Acquired acid resistance of human enamel treated with laser (Er:YAG laser and Co$_2$ laser) and acidulated phosphate fluoride treatment: An *in vitro* atomic emission spectrometry analysis

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Abstract

Background: Dental caries is essentially a process of diffusion and dissolution. If the aspect of dissolution can be curtailed some degree of prevention can be achieved. **Aims:** The present study was carried out to evaluate and compare the effect of Er:YAG laser and Co$_2$ laser irradiation combined with acidulated phosphate fluoride treatment on *in vitro* acid resistance of human enamel. **Design:** An *in vitro* study was carried out on 30 human premolars to evaluate the enamel's acid resistance using an atomic emission spectrometry analysis. **Materials and Methods:** A total of 60 enamel specimens were prepared from 30 human premolars and were randomly assigned to 6 groups: (1) Untreated (control); (2) 1.23% acidulated phosphate fluoride (APF) gel application alone for 4 min; (3) Er:YAG laser treatment alone; (4) Co$_2$ laser treatment alone; (5) Er:YAG laser + APF gel application; (6) Co$_2$ laser + APF gel application. The specimens were then individually immersed in 5 ml of acetate buffer solution (0.1 mol/L, pH 4.5) and incubated at 37°C for 24 h, and the acid resistance was evaluated by determining the calcium ion concentration using the atomic emission spectrometry. **Statistical Analysis:** An ANOVA model was constructed (P value of 0.05), followed by Tukey's test for multiple pair wise comparisons of mean values. **Results:** Significant differences were found between the control group and the test groups ($P < 0.001$). **Conclusions:** Combining acidulated phosphate fluoride with either Er:YAG or Co$_2$ laser had a synergistic effect in decreasing the enamel demineralization more than either fluoride treatment or laser treatment alone.

Keywords: Acidulated phosphate fluoride, acid resistance, Co$_2$ laser, Er:YAG laser

Introduction

During the past few decades, scientific developments in cariology, dental materials, and diagnostic systems have catalyzed evolution in caries management from G.V Black's "extension for prevention" to "minimally invasive."[1] The aim of modern caries prevention must be to identify patients with an elevated risk of caries and give them intensive, individual prophylactic support. In the last few years, new techniques have been studied in this regard and among them, laser irradiation seems to be very promising.[2]

The reduced acid solubility of dental enamel after irradiation with high intensity lasers is related to physical and chemical alterations caused by photo thermal and photo chemical effects. Depending on the temperature achieved by the laser irradiation, different effects occur that change the enamel's solubility.[3] It was demonstrated that the smallest level of acid dissolution of enamel is achieved after heating to 300-350°C. This effect is caused by denaturation and swelling of the organic matrix that leads to the obstruction of the diffusion pathway within the enamel. Above 200°C, a loss of carbonate occurs that could contribute to increased acid resistance. Micro-spaces formed as a consequence of loss of water, carbonate, and organic substances might prevent demineralization by trapping the dissolved ions.[4]

The wavelengths used in studies for caries prevention are Nd: YAG (1.64 μm), Er:YAG (1.94 μm), Er, Cr: YSGG (1.79 μm), Ho: YAG (2.1 μm), Argon (488-514 nm), and Co$_2$ (9.6 and 10.6 μm). Recently, the most frequently studied wavelengths have been those of Co$_2$ and Erbium lasers, because of their high absorption by enamel and thus, the possibility of achieving the high temperatures needed to change the enamel structure and make it less soluble.[5]

Although, the effect of laser irradiation on acid resistance of enamel is recognized the studies on comparisons of acid

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resistance of enamel when irradiated with Co_2 and Er:YAG lasers are scarce. Thus, aim of the present study was to investigate the acid resistance of enamel when irradiated with Er:YAG and Co_2 lasers either alone or in combination with topical fluoride application.

Materials and Methods

A total of 30 human premolars extracted for orthodontic reasons and free of carious and other defects were selected for the study. Teeth were cleaned and kept in 0.1% thymol solution until use (up to 30 days). Teeth were then longitudinally sectioned in mesial to distal direction using water cooled diamond discs and two specimens were obtained from each tooth. Each specimen's surface was coated with acid resistant nail varnish except for a 3.5 mm diameter round window, which was delimited using adhesives [Figure 1]. After the adhesives were removed, the surfaces were cleaned with cotton.

The enamel specimens were randomly allocated to six groups ($n = 10$):
- Group 1: Untreated (control)
- Group 2: 1.23% acidulated phosphate fluoride (APF) gel application alone for 4 min
- Group 3: Er:YAG laser treatment alone
- Group 4: Co_2 Laser treatment alone
- Group 5: Er:YAG laser + APF gel application
- Group 6: Co_2 laser + APF gel application.

The irradiation conditions for Er:YAG laser (Fotona Fidelis Plus III) were: 2.94 µm wavelength, pulse energy of 200 mJ; 1.4 W power; frequency of 7 Hz; 0% air; 0% water. A non-contact hand piece was used. The irradiation was in a scanning style with a distance of 2.5 cm from the tooth surface [Figure 2].

The irradiation conditions for Co_2 laser (sunny surgical laser system, model: PC015-C; Mikro Scientific Instruments Pvt. Ltd.) were: 10.6 µm wavelength; 1 W power; 0.75 s average enamel exposure time, 0.3 mm beam spot size, in pulsed mode. The irradiation was performed by hand, screening the enamel surface with a uniform motion for 30 s [Figure 3]. The fluoride application was performed using 1.23% APF gel during 4 min using a cotton swab and then, samples were washed with deionized water for 1 min and dried with absorbent paper.

The specimens were then individually immersed in 5 ml of acetate buffer solution (0.1 M/L, pH 4.5) and incubated at 37°C for 24 h to simulate oral conditions. After the acid challenge, the teeth were removed from the vials and the acetate buffer solutions from each vial of both the experimental and control groups were collected and analyzed under Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) to determine the parts per million of calcium ion of each solution.

Results

The data acquired from the ICP-AES measurements was imported into SPSS 14 software for statistical analysis. An ANOVA model was constructed (P value of 0.05), followed by Tukey's test for multiple pair wise comparisons of mean values.

The mean value of calcium in Gp 2: APF, Gp4: Co_2, Gp 5: Er:YAG + APF and Gp 6: Co_2 + APF were less than in Group 1 (control) which was statistically significant (P value $= 0.000$, $P < 0.05$). There was no significant difference in calcium dissolution when Gp 3: Er:YAG laser irradiation was used alone when compared to the control group (P value: 1.000, P value > 0.05).

Although, the difference between Group 1 (control) and Group 3 (Er:YAG) was not statistically significant ($P = 1.000$ and $P > 0.05$) there was A 1.4% increase in calcium solubility after Er:YAG laser irradiation. Furthermore, the combination of Er:YAG with APF (Gp 5) and Co_2 + APF (Gp 6) resulted in decreased mean score of calcium when compared to Er:YAG (Gp 3) and Co_2 (Gp 4), which was statistically significant. When Co_2 (Gp 4) laser was used alone it showed 36% reduction in calcium dissolution compared to control, but however was not statistically significant when compared to fluoride treatment alone (Gp 2), which showed a percentage reduction of 43%. Among 6 groups Gp-6 (Co_2 + APF) showed the highest percentage reduction in calcium dissolution of 59.7%.

Discussion

Fluoride is important in enamel demineralizing and remineralizing procedures because it alters the ecology of the bacterial plaque, affecting the acid uric capacity of bacteria and also their production of glucans.[6] Moreover, fluoride inhibits demineralization when present at crystal surfaces during a pH decrease and it enhances remineralization, forming a fluorapatite-like low-solubility veneer on the remineralized crystals.[7] The anti-caries effect of professional F application depends on reaction products formed on enamel during the clinical treatment and their retention over time after the application.[8]

Topical fluoride application results in a deposition of surface crystals of calcium fluoride (CaF_2) that act as a reservoir releasing fluoride in the demineralization process. This may be lost again *in vivo* by back exchange, back diffusion, and migration from the mineral to the surrounding tissue fluid, saliva, or plaque fluid and decreases after short periods of time. Because of that, several applications of topical fluoride are necessary to maintain the anti-caries effect. Considering its strong interaction with dental hard tissues, lasers are also used for caries prevention.[9]

Figure 1: Prepared enamel specimens

Figure 2: Er:YAG laser irradiation of the specimens

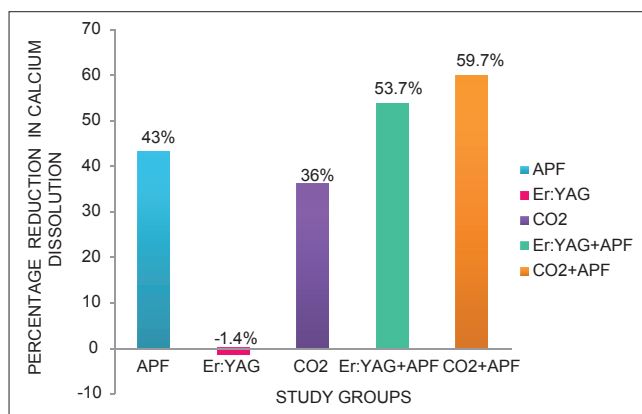**Figure 3:** CO_2 laser irradiation of specimens

Figure 4: Percentage reduction in calcium dissolution compared to control
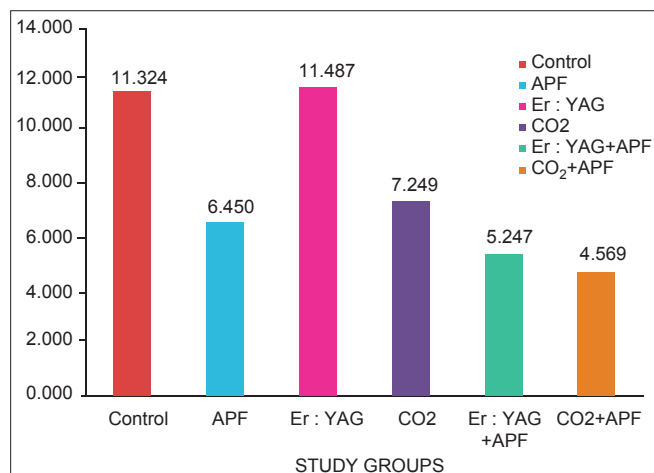

Figure 5: Mean scores of calcium in parts per million

Various mechanisms of acid resistance by lasers have been discussed which included loss of organic matter and carbonate content, change in polarization of enamel components, which favors the retention of fluoride, lowering of critical pH for enamel dissolution from 5.5 to 4.8.[10] Previous *in vitro* studies

have shown that CO_2 laser irradiation inhibits the progression of caries like lesions up to 85%, which is comparable to a daily application of a sodium fluoride dentifrice.[11]

Hydroxyapatite is the main mineral in enamel, dentine and cementum, which presents a maximum of absorption in the region of infrared ranging from 9 μm to 11 μm wavelengths. Therefore, wavelengths must be chosen where absorption is high in regions, which correspond to specific components in dental hard tissues, such as hydroxyapatite and water, which takes place when enamel is irradiated with CO_2 and erbium lasers, respectively.[12]

White *et al*. (1995) proposed that *in vitro* demineralization protocol could be applied as a diagnostic test for modifying effects of laser treatment on enamel and dentine. In order to determine if a laser has the potential for caries prevention, quantitative analysis could be used, which include mineral loss quantification, determination of calcium dissolution, determination of Ca/P ratio in the enamel surface and in the demineralization solution and determination of fluoride uptake.[13] Because of these reasons the present study was designed to investigate the *in vitro* acid resistance of enamel

when irradiated with CO_2 and Er:YAG laser combined with acidulated phosphate fluoride gel and to compare their effects by quantifying the amount of calcium dissolved to the demineralization solution by an AES.

It was reported by Liu *et al.* (2006) that Er:YAG laser without coolant had more effectiveness in caries prevention when compared to Er:YAG laser with water mist.[14] Due to this fact and to reach sufficient temperature at the surface to promote crystallographic changes, all irradiation conditions used in the present study were used without water mist. The present study utilized Er:YAG laser fluencies, which were well below the ablation threshold to avoid mechanical damage on the enamel.

The multiple comparison tests showed that Group 2, 4, 5 and 6 showed a significant decrease in calcium dissolution compared to the control and among these Group 6 (CO_2 + APF) showed the highest decrease in calcium dissolution [Table 1].

The Group 2 (APF) showed 43% reduction in calcium dissolution compared to control [Figure 4]. This result was consistent with the *in vitro* study by Esteves Oliveira *et al.* (2009) who assessed the inhibition of caries lesion depth following APF gel application and they reported a 44% inhibition of caries progression in APF treated enamel specimens compared to the control.[15]

The mean score of calcium in Group 3 (Er:YAG) was 11.487 ppm, which showed a similar value compared to the control group and this was the group, which showed the least percentage reduction in calcium dissolution in fact a negative

percent reduction of - 1.4% compared to control [Figure 5]. This result was consistent with the result obtained by Laura *et al.* (2010) who reported that the acid resistance of enamel due to sub-ablative Er:YAG laser irradiation did not increase significantly compared to control.[16]

Chimello-Sousa *et al.* (2005) explained the Er:YAG laser induced acid resistance of enamel. In this aspect, it had been reported that some chemical alterations occur in enamel due to the crystals liquefaction. During the quick dental tissue cooling, there is an increase of the hydroxyapatite crystals, which present with reduction of carbonate content and formation of pyrophosphate and metaphosphate, which have been described as more stable and less soluble components; thus, reducing dental susceptibility to acid attack. The ablation is via thermo mechanical interaction where majority of incident radiation is consumed in the ablation process, leaving very little residual energy for adverse thermal interactions with the pulp tissue and surrounding soft in hard structures.[17] Intending not to ablate or melt the surface but only change its structure or chemical composition, the energy density of the laser should be below the ablation threshold.[18]

At the ablation threshold, Er:YAG laser can provide temperature rises up to 300°C, promoting the water evaporation and loss of carbonate. Therefore, reaching this temperature range, Er:YAG has theoretical potential for inducing acid resistance in enamel. When enamel is irradiated at the sub-ablative fluences used in the present study, the peak temperature rise was expected to be below 100°C, and it seemed to be not sufficient to induce crystallographic

Table 1: Comparison of acid solubility in different groups

Groups	Ca concentration (ppm) (mean±standard error)	Standard deviation	Percentage reduction of acid solubility versus group 1	Comparison of different groups
Control	11.324±0.628	1.987		1 versus 2: *P*<0.05 (S)
				1 versus 3: *P*>0.05 (NS)
				1 versus 4: *P*<0.05 (S)
				1 versus 5: *P*<0.05 (S)
				1 versus 6: *P*<0.05 (S)
APF	6.450±0.656	2.073	43%	2 versus 3: *P*<0.05 (S)
				2 versus 4: *P*>0.05 (NS)
				2 versus 5: *P*>0.05 (NS)
				2 versus 6: *P*<0.05 (S)
Er:YAG	11.487±0.400	1.265	−1.4%	3 versus 4: *P*<0.05 (S)
				3 versus 5: *P*<0.05 (S)
				3 versus 6: *P*<0.05 (S)
CO_2	7.249±0.319	1.008	36%	4 versus 5: *P*<0.05 (S)
				4 versus 6: *P*<0.05 (S)
Er:YAG+APF	5.247±0.242	0.765	53.7%	5 versus 6: *P*>0.05 (NS)
CO_2+APF	4.569±0.212	0.672	59.7%	6 versus 5: *P*>0.05 (NS)

APF: Acidulated phosphate fluoride; Er:YAG: Erbium YAG laser

changes on enamel surface.[19] This might be the reason for decreased acid resistance of enamel specimens when treated with sub ablative Er:YAG fluencies.

Cameron *et al*. (2003),[20] in an *in vitro* study assessed the effectiveness of Q-switched laser of 355 nm in increasing the shear bond strength of enamel to composite and its ability to increase the resistance to acid dissolution when used alone or in combination with topical fluoride application. They concluded that laser treatment alone did not inhibit dissolution of mineral and actually increased the dissolution rate by 14% as indicated by a percent inhibition of - 14%.

The specimens treated with CO_2 laser (Group 4) showed a 36% reduction in calcium dissolution. The CO_2 laser with emission wavelength of 10.6 μm is very close to the phosphate and carbonate absorption bands, which may be absorbed more efficiently by dental enamel, causing a loss of carbonate and a reduction of reactivity at a sufficiently high temperature thereby increasing the acid resistance of superficial enamel confined to 5-10 μm without affecting the underlying enamel at depth of 50 μm or greater and very importantly the underlying dentine or pulp.[21]

Group 5 (Er:YAG + APF) specimens showed a 53.7% reduction in calcium dissolution compared to control. On the enamel surface, Er:YAG laser treatment combined with APF resulted in the lowest decrease of surface micro hardness and the Er:YAG laser influenced the deposition of CaF_2 on the enamel and showed a superficial anti-cariogenic action, but not in depth.[22]

The specimens treated with CO_2 + APF (Group 6) showed the highest percentage reduction in calcium dissolution of 59.7%. These results were consistent with the results obtained by Nancy *et al.* (1999) who reported 87% dissolution rate reduction for laser and fluoride combination.[23] High F concentrations were incorporated into the laser treated samples, producing marked dissolution rate reductions, which most likely might be related to the formation of fluorapatite (FAP).

Yong Hoon *et al*. (2005) compared the acquired acid resistance in dental enamel after Er:YAG and CO_2 laser irradiation *in vitro* with additional fluoride treatment. They found that the crystallinity of enamel was much improved after Er:YAG laser ablation. The CO_2 laser irradiation in the fluoride-treated laser enamel formed α-Tricalcium phospate (TCP) and fluorapatite. They concluded that additional fluoride treatment both after Er:YAG and before CO_2 laser irradiation improved the acid resistance of enamel.[24]

Hsu *et al*. (2001) conducted an *in vitro* study to evaluate the role of CO_2 laser irradiation and fluoride treatment in inhibiting enamel demineralization before or after removal of organic matrix from the enamel. The combined fluoride-laser treatment led to 98.3% and 95.1% reductions in mineral loss for enamel with and without organic matrix, respectively, when compared with sound enamel.[25]

Sato had suggested that the heat-induced melting and swelling of the organic matter might block the diffusion pathway and thus, account for decreased calcium loss and they also suggested that this organic blocking effect reached a maximum between 300°C and 400°C and decreases after the complete decomposition of organic matrix above 400°C.[26]

Laser treatment would remove the carbonate and increase the crystalline stability making the enamel less vulnerable to acid attack. Synergistic mechanism of laser with fluoride in improving the acid resistance of enamel might be due to an increased fluoride uptake, because removing the organic matrix would render a greater surface area for the binding of ions, including fluoride and calcium.[25]

Thus, from the present study, it could be concluded that fluoride treatment after both Er:YAG and CO_2 laser irradiation improved the acid resistance of enamel significantly and when used alone the sub-ablative Er:YAG laser irradiation did not increase the resistance to acid dissolution. On comparing the effects of 6 groups, Group 6 (CO_2 + APF) showed the lowest mean score of calcium (highest acid resistance) followed by Group 5 (Er:YAG + APF); however, the difference between these groups were statistically not significant.

Work is proceeding in an attempt to establish the precise morphological and chemical changes that occur on sound enamel in order to understand the ablation mechanism that is believed to be a mixture of photochemical and photo thermal dissociation and to verify the potential of lasers in dentistry. Laser technique might be useful for treatment of carious and non-carious (preventive treatment) of pits and fissures that are hard to reach with conventional technique. Further, studies are necessary to investigate the reduction on the progression of caries lesions in depth and to understand the involved mechanisms of induced acid solubility when dental enamel is irradiated with CO_2 and Er:YAG laser at sub-ablative fluencies.

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