Original Article

Assessment of Inhibition of Mineral Loss from Human Tooth Enamel by Carbon Dioxide Laser and 1.23% Acidulated Phosphate Fluoride

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Abstract

Aims and Objectives: The efficacy of carbon dioxide (CO_2) laser irradiation combined with fluoride in inhibiting enamel demineralization has been demonstrated by several laboratory investigations. However, there are very few reports about the *in situ* or *in vivo* caries preventive effect of CO_2 laser combined with topical fluoride on dental enamel. Hence, an *in situ* study was designed and carried out to assess inhibition of mineral loss from human tooth enamel by CO_2 laser and 1.23% acidulated phosphate fluoride (APF).

Material and Methods: Impressions of upper and lower arch of the volunteers were made in alginate impression material. Study models were poured, duplicated, and duly labeled. On the working model, appliances were fabricated in acrylic resin to fit the upper dental arch of the volunteers. Four enamel slabs (one from each group) were fitted on the palatal surface of the appliance as close as possible to posterior teeth. Surfaces of slabs were kept below the outer surface of acrylic. The analysis was done using SPSS version 15 (SPSS Inc., Chicago, IL, USA) Windows software program.

Results: Statistically significant increase in inhibition of mineral loss of enamel slabs when treated individually or in a combination of low power CO_2 laser and 1.23% APF solution. The application of 1.23% APF solution after low power CO_2 laser treatment showed maximum inhibition of mineral loss.

Conclusion: The combined use of this specific laser treatment plus fluoride was more successful than either laser treatment or fluoride alone in the inhibition of mineral loss in the mouth. The results of this study also suggest that the combination of low power laser treatment with fluoride therapy may be effective as a caries inhibition treatment.

Keywords: *Assessment, carbon dioxide laser, dental caries, fluoride, mineral loss*

Introduction

The greatest challenge in managing dental caries process is to sort how the contributing factors interact in a way that translates into effective strategies for disease diagnosis, prevention, and therapy. The focus of the management of dental caries has shifted from intervention to prevention in the past few decades. The aim of prevention is to diagnose the disease before any irreversible carious lesion has been established. However, because of the nature of this disease process, there can be no single "preventive program" to be superimposed uncritically on all populations.[1] The important question remains how to control caries lesion as cost-effectively as possible. The severe "old" recommended preventive programs are no longer effective, not just because the caries incidence rate has changed but as the environment and changing trends among the population.[2,3]

Among various means for prevention of dental caries, fluoride has emerged among a plethora of elements as

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the redeeming messiah to the plague of dental caries. It has been postulated that the ability to conquer this indomitable disease lies within the folds of the widespread and judicious use of fluoride.[4] In fact, the most developed countries have benefited from the use of fluoride.[5,6] Over the years, topical fluoride application has managed to give a consistent decline in the prevalence of dental caries. Among the professionally applied topical fluorides, 1.23% acidulated phosphate fluoride (APF) has been found to be promising.[7,8] However, it has been postulated that 4 min application of 1.23% APF is not sufficient for the adequate effect and fluoride should be in contact with the tooth at least for 2–6 h to have optimum effect.[9,10]

Of all the other avenues open to caries prevention, prevention through increased acid resistance and inhibition to mineral losses a result of laser treatment is one of the most fascinating and promising. Different explanations for the increased acid resistance of the laser treated enamel have been suggested, such as decreased enamel permeability,[11] alterations in chemical composition, or a combination of both.[12] Carbon dioxide (CO_2) laser irradiation of human dental enamel at 10.6 μm wavelength and continuous as well as pulsed[13-15] wave modeled to significantly reduced dissolution rates. Instantaneous transformation of hydroxyapatite to fluorapatite by irradiation with a high-energy CO_2 laser in the presence of sodium fluoride has been demonstrated *in vitro*.[16] Furthermore, 1.23% APF application after laser irradiation has been shown to have greater fluoride uptake and acid resistance as compared to sodium fluoride application after laser treatment.[17]

While laser treatment in combination with fluoride application appears to have several advantages over fluoride application alone, some untoward effects are observed on enamel surfaces after laser treatment, such as surface cracking and melted areas.[18] In order to overcome these untoward effects of laser, laser application through fluoride solution has been tried, but very few studies are available. The efficacy of CO_2 laser irradiation combined with fluoride in inhibiting enamel demineralization has been demonstrated by several laboratory investigations. However, there are very few reports about the *in situ* or *in vivo* caries preventive effect of CO_2 laser combined with topical fluoride on dental enamel.[19,20] Hence, an *in situ* study was designed and carried out to assess inhibition of mineral loss from human tooth enamel by CO_2 laser and 1.23% APF.

Materials and Methods

The present *in situ* study was carried out in the Department of Pediatric and Preventive Dentistry, in collaboration with the Department of Sophisticated Analytical Instrument Facility and Department of Metallurgical and Material Sciences, Indian Institute of Technology to assess inhibition of mineral loss from human tooth enamel by CO_2 laser and 1.23% APF.

Before starting the study, the necessary approval was obtained from the Local Ethical Committee (Letter no: GDCB/25/2017), and permission was sought from volunteers participating in the study. Volunteers in the age group of 18–25 years were selected for the study. A written informed consent was obtained after explaining the study details, schedule, and possible risks involved in the study. Complete medical and dental history of volunteers was elicited.

Methodology

Preparation of enamel slabs

Eighty sound premolars extracted for orthodontic reasons were used in the study. The teeth were washed with water and stored in prepared thymol solution under refrigeration till use. The selected teeth were checked for surface cracks, white spots, or mottling using contrast light. Using water-cooled diamond disc, enamel slabs of 4 mm × 4 mm × 1.5 mm were cut from buccal surface of each tooth. The dimensions were standardized using vernier caliper.

Preparation of 1.23% acidulated phosphate fluoride solution

The topical fluoride solution was prepared by adding 4 g of pure sodium fluoride in 200 ml of 0.1 M phosphoric acid in a plastic beaker. To this solution, 48% hydrofluoric acid was added to adjust the pH between 3 and 3.5 using Eutech pH meter and fluoride concentration at 1.23%. The solution was prepared under constant stirring using a magnetic stirrer and fischer.

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 10.6 μm wavelength.

Lasing the samples

The entire enamel surface of the samples was irradiated for 15 s by the single operator by moving the laser probe tip continuously at a distance <1 mm from the sample surface.

The groups studied in this investigation were as follows:

- Group A: control samples, sound tooth enamel slabs receiving no treatment
- Group B: Enamel slabs treated with 1.23% APF solution for 4 min

- Group C: Enamel slabs lased for 15 s 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 D: Enamel slabs which were treated with 1.23% APF solution for 4 min after lasing.

Acrylic palatal appliance

Impressions of upper and lower arch of the volunteers were made in alginate impression material. Study models were poured, duplicated, and duly labeled. On the working model, appliances were fabricated in acrylic resin to fit the upper dental arch of the volunteers. Four enamel slabs (one from each group) were fitted on the palatal surface of the appliance as close as possible to posterior teeth. Surfaces of slabs were kept below the outer surface of acrylic.

Study design

The volunteers were given a commercially available nonfluoridated dentifrice to use for 2 weeks (washout period) before the actual study. The volunteers were recalled at the end of the 2 weeks of washout period for delivering the acrylic palatal appliance with the incorporated four enamel slabs. They were instructed to continue the normal tooth brushing with the same nonfluoridated dentifrice for the next 4 weeks of study. Before the commencement of the study, the volunteers were demonstrated the correct brushing technique to brush their teeth.

Volunteers were instructed to wear acrylic palatal appliance all the time, including at night. They were allowed to remove the appliances only during meals and when performing oral hygiene. They were also instructed to remove the appliance and drip one drop of 20% sucrose solution onto all 4 enamel slabs 4 times a day at a fixed time interval of 6 h. Before the palatal appliance was replaced in the mouth, a 5 min waiting time was standardized for sucrose diffusion into the dental plaque. The appliances were extraorally brushed, except the enamel slabs, and volunteers were asked to brush carefully to avoid disturbing the plaque.

At the end of the study period of 4 weeks, the appliances were retrieved, and the slabs were removed from the appliances, washed with deionized water and stored in Eppendorf tubes duly labeled till further analysis.

The enamel slabs were analyzed using,

a. Wet Chemical Analysis
b. Surface Microhardness Analysis
c. Polarized Light Microscopy (PLM).

Following is the schematic representation of the study design:

- Total slabs used for study = 80
- Wet chemical analysis of 10 slabs from each group was done
- Surface microhardness testing of 5 slabs from each group was done before and after the study period
- 5 enamel slabs from each group were assessed using PLM.

The results obtained were tabulated and following statistical tests were applied:

- Mean and Standard deviation
- ANOVA test
- Tukey test.

Results

The mean total calcium content in Group A, B, C, and D were, respectively, 388.77 ± 32.35 μg, 284.74 ± 24.15 μg, 299.99 ± 16.28 μg, and 206.92 ± 29.65 μg [Table 1]. The mean enamel weight leached away was found to be 1039.48 ± 86.49 μg, 761.35 ± 64.56 μg, 802.11 ± 43.53 μg, and 553.25 ± 79.27 μg respectively in Group A, B, C, and D [Table 2]. The mean enamel thickness removed was found to be 22.02 ± 1.83 μm, 16.13 ± 1.37 μm, 16.99 ± 0.92 μm, and 11.72 ± 1.68 μm in Group A, B, C, and D, respectively [Table 3]. In a comparison of all the groups, lowest values for all the parameter were found in group D.

In the intergroup comparison of relative acid resistance using the weight of enamel etched away (WE) as a parameter the difference in Groups A and B, A and C, A and D, B and D, C and D was found to be statistically significant with $P < 0.05$. Whereas the difference between Group B and C was marginally less and the difference was not found to be statistically significant with $P > 0.05$ [Tables 4-6].

Table 1: Calcium content in solution among various groups

Groups	Calcium content, mean±SD (ug)	*P*
Group A	388.77±32.35	0.01*
Group B	284.74±24.15	
Group C	299.99±16.28	
Group D	206.92±29.65	

Test applied one-way ANOVA, *Statistically significance at $P \leq 0.05$. SD=Standard deviation

Table 2: WE leached away in solution (ug)

Groups	WE leached away in solution, mean±SD (ug)	*P*
Group A	1039.48±86.49	0.05*
Group B	761.35±64.56	
Group C	802.11±43.53	
Group D	553.25±79.27	

Test applied one-way ANOVA, *Statistically significance at $P \leq 0.05$. SD=Standard deviation, WE=Weight of enamel

Among all the groups, Group D showed lowest values of mean calcium content in prepared solution (206.92 ± 29.65 µg), mean WE (553.25 ± 79.27 µg), and mean thickness of enamel removed (11.72 ± 1.68 µm) suggestive of maximum acid resistance.

Surface microhardness analysis

The mean surface microhardness value of enamel slabs from all the four groups before the study period were 320.60 ± 9.21, 320.60 ± 7.89, 318.60 ± 10.92, and 321.807 ± 0.22, respectively [Table 7]. The difference in microhardness value among the study group was not statistically significant as the $P > 0.05$. After the end of the study period of 4 weeks, the value was found to be 112 ± 4.30, 165.60 ± 4.77, 167.40 ± 6.58, and 224.80 ± 6.61, respectively [Table 8]. There was decrease in the value after the study period, and the difference was found to be statistically different in all the groups.

When the intergroup comparison of the change in surface microhardness between Group A and B was done, the value in B was found to be <A. The difference of decrease in surface microhardness of these two groups was statistically significant with $P < 0.05$. The difference of decrease in surface microhardness from Group A to C and A to D was found to be statistically significant with $P < 0.05$. The difference in surface microhardness of both Groups B and C was statistically not significant with $P > 0.05$. The difference of decrease in surface microhardness of these two Groups B and D, C and D was statistically significant with $P < 0.05$ [Table 9]. Among all the groups, Group D showed least change in surface microhardness, i.e., only 46.50% of the Group A suggestive of maximum inhibition of mineral loss.

Polarized light microscopy

The mean depth of demineralized lesions in enamel slabs were 220.60 ± 7.02 µm, 160.60 ± 5.73 µm, 167.20 ± 5.45 µm, and 120.20 ± 5.07 µm in Group A, Group B, Group C, and Group D, respectively [Table 10]. In the intergroup comparison of depth of demineralized lesion, the difference in mean of the depth of demineralized lesion of these Groups A and B, A and C, A and D, B and D, C and D was statistically significant with $P < 0.05$ [Table 11]. The difference in mean of the depth of demineralized lesion

Table 3: Thickness of enamel removed (um)

Groups	Thickness of enamel removed (um), mean±SD	*P*
Group A	22.02±1.83	0.004*
Group B	16.13±1.37	
Group C	16.99±0.92	
Group D	11.72±1.68	

Test applied one-way ANOVA, *Statistically significance at $P \leq 0.05$. SD=Standard deviation

Table 4: Intergroup comparison of total calcium in solution

Comparison	*P*≤0.05
Group A versus Group D	Yes
Group A versus Group B	Yes
Group A versus Group C	Yes
Group C versus Group D	Yes
Group B versus Group D	Yes
Group B versus Group C	No

Test applied one-way ANOVA with *post hoc* Tukey's test

Table 5: Intergroup comparison of WE leached away in solution

Comparison	*P*≤0.05
Group A versus Group D	Yes
Group A versus Group B	Yes
Group A versus Group C	Yes
Group C versus Group D	Yes
Group B versus Group D	Yes
Group B versus Group C	No

Test applied one-way ANOVA with *post hoc* Tukey's test. WE=Weight of enamel

Table 6: Intergroup comparison of the thickness of enamel removed

Comparison	*P*≤0.05
Group A versus Group D	Yes
Group A versus Group B	Yes
Group A versus Group C	Yes
Group C versus Group D	Yes
Group B versus Group D	Yes
Group B versus Group C	No

Test applied one-way ANOVA with *post hoc* Tukey's test

Table 7: Microhardness (Vicker's hardness number) before the study period

Groups	VHN before, mean±SD	*P*
Group A	320.60±9.21	0.14
Group B	320.60±7.89	
Group C	318.60±10.92	
Group D	321.807±0.22	

Test applied one-way ANOVA, statistically significance at $P \leq 0.05$. VHN=Vicker's hardness number, SD=Standard deviation

Table 8: Microhardness (Vicker's hardness number) after the study period

Groups	VHN after, mean±SD	*P*
Group A	112±4.30	0.05*
Group B	165.60±4.77	
Group C	167.40±6.58	
Group D	224.80±6.61	

Test applied one-way ANOVA, *Statistically significance at $P \leq 0.05$. VHN=Vicker's hardness number, SD=Standard deviation

Table 9: Intergroup comparison of Microhardness (Vicker's hardness number) after the study period

Comparison	*P*≤0.05
Group A versus Group D	Yes
Group A versus Group B	Yes
Group A versus Group C	Yes
Group C versus Group D	Yes
Group B versus Group D	Yes
Group B versus Group C	No

Test applied one-way ANOVA with *post hoc* Tukey's test

Table 10: Depth of demineralized lesions in um (polarized light microscopy)

Groups	Depth of demineralized lesions in um (PLM), mean±SD (ug)	*P*
Group A	220.60±7.02	0.05*
Group B	160.60±5.73	
Group C	167.20±5.45	
Group D	120.20±5.07	

Test applied one-way ANOVA, *Statistically significance at *P*≤0.05. PLM=Polarized light microscopy, SD=Standard deviation

Table 11: Intergroup comparison of depth of demineralized lesions in um (polarized light microscopy)

Comparison	*P*≤0.05
Group A versus Group D	Yes
Group A versus Group B	Yes
Group A versus Group C	Yes
Group C versus Group D	Yes
Group B versus Group D	Yes
Group B versus Group C	No

Test applied one-way ANOVA with *post hoc* Tukey's test

of the Groups B and C was statistically not significant with $P > 0.05$. Among all the groups, Group D showed the least depth of demineralized lesion suggestive of maximum inhibition to demineralization followed by Group B and Group C.

Discussion

Of all the avenues open to combat the menace of dental caries, use of fluorides has been promising. Fluoride influences dental caries process in two ways: (i) inhibition of demineralization at the crystal surface within the tooth, and (ii) the enhancement of subsurface remineralization resulting in arrest or reversal of carious lesions.[21] Another way for prevention of dental caries, prevention through increased acid resistance as a result of laser treatment has been investigated ever since the introduction of lasers to the field dentistry by Stern and Sognnaes.[22] However, the use of high power laser systems did result in some unwanted effects such as surface cracking and melted areas, surface crazing, and cratering.[18]

The question, how to avoid the undesirable effect of the surface cracks and craters with the preventive potential of laser treatment, captured the minds of some investigators. Thus, many *in vitro* studies were carried out to possibly avoid unwanted effects of the laser by use of topically applied fluoride solution and laser treatment.[23] These studies have shown a beneficial effect by increasing acid resistance and fluoride uptake. Despite the promising results of these studies, very few *in situ* studies have been carried out to study the effect of topically applied fluoride and CO_2 laser.[20] The present study was designed and carried out to assess the inhibition of mineral loss from human tooth enamel by CO_2 laser and 1.23% APF.

As stated by Paes Leme *et al*.[24] this enamel slab model used in the study was able to simulate a high caries-risk situation. At the end of the study period of 4 weeks, the appliances were retrieved, and the slabs were removed from the appliance, washed with deionized water, and stored in Eppendorf tubes duly labeled till further analysis. Recent papers confirm that topical fluoride application is more effective in caries prevention than systemic fluoride application.[25]

Jiang *et al*.[26] evaluated the effect of a bi-annual professional application of APF foam on caries increment in the primary dentition over a 2-year period and found it effective in reducing the increment of dental caries in the primary teeth. However, at the same time, time of application of professionally applied topical fluorides play a significant role and minimum of 6 h contact time is necessary for the optimum formation of fluorapatite or fluorohydroxyapatite.[16] Furthermore, using synthetic hydroxyapatite, it has been shown that high energy CO_2 laser treatment could transform hydroxyapatite into fluorapatite in the presence of fluoride.[27] Thus, APF solution was used in the present study to check whether any beneficial effect could be obtained when it is used along with CO_2 laser.

Dental hard tissues strongly absorb light in certain regions of infrared spectrum, because of phosphate, carbonate, and hydroxyl groups in the crystal structure.[13] The CO_2 laser produces radiation in the infrared region that coincides closely with the apatite absorption bands.[28] For this reason, CO_2 laser was used in the present study.

CO_2 laser when used at continuous wave mode or pulsed wave mode have shown increased acid resistance. In pulsed wave mode, if the pulse duration is short enough, nearly all energy is dissipated at this region, whereas if it is longer, some heat is deposited in upper layers, and the remaining energy is transmitted deeper into the tissue. Thus, a large amount of energy is delivered in a short time to achieve optimum effect. Hence, pulsed wave

CO_2 laser was preferred in the present study over the continuous wave CO_2 laser.

Low-power surgical laser system (Dream Pulse III, Daeshin Enterprise, Korea) was used in this study. The test groups were lased with CO_2 laser at 10.6 μm wavelength, energy level of 5 J/cm^2 for 15 s with pulse duration of 800 μs and each pulse separated by 100 ms. The use of low power laser system for the evaluation of possible acid resistance has also been carried out by Hsu *et al*. 2000,[29] Whitters and Strang 2000,[30] and Esteves-Oliveira *et al*. 2009.[31]

The 9.6 μm wavelength has 10 times higher absorption in enamel (8000^{-1}) than 10.6 μm wavelength (825^{-1}) and has therefore been considered the most promising for use in caries prevention.[27] However, the lower absorption of the 10.6 μm wavelength results in a higher penetration depth and can, therefore, affect thicker layer of enamel. For this reason, it has been suggested that caries preventive effect of 10.6 μm could be longer lasting.[32] Considering these facts, CO_2 laser with 10.6 μm was selected for lasing the samples.

Effects of laser irradiation on the pulp were not investigated in the present study. Anić *et al*. 1992[33] noted that the use of a continuous wave CO_2 laser for 15 s with power output of 6 W and 1 mm spot size caused a temperature rise of 3.5°C. In the present study, the samples were lased at a power output of 5 W with pulse duration of 800 μs, each pulse separated by 100 ms for 15 s. Hence, the irradiation conditions applied here are safe.

The test and control samples were evaluated for relative acid resistance by wet chemical analysis using Inductively Coupled Plasma Atomic Emission Spectrometer. The resultant calcium levels were taken into consideration to calculate the Weight of Enamel (WE) etched away, enamel depth penetration. The use of calcium levels as a parameter to evaluate the acid resistance of tooth enamel has also been studied by Tagomori and Morioka 1989,[17] Kakade *et al*. 1996.[34]

As compared to the control group, i.e., Group A samples, Group B (1.23% APF Group) samples showed a reduction in enamel solubility. Enamel weight (WE) etched away for Group B samples was 761.35 ± 64.56 μg. When this was compared with the WE of control group, i.e., 1039.48 ± 86.49 μg, the difference was statistically significant suggesting increased resistance to acid. Other authors who have found increased acid resistance after 1.23% APF treatment are Delbem and Cury 2002.[35]

After the use of low power surgical laser system (5 J/cm^2), WE of samples in Group C was 802.11 ± 43.53 μg which was significantly less than that of Group A samples, i.e., 1039.48 ± 86.49 μg. Significant resistance to acid attack after exposure to low power CO_2 laser has also been observed by Esteves-Oliveira *et al*. 2009.[31]

WE of Group D was 553.25 ± 79.27 μg which was significantly less as compared to control Group, i.e., 1039.48 ± 86.49 μg. Group D exhibiting more acid resistance as compared to other groups. These findings are in agreement with Flaitz *et al*. 1995.[36]

Microhardness determinations provide indirect evidence of mineral loss or gain, i.e., demineralization or remineralization of enamel. Two types of hardness measurement techniques have been used in the past to evaluate demineralization and remineralization of enamel.

i. Cross-sectional microhardness: The indenter load is parallel to the polished tissue surface
ii. Surface microhardness: The indenter load is perpendicular to the polished tissue surface.

The results obtained by cross-sectional microhardness measurements are accurate. It has been observed that there is a linear relation between the Knoop hardness number of the enamel and the mineral concentration.

The method of surface microhardness measurement is widely used because of its simplicity. It has the advantage which allows evaluation of the enamel slabs before and after the experimental period as it is nondestructive.[37] Whereas, cross-sectional microhardness is a destructive method as sections are prepared and the sample is destroyed. Multiple evaluations of the same slab are not possible with cross-sectional microhardness. In the present study, Vickers indenter was used because of unavailability of the Knoop indenter. The main difference between the Knoop and Vickers hardness measurement is the indentation length. For an indentation length of approximately 100 μm the Knoop indenter penetrates about 3.5 μm, whereas the Vickers indenter reaches a depth of about 14 μm.

In the present study, the mean surface microhardness values of Groups A, B, C, and D were 320.60 ± 9.21, 320.60 ± 7.89, 318.60 ± 10.92, and 321.807 ± 0.22 respectively before the study period. After a study period of 4 weeks, the mean surface microhardness values of groups A, B, C, and D were reduced to 112 ± 4.30, 165.60 ± 4.77, 167.40 ± 6.58, and 224.80 ± 6.61. Difference between microhardness values before and after study period was 208.60 ± 8.50, 155 ± 11.68, 151.2 ± 14.39, and 97 ± 2 in Group A, B, C, and D, respectively. This difference in microhardness was statistically significant and suggestive of there were maximum inhibition of mineral loss from enamel slabs of Group D followed by Group C and Group B.

These findings are in agreement with, Rodrigues *et al*.[20] Authors assessed *in situ* effect of a Transversely Excited Atmospheric pressure CO_2 laser (wavelength 9.6 um) and the use of fluoridated dentifrice on enamel demineralization. Results suggested that the specimen treated with laser and/or fluoridated dentifrice presented a significantly lower mineral loss.

PLM has been used in the past to evaluate the demineralization and remineralization of carious lesions of enamel qualitatively as well as quantitatively by Bertassoni *et al*.[38]

All crystals and anisotropic materials except cubic materials exhibit the property of birefringence. This means that when an unpolarized beam of light enters such a crystal or materials, it spills into two plane polarized rays – the ordinary and extraordinary rays – vibrating at right angles to each other. For these two rays, the material exhibits two different refractive indices; n_o and n_e, respectively. The birefringence is then calculated by the difference (n_e-n_o) 10.[14]

In enamel, the total birefringence is the sum of intrinsic and form birefringence. Intrinsic birefringence is due to the mineral content of the tooth whereas; form birefringence is due to the porosities of the enamel filled with a medium having a refractive index different from that of the mineral. When sections of demineralized enamel are imbibed in water and viewed under polarized light, the subsurface lesion appears positively birefringent, and the surface zone appears negatively birefringent.

The mean depth of demineralized lesions in enamel slabs was 220.60 ± 7.02 μm, 160.60 ± 5.73 μm, 167.20 ± 5.45 μm, and 120.20 ± 5.07 μm in Group A, Group B, Group C, and Group D, respectively. Among all the groups, Group D showed the least depth of demineralized lesion suggestive of maximum inhibition to mineral loss followed by Group B and Group C. These findings are in agreement with the following studies. Oliveira *et al*. (2009) evaluated the effect of APF application time (1 and 4 min) on caries-like lesion formation in enamel, and the results showed that APF treatment before lesion formation resulted in a significant reduction in lesion depth.

Conclusion

Low power CO_2 laser treatment and topically applied 1.23% APF used individually or in combination, inhibits subsequent mineral loss in an *in situ* high caries challenge situation. In particular, the combined use of this specific laser treatment plus fluoride was more successful than either laser treatment or fluoride alone in the inhibition of mineral loss in the mouth. The results of this study also suggest that the combination of low power laser treatment with fluoride therapy may be effective as a caries inhibition treatment. However, safety parameters for CO_2 laser in terms of frequency, pulse duration, energy output, time of application, along with effect on pulp needs to be carefully studied and established, and more *in situ* and *in vivo* studies need to carry out in the same context. Nevertheless, synergistic effect of CO_2 laser and topically applied fluoride can be the solution for the effective control of the disease process – "Dental Caries."

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Conflicts of interest

There are no conflicts of interest.

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