Surface and Mineral Changes in Enamel when Treated with Acidulated Phosphate Fluoride Gel (APF) and CO_2 Laser

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Purpose: To compare in vitro the surface and mineral changes of enamel treated with acidulated phosphate fluoride gel (APF), CO_2 laser, and a combination of both.

Materials and Methods: Forty extracted maxillary single-rooted teeth were randomly selected. Roots were resected and teeth were divided into 4 groups (n = 10). Group 1: control; group 2: APF gel applied; group 3: irradiated with CO_2 laser; group 4: irradiated with CO_2 laser and APF gel applied. A single application of APF gel (pH 3.5) was performed using a brush for 4 min. A pulsed CO_2 laser with 10.6 μm wavelength was used with the following parameters: 1 W, 50-μs pulse duration, 1-Hz repetition rate, and a 0.8-mm beam diameter. X-ray diffraction (XRD) analysis of all specimens was performed to determine the mineral changes, and surface changes were compared using SEM.

Results: XRD analysis of groups 1 and 2 showed only the presence of hydroxyapatite, whereas group 3, which was irradiated with CO_2 laser, showed the presence of hydroxyapatite along with calcium hydrogen phosphate. Group 4 showed the presence of fluorapatite along with hydroxyapatite. SEM analysis revealed more fluoride retention in group 4 than in all the other groups. The SEM images confirm that fluoride application after CO_2 laser irradiation presented a surface with fewer craters than the surface irradiated with CO_2 alone. The XRD analysis revealed the presence of fluorapatite in groups treated with CO_2 and APF gel, which was not to be seen in any other groups.

Conclusion: The combination of laser and fluoride treatment showed increased fluoride uptake, with the formation of fluorapatite. The adjunct use of these two treatments may be effective in preventing caries.

Keywords: APF, fluoride, hydroxyapatite, fluorapatite, laser, enamel, acid resistance.

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Many attempts have been made to alter tooth enamel to increase its resistance to caries. If it were possible to eliminate the pits and fissures from the enamel surface by fusing or "welding" the enamel, the susceptibility of teeth to caries might be considerably decreased. Laser technology was first used in dentistry for the removal of carious tissue and preparation of cavities. Attention has been focused on the laser's potential to enhance enamel's hardness and resistance to acid.[1,2]

Of all the techniques used in dentistry to enhance enamel's resistance to acid attack, fluoride application and CO_2 laser have shown the most satisfactory outcome. Different types of lasers, such as ruby, CO_2, Nd:YAG, and argon, with different operational modes and energy outputs, have been used to investigate the possibility of preventing dental caries.[3-15] Oliveira et al[16] has shown that CO_2 laser was able to decrease the enamel caries progression by causing surface and subsurface thermal damage.

Pulsed CO_2 laser irradiation of enamel caused marked surface fusion and inhibited the progress of subsurface caries-like lesions by as much as 50%.[7] Research suggests that pulsed CO_2 laser treatment of pitted and fissured occlusal surfaces, where fluoride is not as effective as it is on smooth surfaces, might be an effective caries-preventive measure.[17,18]

The anticaries effect of professional fluoride application depends on reaction products formed on enamel during the clinical treatment and their retention over time after the application. The formation and retention of fluoride compounds on enamel is dependent on the concentration of fluoride applied and the pH of the commercial product used. Professional acidulated phosphate fluoride (APF) application is a well-known method used for caries prevention, and its efficacy is evidence-based.[3]

MATERIALS AND METHODS

Extracted maxillary single-root teeth were collected and stored in saline. Of these, 40 teeth were randomly selected. Roots were resected and the crowns were stored in saline at ambient temperature and washed in distilled water.

The teeth were divided into 4 groups (n = 10). Group 1: untreated teeth (control group); group 2: APF gel application; group 3: CO_2 laser irradiation; group 4: CO_2 laser irradiation was performed followed by the application of APF gel.

Application of the APF gel and CO_2 irradiation were done on the labial surface of the tooth.

Fluoride Application

A single application of APF gel (1.23 APF Gel, Pascal; Bellevue, WA, USA), pH 3.5, was performed for 4 min using a brush. The fluoridated samples were wiped with cotton, rinsed, and stored in artificial saliva to imitate the clinical protocol. The samples were subjected to analysis after 6 h of application of APF gel.

Laser Irradiation Conditions

A pulsed CO_2 laser (Sunny Surgical Laser, model number-PC015C; Shanghai, China) at 10.6 µm wavelength was used with the following parameters: 1 W, 50-µs pulse duration, 1-Hz repetition rate and a 0.8-mm beam diameter.

A distance of 5 mm from the tip of the handpiece to the specimen was maintained during irradiation using a device made with orthodontic wire and fixed to the laser tip.[19] Laser irradiation was carried out by exposing the enamel of each specimen for approximately 60 s by moving the laser tip manually. All the samples were randomly selected for x-ray diffraction (XRD) analysis and the samples with the best XRD results were subjected to scanning electron microscopy (SEM).

XRD Analysis

X-ray diffraction measurements were performed using a powder diffractometer operating at 45 KV and 40 mA with Cu-Kα radiation and a diffracted beam monochromator. Data were collected in the 2 θ range of 8 to 90 degrees with a step size of 0.02 and a counting time of 20 s at each step. The data bank from the International Center for Diffraction Data (ICDD) was used in a search/match program for phase identification.

RESULTS

XRD Analysis (Table 1)

Control group: untreated enamel

The x-ray diffraction pattern of the control group revealed the presence of hydroxyapatite crystals (Fig 1).

Group 2: APF gel application

The x-ray diffraction pattern of this group, treated with fluoride, also showed the presence of hydroxyapatite crystals. However, it did not show the presence of fluorapatite (Fig 2).

Table 1 Compounds found in XRD analysis				
Group	Chemical formula	Compound name	Scale factor*	Displacement[°2Th.]*
1	$Ca_{10}(OH)_2(PO_4)_6$	Hydroxyapatite	0.461	0.583
2	$Ca_{10}(OH)_2(PO_4)_6$	Hydroxyapatite	0.424	0.678
3	1. $Ca_{10}(OH)_2(PO_4)_6$ 2. $CaHPO_4$	1. hydroxyapatite, 2. calcium hydroxide phosphate	0.462 0.384	0.484 -0.080
4	1. $Ca_{10}(OH)_2(PO_4)_6$ 2. $Ca_{10}(PO_4)_6F_2$	1. hydroxyapatite, 2. fluorapatite	0.374 0.526	0.381 0.254

*Calculated by PANalytical X'Pert HighScore Plus software; Almelo, The Netherlands.

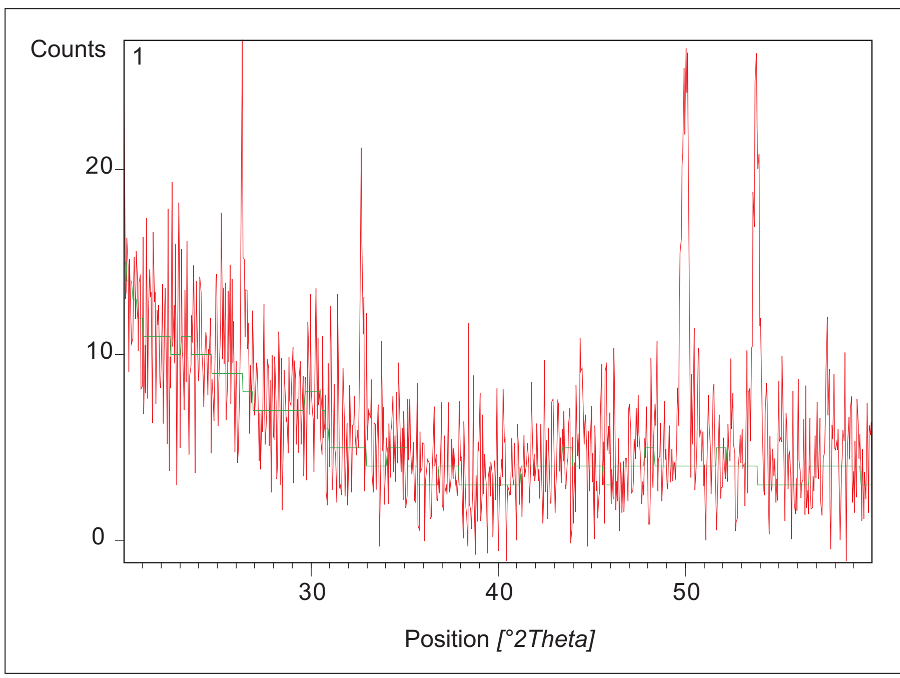

Fig 1 X-ray diffraction pattern of the control group revealed the presence of hydroxyapatite crystals.

Group 3: CO_2 laser irradiation

The group treated with laser alone showed the dissolution of hydroxyapatite to calcium hydroxide phosphate, which was not seen in groups 1 and 2 (Fig 3).

Group 4: Combination of CO_2 laser and APF gel

Irradiation with laser followed by fluoride application revealed the presence of fluorapatite, which was not seen in any other groups (Fig 4).

SEM Analysis

Untreated enamel (control group) showed unchanged hydroxyapatite crystals which presented a smooth surface in SEM (Fig 5). In group 2, fluoride particles were observed on the APF-treated enamel (Fig 6). Micrographs of group 3 specimens (CO_2 laser ap-

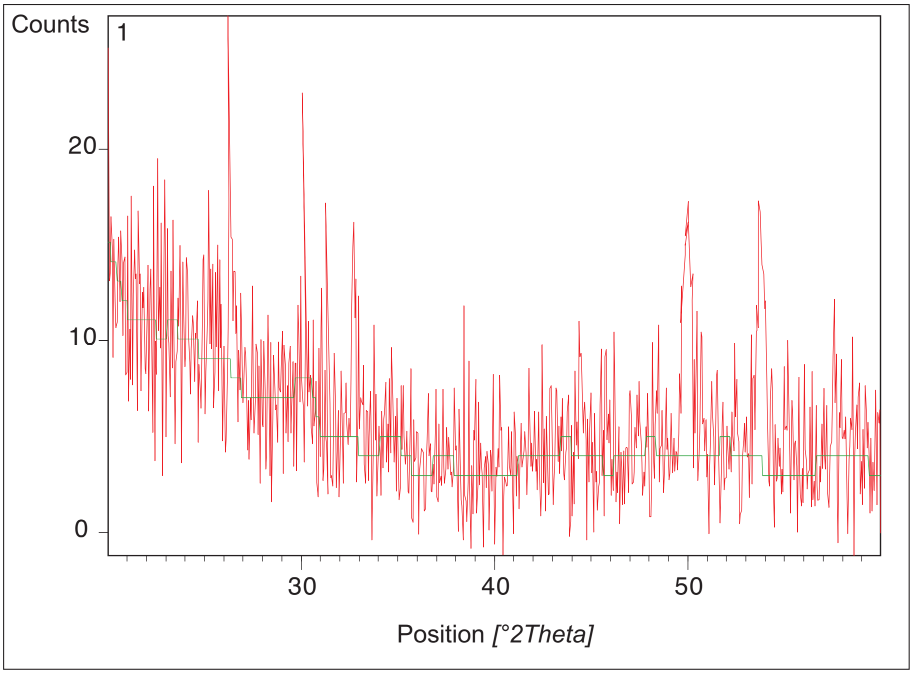

Fig 2 X-ray diffraction pattern of the group treated with fluoride alone also showed the presence of hydroxyapatite crystals.

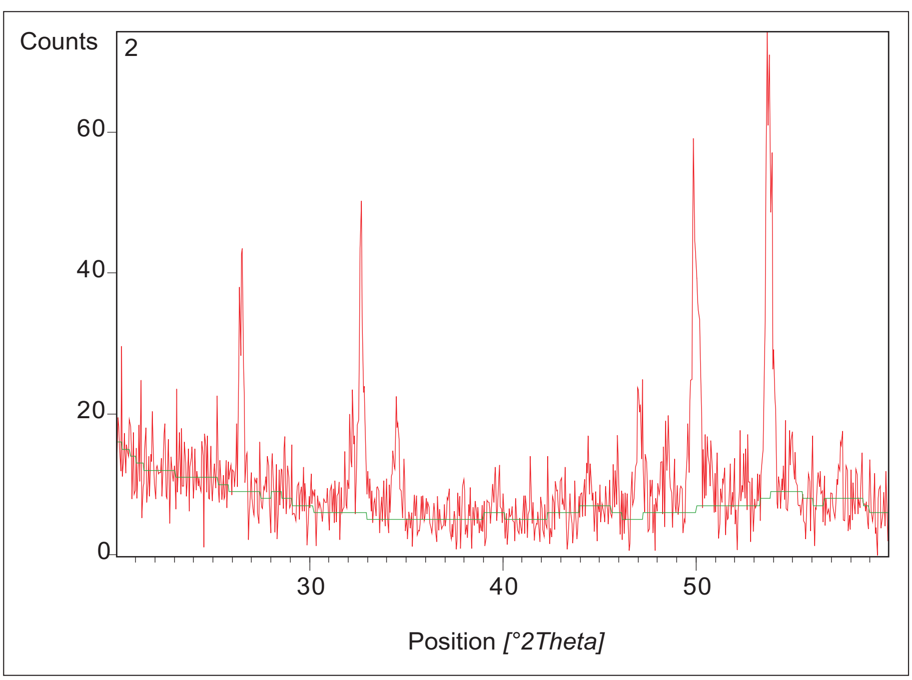

Fig 3 The group treated with laser alone showed the formation of calcium hydroxide phosphate, which was not seen in groups 1 and 2.

plication only) showed evidence of melting and fusion (Figs 7 and 8). In group 4 – combined CO_2 laser and APF – etched prism pits were filled with small fluoride particles (Fig 9).

DISCUSSION

The motivation and search for new methods for the prevention of dental caries by increasing the acid resistance of the enamel is a very sophisticated way of preventing tooth decay. With advancements in today's

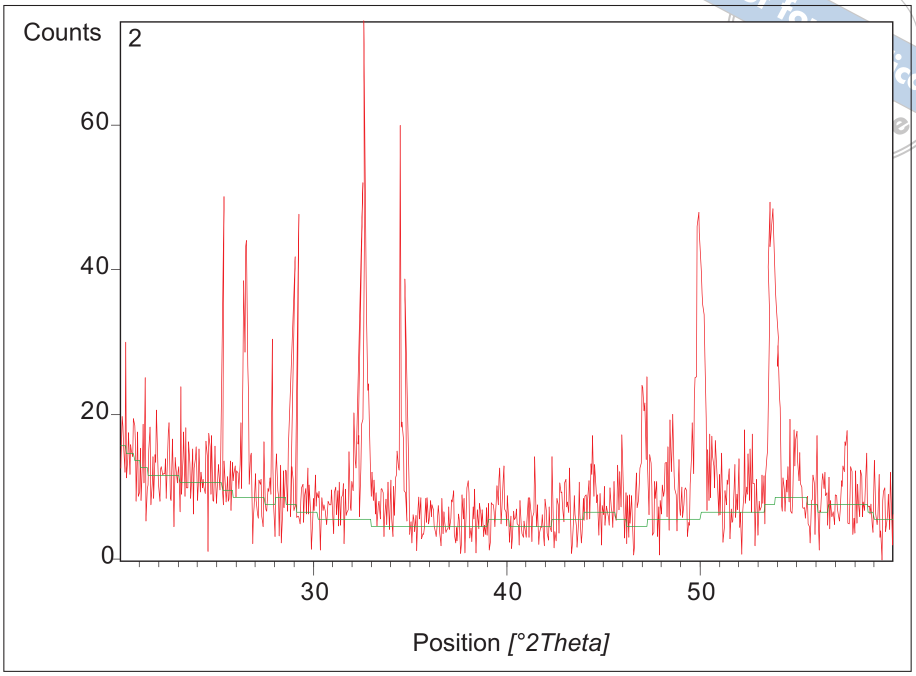

Fig 4 Irradiation with laser plus fluoride application revealed the presence of fluorapatite, which was not seen in any other groups.

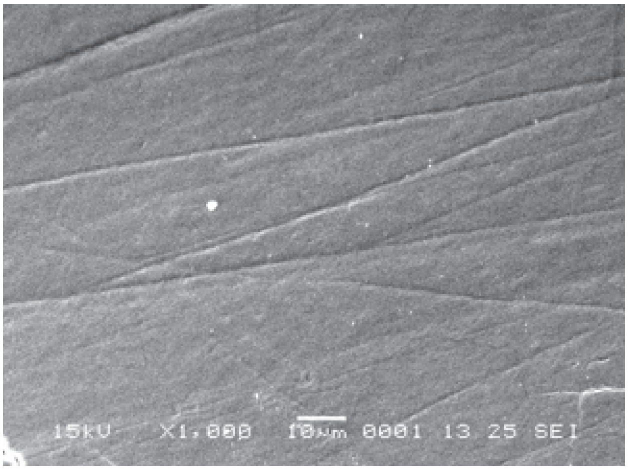

Fig 5 SEM of control specimen: untreated enamel surface.

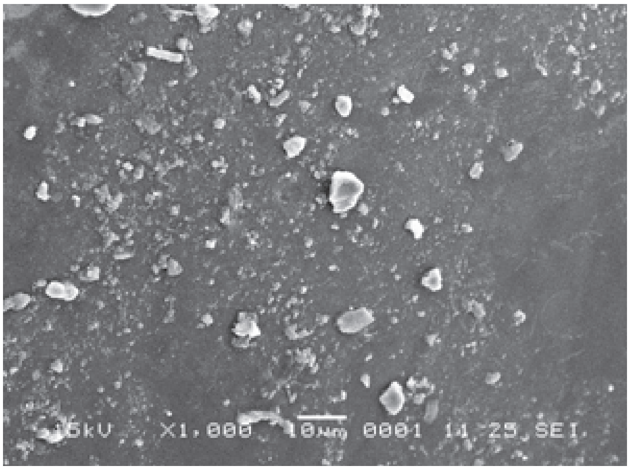

Fig 6 SEM of group 2 specimen: enamel surface treated with APF gel.

dental equipment, eg, lasers, the scope of potentially preventive methods is greatly increased.

Several studies have shown the resistance of enamel to acid attack by irradiation with CO_2 laser and fluoride application. Tagliaferro et al[19] showed that CO_2 laser irradiation alone, as well as in combination with APF, significantly inhibited demineralization progression in carious primary dental enamel, but fluoride retention on CO_2 irradiated enamel was never reported. Tepper et al[20] also evaluated the effect of the association of CO_2 laser and amine fluoride solution on the inhibition of enamel dissolution. Although they did not observe any statistically significant difference between fluoride or laser treatments and the combined therapy, they believed that a synergism occurred between the treatments. Hence, this study was undertaken to evaluate the fluoride retention and conversion of hydroxyapatite to fluorapatite.

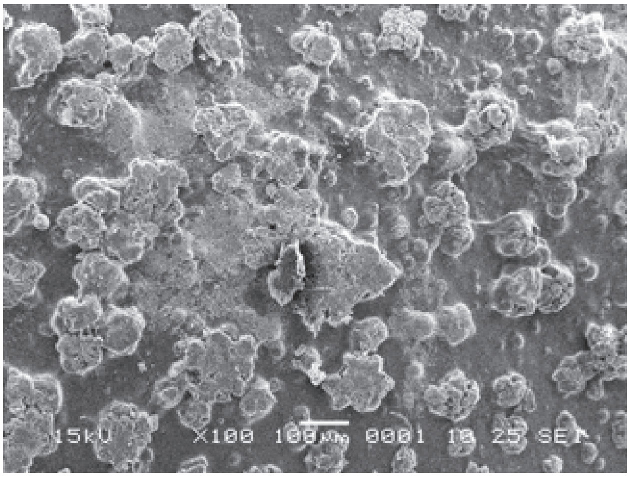

Fig 7 SEM of group 3 specimen: enamel surface irradiated with CO_2 (magnification 100X).

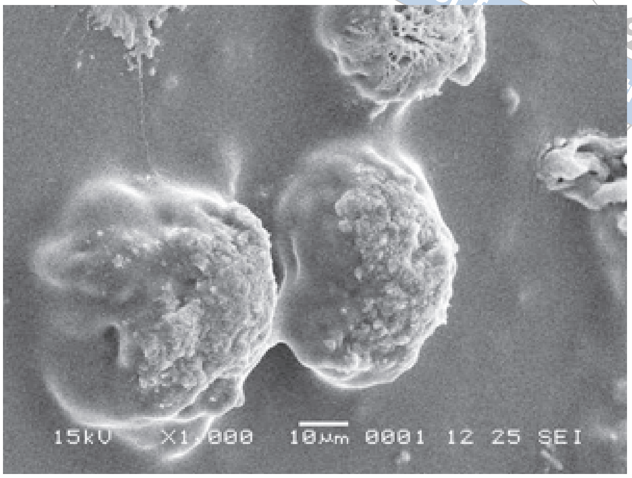

Fig 8 SEM of group 3 enamel surface irradiated with CO_2 laser (magnification 1000X).

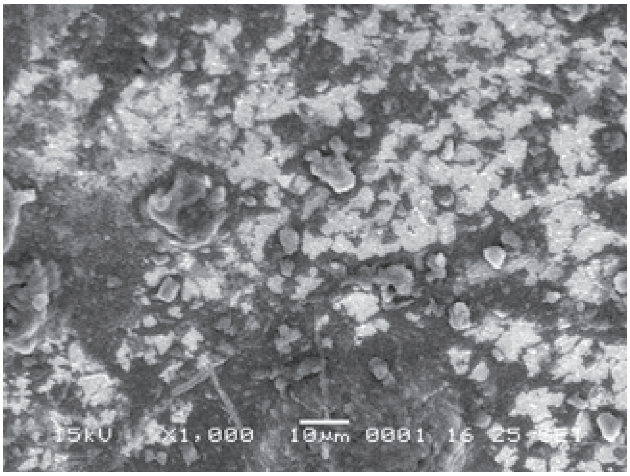

Fig 9 SEM of group 4 enamel surface irradiated with CO_2 laser, followed by application of APF gel at 1000X.

The topical application of APF gel has been shown to reduce dental caries by an average of 25%.[21,22] APF gel contains 1.23% F (as sodium fluoride and HF), phosphoric acid, and carboxymethyl cellulose as the gelling base. The pH is usually between 3.0 and 4.5, and the viscosity 7000 to 20,000 centipoises. Therefore, APF gel was used in this study because of its etching property on the tooth surface, thereby retaining more fluoride and its acidic pH of 3.5.

The XRD analysis of untreated enamel showed unchanged hydroxyapatite crystals, which presented a smooth surface in SEM. Although SEM confirmed the presence of fluoride particles on the APF treated enamel, XRD analysis revealed that there was no change in the apatite structure. Nelson[23] suggested that the particle size of CaF_2 deposits in the surface coating produced by topical fluoride agents may be an important factor in determining the effectiveness of a topical fluoride agent. As the solubility of crystals decreases with their increasing size, CaF_2 particles occurring as a result of APF application will dissolve slower than those of NaF application.[19] According to Cruz,[24] low pH increases the amount of CaF_2 deposited on enamel during topical fluoride application. In addition, the small CaF_2 particles formed at low pH have a lower solubility than CaF_2 particles formed at high pH.[25]

By irradiating the whole labial surface rather than irradiating a single spot, we better simulated clinical conditions. The use of CO_2 laser has been extensively proven to resist acid attack and prevent demineralization of the dental hard tissues.[19] The emission wavelength of 10.6 μm of the CO_2 laser is very close to the phosphate and carbonate absorption bands of dental enamel apatite, and is thus absorbed more efficiently by dental enamel,[8] causing a loss of carbonate.[8,26] The SEM observations showed evidence of melting and fusion in specimens, and the dissolution of hydroxyapatite to calcium hydroxide phosphate was confirmed by the XRD analysis in the CO_2 laser-treated group.

When APF gel was applied after CO_2 laser, etched prism pits were filled with small fluoride particles as seen with SEM. This was not observed in the group where only APF gel was applied. Fluoride ions are generated as the CaF_2 in these pits dissolves. This may result in subsequent enhanced remineralization of enamel[11] or dissolution inhibition of enamel crystallites.[10] The surface deposits formed on the enamel surface following 4 min of a common topical fluoride treatment have a mountain-like morphology. This observation is consistent with the findings of previous SEM studies,[23] which reported a mean diameter of calcium fluoride of approximately 1 μm.

A CO_2 laser treatment can even transform hydroxyapatite (HA) into fluorapatite (FA) in the presence of fluoride,[27] which was confirmed in our study by the XRD analysis. Both the incorporation of fluoride into the HA structure and an increase in apatite crystallinity were shown to be energy-dependent.

It should be emphasized that this study deals entirely with changes from an apatite structure to any different structure, and not changes, if any, within the apatite series of structures. Finally, the combination of laser and fluoride treatment showed increased fluoride uptake, with the formation of fluorapatite.

CONCLUSION

Within the limitations of this study it can be concluded that more fluoride retention was seen on the enamel surface irradiated with CO_2 laser.

The SEM images confirm that fluoride application after CO_2 irradiation presented a surface with fewer craters than the surface irradiated with CO_2 alone. The XRD analysis revealed the presence of fluorapatite in group 4, which was not to be seen in any other groups.

For years, a fluoride regimen has been the gold standard to make teeth resistant to acid attack. Today, CO_2 laser can be implemented along with a fluoride regimen in daily clinical practice to increase the retention of fluoride on the tooth surface and even transform hydroxyapatite into fluorapatite.

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