Table 3: Calcium content, WE etched away, enamel depth, fluoride content in solution, and fluoride content in tooth enamel slabs of group III

Group III						
Sample no.	*Ca in solution (ppm)*	*WE in solution (ppm)*	*Calculated depth (μm)*	*Fluoride in solution (ppm)*	*Total fluoride content in solution (μg)*	*Calculated fluoride in tooth (ppm)*
1	18.75	50.13	28.40	0.05	0.54	1081
2	19.98	53.42	30.26	0.04	0.42	791
3	20.3	54.28	30.75	0.02	0.18	336
4	20.57	55.00	31.16	0.08	0.75	1370
5	20.43	54.63	30.94	0.06	0.60	1104
6	19.97	53.40	30.25	0.04	0.38	717
7	17.1	45.72	25.90	0.09	0.85	1867
8	19.87	53.13	30.10	0.06	0.55	1041
9	15.2	40.64	23.02	0.07	0.65	1607
10	16.2	43.32	24.54	0.06	0.62	1436
Mean	18.84 ± 1.96	50.37 ± 5.24	28.53 ± 2.97	0.06 ± 0.02	0.56 ± 0.20	1141.00 ± 480.28

Table 4: Calcium content, WE etched away, enamel depth, fluoride content in solution, and fluoride content in tooth enamel slabs of group IV

Group IV						
Sample no.	*Ca in solution (ppm)*	*WE in solution (ppm)*	*Calculated depth (μm)*	*Fluoride in solution (ppm)*	*Total fluoride content in solution (μg)*	*Calculated fluoride in tooth (ppm)*
1	19.81	52.97	30.01	0.84	8.39	15844
2	20.25	54.14	30.67	1.44	14.38	26555
3	18.04	48.24	27.32	0.79	7.86	16299
4	21.27	56.87	32.22	0.89	8.87	15591
5	20.69	55.32	31.34	0.69	6.86	12406
6	21.17	56.60	32.07	0.89	8.89	15709
7	22.26	59.52	33.72	0.69	6.86	11531
8	15.96	42.67	24.17	0.61	6.06	14204
9	18.86	50.43	28.57	0.63	6.32	12537
10	20.19	53.98	30.58	0.70	7.04	13045
Mean	19.85 ± 1.82	53.07 ± 4.88	30.07 ± 2.77	0.82 ± 0.24	8.15 ± 2.41	15372.10 ± 4277.46

Table 5: Calcium content, WE etched away, enamel depth, fluoride content in solution, and fluoride content in tooth enamel slabs of group V

Group V						
Sample no.	*Ca in solution (ppm)*	*WE in solution (ppm)*	*Calculated depth (μm)*	*Fluoride in solution (ppm)*	*Total fluoride content in solution (μg)*	*Calculated fluoride in tooth (ppm)*
1	13.03	34.84	19.74	0.53	5.30	15218
2	14.65	39.17	22.19	0.61	6.13	15654
3	16.31	43.61	24.70		6.26	14359
4	17.66	47.22	26.75	0.62	6.21	13155
5	16.53	44.20	25.04	0.56	5.61	12694
6	18.11	48.42	27.43	0.64	6.36	13139
7	15.73	42.06	23.83	0.69	6.89	16387
8	17.86	47.75	27.05	0.64	6.36	13323
9	20.33	54.36	30.79	0.74	7.40	13617
10	15.63	41.79	23.67	0.73	7.33	17544
Mean	16.58 ± 2.03	44.34 ± 5.44	25.12 ± 3.08	0.64 ± 0.07	6.39 ± 0.67	14509.00 ± 1625.53

Table 6: Calcium content, WE etched away, enamel depth, fluoride content in solution, and fluoride content in tooth enamel slabs of group VI

Group VI						
Sample no.	*Ca in solution (ppm)*	*WE in solution (ppm)*	*Calculated depth (μm)*	*Fluoride in solution (ppm)*	*Total fluoride content in solution (μg)*	*Calculated fluoride in tooth (ppm)*
1	26.77	71.58	40.55	1.00	10.03	14011
2	29.44	78.72	44.59	1.10	10.97	13940
3	32.34	86.47	48.98	1.00	9.97	11531
4	30.17	80.67	45.70	1.00	9.97	12361
5	29.1	77.81	44.08	1.05	10.47	13459
6	28.36	75.83	42.96	1.20	11.98	15792
7	27.42	73.32	41.53	1.20	12.05	16434
8	28.68	76.68	43.44	1.07	10.74	14004
9	26.96	72.09	40.84	1.10	11.05	15328
10	27.91	74.63	42.27	1.15	11.50	15409
Mean	28.72 ± 1.68	76.78 ± 4.48	43.49 ± 2.54	1.09 ± 0.08	10.87 ± 0.79	14226.90 ± 1543.75

Table 7: Table of means with standard deviation

Group	*Ca in solution (ppm)*	*WE in solution (ppm)*	*Calculated depth (μm)*	*Fluoride in solution (ppm)*	*Total fluoride content in solution (μg)*	*Calculated fluoride in tooth (ppm)*
Group I	25.50 ± 1.94	68.17 ± 5.19	38.62 ± 2.94	0.03 ± 0.01	0.27 ± 0.06	399.90 ± 112.86
Group II	19.66 ± 2.17	52.57 ± 5.79	29.78 ± 3.28	0.37 ± 0.09	3.37 ± 1.25	6546.82 ± 2659.15
Group III	18.84 ± 1.96	50.37 ± 5.24	28.53 ± 2.97	0.06 ± 0.02	0.56 ± 0.20	1141.00 ± 480.28
Group IV	19.85 ± 1.82	53.07 ± 4.88	30.07 ± 2.77	0.82 ± 0.24	8.15 ± 2.41	15372.10 ± 4277.46
Group V	16.58 ± 2.03	44.34 ± 5.44	25.12 ± 3.08	0.64 ± 0.07	6.39 ± 0.67	14509.00 ± 1625.53
Group VI	28.72 ± 1.68	76.78 ± 4.48	43.49 ± 2.54	1.09 ± 0.08	10.87 ± 0.79	14226.90 ± 1543.75

to a low-power CO_2 laser has been reported by Hsu,[27] Whitters and Strang,[28] Tepper et al.,[11] and Oliveira et al.[29]

Also, there was statistically significant difference between WE of groups II, III, and IV (52.57 ± 5.79 ppm, 50.37 ± 5.24 ppm, and 53.07 ± 4.88 ppm, respectively) and group V (44.34 ± 5.44 ppm); group V reported more acid resistance than other groups. These findings are in agreement with Tagomori and Morioka,[42] Kakade et al.,[17] and Flatiz et al.[43]

An interesting finding was seen when WE of group VI (76.78 ± 4.48 ppm) was compared with WE of all other groups. There was a significant difference between WE of group VI and all other groups suggesting that the acid resistance of enamel slabs had decreased after exposure to a low-power laser system through topically applied fluoride solution. Meurman et al.[19] showed that phase transformation of hydroxyapatite into fluorapatite is possible when a CO_2 laser was used with the threshold energy density of 38 J/cm^2 through topically applied sodium fluoride solution.

A possible explanation for decreased acid resistance after lasing through applied fluoride solution in the present study can be attributed to the use of insufficient power of the laser. Due to presence of the water content of 1.23% APF solution on the tooth enamel slab, the low-power laser failed to raise the surface temperature adequately and may have led to increased permeability of the enamel rendering it less resistant to acid attack.

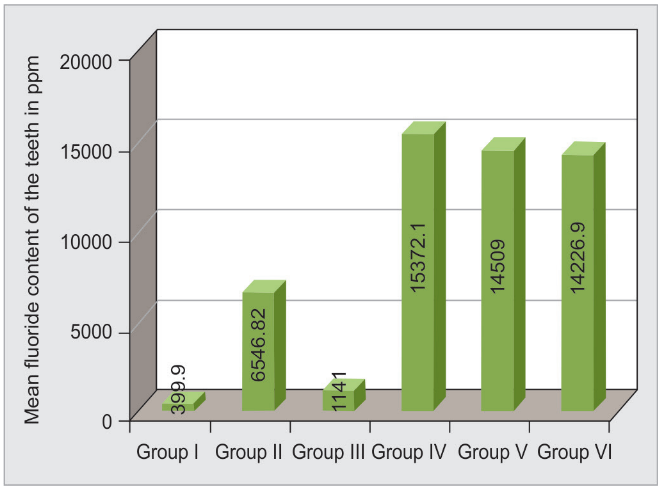

Fig. 5: Comparison of mean fluoride content of the teeth in different groups

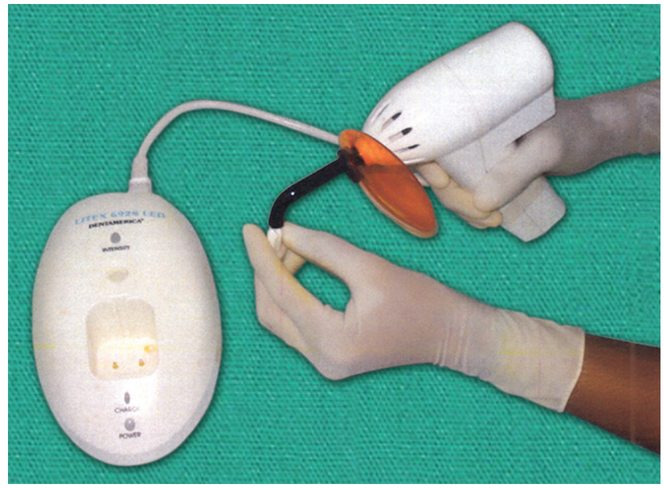

Fig. 6: Tooth being checked for surface cracks, white spots, or mottling using contrast light

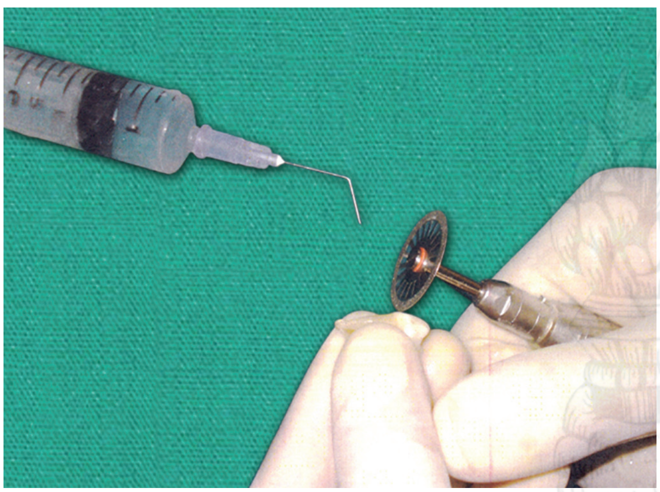

Fig. 7: Preparation of enamel slabs

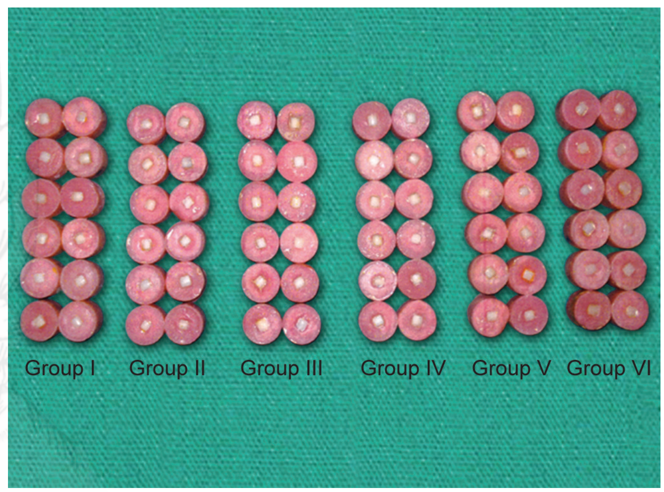

Fig. 8: Enamel slabs mounted in acrylic resin

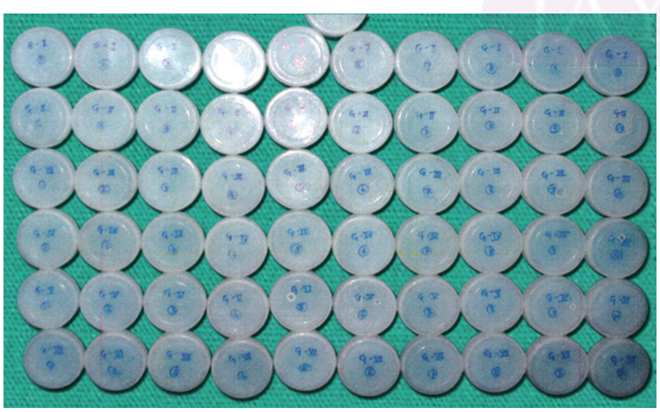

Fig. 9: Samples prepared for fluoride analysis and wet chemical analysis

The heat generated by exposure to the CO_2 laser may have been dissipated in the applied solution itself rendering increased activity of H^+ and F^- ions in solution that has led to dissolution of apatite crystals and formation of soluble calcium fluoride-like deposits.

Moreover, formation of calcium phosphate and conversion of hydroxyapatite to fluorapatite or fluorohydroxytapatite are spontaneous reactions and are exothermic in nature. But conversion of fluorapatite to calcium fluoride and calcium phosphate is an endothermic reaction. Heat generated after CO_2 laser treatment through applied 1.23% APF solution might have been used for conversion of fluorapatite to calcium fluoride and calcium phosphate with resultant decrease in acid resistance. There was marked increase in uptake of fluoride in group II tooth enamel slabs, difference being statistically significant. These findings are in agreement with Mellberg[32] and Mellberg and Loertscher.[33] The fluoride content of group III enamel slab was 1141.00 ± 480.28 ppm while that of the control group was 399.90 ± 112.86 ppm. There has been an increase in the fluoride content of the surface enamel suggesting redistribution of fluoride that was already present in the human tooth enamel slabs.

There has been an overall increase in fluoride uptake of group IV, group V, and group VI (15372.10 ± 4277.46 ppm, 14509.00 ± 1625.53 ppm, and 14226.90 ± 1543.75 ppm, respectively) human tooth enamel slabs as compared to the control group (399.90 ± 112.86 ppm) human tooth enamel slabs, difference being statistically highly significant. This suggests that there is definite increase in fluoride uptake of human tooth enamel after treatment with a CO_2 laser. Other authors who have reported increased fluoride uptake after laser treatment are Tepper et al.[11] and Tagomori and Morioka.[42] However, Hattab[35] did not notice any significant difference in fluoride uptake by lased and unlased human tooth enamel. The observed difference might be attributed to the use of an argon laser by the author that has poor absorption in the human tooth enamel.

Observed increase in fluoride uptake of the tooth enamel after lasing with a CO_2 laser through topically applied fluoride solution in the present study is in accordance with Meurman et al.[19] and Tepper et al.[11] But the resultant decrease in acid resistance of group VI human tooth enamel slabs in the present study suggests that increased fluoride uptake may not be necessarily associated with increased acid resistance. As per findings of Sterns and Berndt,[44] reliance on fluoride uptake data alone in considering the reaction of human apatite with 1.23% APF solutions can lead to false or misleading conclusions. Often high fluoride uptake data are interpreted as indicating a high conversion of fluorapatite, whereas they could indicate considerable destruction of the apatite to calcium fluoride. Occurrence of this phenomenon in group VI human tooth enamel slabs of the present study can be possible considering the decrease in their acid resistance.

Conclusion

The present study concludes that a low-power CO_2 laser can be used effectively in combination with topically applied 1.23% APF solution in order to make enamel more resistant to acid attack, thereby helping in controlling dental caries process. However, clinical safety of the CO_2 laser in caries prevention is yet to be proven. Also, "does the same amount of acid resistance is obtained after the combination of CO_2 laser and topically applied fluoride in oral cavity?" is yet to be determined.

Acknowledgments

We would sincerely like to thank Prof Dhanesh Manik, Department of Mechanical Engineering, IIT, Powai; Mr Arijit Laik, Department of Material Science Division, Bhabha Atomic Research Centre, Trombay; and Mrs Shilpa, Earth and Science Department, in providing insight and expertise that greatly assisted our research study.

References

1. Bibby BG. Use of fluorides in prevention of dental caries III. A consideration of the effectiveness of various mixtures. J Am Dent Assoc 1947;34(1):26–32. DOI: 10.14219/jada.archive.1947.0009.
2. Brudevold F, De Paola PF. Studies on topically applied acidulated phosphate fluoride at Forsyth dental center. Dent Clin North Am 1966; 299–308.
3. Horowitz HS, Doyle J. The effect on dental caries of topically applied acidulated phosphate-fluoride: results after three years. J Am Dent Assoc 1971;82:359–365. DOI: 10.14219/jada.archive.1971.0063.
4. Benediktsson S, Retief DH, et al. The effect of contact time of acidulated phosphate fluoride on fluoride concentration in human enamel. Arch Oral Biol 1982;27(7):567–572. DOI: 10.1016/0003-9969(82)90071-1.
5. Stern RH, Sognnaes RF, et al. Laser effect on *in vitro* enamel permeability and solubility. J Am Dent Assoc 1966;73(4):838–843. DOI: 10.14219/jada.archive.1966.0319.
6. Featherstone JD, Nelson DG. Laser effects on dental hard tissues. Adv Dent Res 1987;1(1):21–26. DOI: 10.1177/08959374870010010701.
7. Nelson DG, Wefel JS, et al. Morphology, histology and crystallography of human dental enamel treated with pulsed low-energy infrared laser radiation. Caries Res 1987;21(5):411–426. DOI: 10.1159/000261047.
8. Lobene RR, Bhussry BR, et al. Interaction of carbon dioxide laser radiation with enamel and dentin. J Dent Res 1968;47(2):311–317. DOI: 10.1177/00220345680470021901.
9. Fox JL, Yu D, et al. Combined effects of laser irradiation and chemical inhibitors on the dissolution of dental enamel. Caries Res 1992;26(5):333–339. DOI: 10.1159/000261464.
10. Fox JL, Yu D, et al. Initial dissolution rate studies on dental enamel after CO_2 laser irradiation. J Dent Res 1992;71(7):1389–1398. DOI: 10.1177/00220345920710070701.
11. Tepper SA, Zehnder M, et al. Increased fluoride uptake and acid resistance by CO_2 laser-irradiation through topically applied fluoride on human enamel *in vitro*. J Dent 2004;32:635–641. DOI: 10.1016/j.jdent.2004.06.010.
12. Stern RH, Vahl J, et al. Lased Enamel: Ultrastructural observations of pulsed carbon dioxide laser effects. J Dent Res 1972;51:455–460. DOI: 10.1177/00220345720510023501.
13. Kantorowitz ZVI, Featherstone JDB, et al. Caries prevention by CO_2 laser treatment: dependency on the number of pulses used. J Am Dent Assoc 1998;129:585–591. DOI: 10.14219/jada.archive.1998.0276.
14. Featherstone JDB, Barrett-Vespone NA, et al. CO_2 Laser inhibition of artificial caries-like lesion progression in dental enamel. J Dent Res 1998;77:1397–1403. DOI: 10.1177/00220345980770060401.
15. Lakshmi A, Shobha D, et al. Prevention of caries by pulsed Co_2 laser pre treatment of enamel: An *in vitro* study. J Indian Soc Pedod Prev Dent 2001;19(4):152–156.
16. Tagomori S, Morioka T. Combined effects of laser and fluoride on acid resistance of human dental enamel. Caries Res 1989;23(4):225–231. DOI: 10.1159/000261182.
17. Kakade A, Damle SG, et al. Combined effect of carbon-dioxide laser and neutral 2% NaF on acid resistance of human tooth enamel. J Indian Soc Pedod Prev Dent 1996;14(1):26–30.
18. Hsu Chin-Ying S, Xiaoli G, et al. Effects of CO_2 laser on fluoride uptake in enamel. J Dent 2004;32(2):161–167. DOI: 10.1016/j.jdent.2003.10.004.
19. Meurman JH, Hemmerlé J, et al. Transformation of hydroxyapatite to fluorapatite by irradiation with high-energy CO_2 laser. Caries Res 1997;31(5):397–400. DOI: 10.1159/000262425.
20. McCormack SM, Fried D, et al. Scanning electron microscope observation of CO_2 laser effects on dental enamel. J Dent Res 1995;74(10):1702–1708. DOI: 10.1177/00220345950740101201.
21. Hattab F, Frostell G. The release of fluoride from two products alginate impression materials. Acta Odontol Scand 1980;38:385–395. DOI: 10.3109/00016358009033609.
22. Featherstone JDB, Glena R, et al. Dependence of *in vitro* demineralization of apatite and remineralization of dental enamel on fluoride concentration. J Dent Res 1990;69(Spec Issue):620–625. DOI: 10.1177/00220345900690S121.
23. Stern RH, Sognnaes RF. Laser beam effects on dental hard tissues. J Dent Res 1964;43:873.
24. Ferreira JM, Palamara J, et al. Effects of continuous-wave CO_2 laser on the ultrastructure of human dental enamel. Arch Oral Biol 1989;34(7):551–562. DOI: 10.1016/0003-9969(89)90094-0.
25. Kantorowitz Z, Featherstone JDB, et al. Augmentation of CO_2 laser inhibition of *in vitro* caries by fluoride. J Dent Res 1996; 75(Spec Issue):25.
26. Fried D, Ragadio J, et al. Dental hard tissue modification and removal using sealed transverse excited atmospheric pressure lasers operating at 9.6 and 10.6 µm. J Biomed Opt 2001;6(2):231–238. DOI: 10.1117/1.1344192.
27. Hsu CYS, Jordan TH, et al. Effects of low-energy CO_2 laser irradiation and the organic matrix on inhibition of enamel demineralization. J Dent Res 2000;79(9):1725–1730. DOI: 10.1177/00220345000790091401.

28. Whitters CJ, Strang R. Preliminary investigation of a novel carbon dioxide laser for applications in dentistry. Lasers Surg Med 2000;26:262–269. DOI: 10.1002/(SICI)1096-9101(2000)26:3<262::AID-LSM3>3.0.CO;2-7.
29. Esteves-Oliveira M, Zezell DM, et al. CO_2 laser (10.6 µm) parameters for caries prevention in dental enamel. Caries Res 2009;43(4):261–268. DOI: 10.1159/000217858.
30. Zulerian MJ, Fried D, et al. Optical properties of dental enamel in the mid-IR determined by pulsed photothermal radiometry. IEEE J Sel Top Quant 1999;5:1083–1089. DOI: 10.1109/2944.796333.
31. Bosch T, Booij M. Quantitative comparison of methods measuring fluoride in solutions or in enamel. J Dent Res 1992;71(Spec Issue): 944–948.
32. Mellberg JR. Fluoride uptake by intact human tooth enamel from acidulated fluoride-phosphate preparations. J Dent Res 1966;45(2):303–307. DOI: 10.1177/00220345660450021501.
33. Mellberg JR, Loertscher KL. Fluoride acquisition *in vitro* by etched enamel from acidulated phosphate-fluoride preparations. J Dent Res 1973;52:447–450. DOI: 10.1177/00220345730520031001.
34. Margill S. Influence of fluoride on rate of dissolution of hydroxyapatite in acidic buffer solution. Caries Res 1975;9:45–49. DOI: 10.1159/000260141.
35. Hattab FN. *In vitro* fluoride uptake by lased and unlased ground human enamel. ASDC J Dent Child 1987;54(1):15–17.
36. Crall JJ, Bjerga JM. Enamel fluoride retention after DCPD and APF application and prolonged exposure to fluoride *in vitro*. J Dent Res 1986;65(3):387–389. DOI: 10.1177/00220345860650030201.
37. Dijkman AG, Tak J, et al. Influence of HClO4 strength and Etching time on rate of etching and surface roughness of human enamel. Cries Res 1983;17:14–22. DOI: 10.1159/000260644.
38. Jetpurwala A, Damle SG. Study to evaluate the remineralization ability of casein phosphopeptide – amorphous calcium phosphate on artificial caries like lesion using X-ray fluorescence, inductively coupled plasma atomic emission spectrometer and scanning electron microscopy with energy dispersive X-ray analysis – an *in situ* study, MDS Thesis, Bombay University; 2008.
39. Garcia-Godoy F, Hicks MJ, et al. Acidulated phosphate fluoride treatment and formation of caries-like lesions in enamel: effect of application time. J Clin Pediatr Dent 1995;19(2):105–110.
40. Delbem AC, Cury JA. Effect of application time of APF and NaF gels on microhardness and fluoride uptake of *in vitro* enamel caries. Am J Dent 2002;15(3):169–172.
41. Ohoi T, Morioka T. A possible mechanism of acquired acid resistance of human dental enamel by laser irradiation. Caries Res 1990;24: 86–92. DOI: 10.1159/000261245.
42. Tagomori S, Morioka T. Combined effects of laser and fluoride on acid resistance of human dental enamel. Caries Res 1989;23(4): 225–231. DOI: 10.1159/000261182.
43. Flaitz CM, Hicks MJ, et al. Argon laser irradiation and acidulated phosphate fluoride treatment in caries-like lesion formation in enamel: an *in vitro* study. Pediatr Dent 1995;17(1):31–35.
44. Sterns RI, Berndt AF. Reaction of acidulated phosphate fluoride solutions with human apatite. J Dent Res 1973;52(6):1253–1260. DOI: 10.1177/00220345730520061601.