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Maryam Poosti, Farzaneh Ahrari, Horieh Moosavi & Hoda Najjaran

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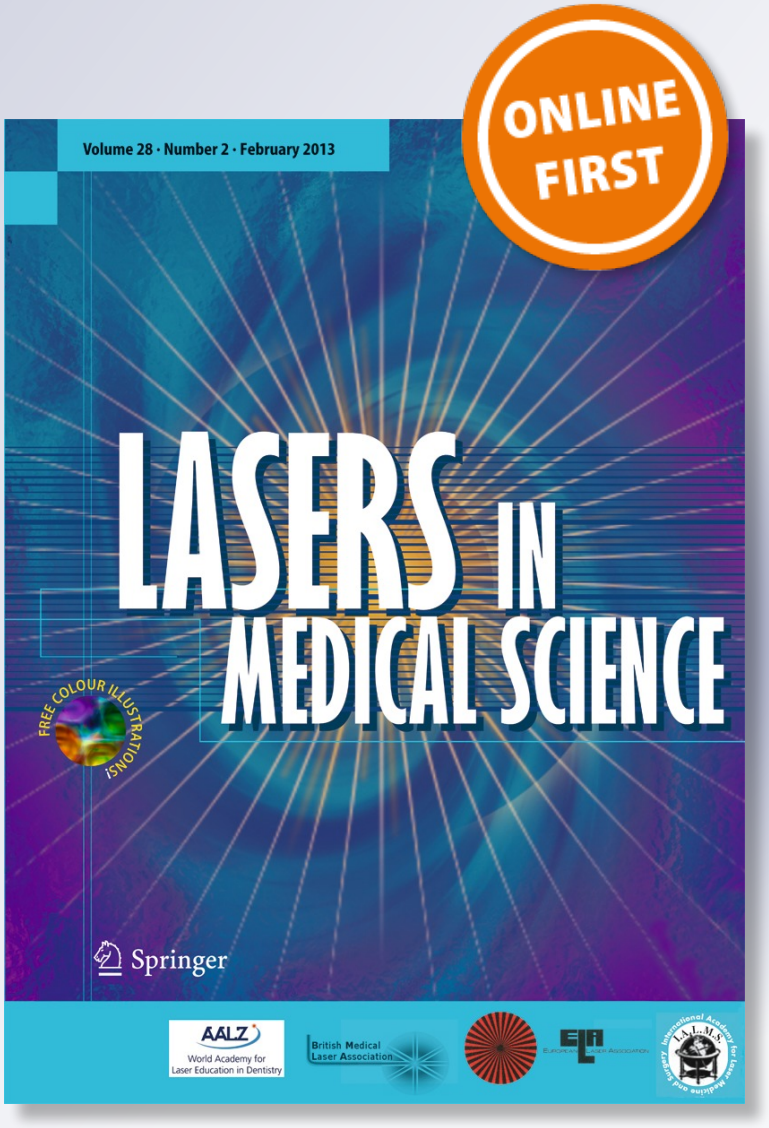

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ORIGINAL ARTICLE

The effect of fractional CO_2 laser irradiation on remineralization of enamel white spot lesions

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Abstract This study investigated the combined effect of fractional CO_2 laser irradiation and fluoride on treatment of enamel caries. Sixty intact premolars were randomly assigned into four groups and then stored in a demineralizing solution to induce white spot lesions. Tooth color was determined at baseline (T1) and after demineralization (T2). Afterwards, the teeth in group 1 remained untreated (control), while group 2 was exposed to an acidulated phosphate fluoride (APF) gel for 4 min. In groups 3 and 4, a fractional CO_2 laser was applied (10 mJ, 200 Hz, 10 s) either before (group 3) or through (group 4) the APF gel. The teeth were then immersed in artificial saliva for 90 days while subjected to daily fluoride mouthrinse and weekly brushing. Color examinations were repeated after topical fluoride application (T3) and 90 days later (T4). Finally, the teeth were sectioned, and microhardness was measured at the enamel surface and at 30 and 60 μ from the surface. In both lased groups, the color change between T1 and T4 stages (ΔE_{T1-T4}) was significantly lower than those of the other groups ($p<0.05$). Laser irradiation followed by fluoride application (group 3) caused a significant increase in surface microhardness compared to APF alone and control groups ($p<0.05$). Microhardness at depths of 30 and 60 μ was also significantly greater in group 3 compared to those of all other groups ($p<0.05$). Application of a fractional CO_2 laser before fluoride therapy is suggested for recovering the color and rehardening of demineralized enamel.

Keywords Enamel · Remineralization · CO_2 · Laser · Fractional · Colorimetry · Microhardness · White spot · Tooth color · Caries · Fraxel

Introduction

Enamel demineralization is an inevitable risk associated with fixed orthodontic treatment, which can, nevertheless, jeopardize the esthetic benefits of the therapy and make the tooth more susceptible to future restoration. At the early stages of demineralization, enamel caries is reversible via a remineralization process involving the diffusion of calcium and phosphate ions into the subsurface lesion to restore the lost tooth structure [1]. It has been demonstrated that the remineralization process is enhanced in the presence of fluoride-containing products [2]. Fluoride helps to reharden the softened tooth structure by increasing the percentage of mineral deposition, but there is controversy regarding whether this treatment improves the milky color of the porous enamel, or it just rehardens the surface layer with less effect on its appearance.

Several types of lasers, such as erbium-doped yttrium aluminum garnet (Er:YAG) [3–5], neodymium-doped yttrium aluminum garnet (Nd:YAG) [4, 6], and carbon dioxide (CO_2) [6–10], with different parameter settings, have been used for caries inhibition. The CO_2 laser (9.3, 9.6, 10.3, and 10.6 μ

M. Poosti
Department of Orthodontics, School of Dentistry,
Islamic Azad University, Tehran, Iran

F. Ahrari (✉)
Dental Research Center, School of Dentistry,
Mashhad University of Medical Sciences, Vakilabad Boulevard,
Mashhad, Iran
e-mail: Farzaneh.Ahrari@gmail.com

F. Ahrari
e-mail: AhrariF@mums.ac.ir

H. Moosavi
Dental Material Research Center, School of Dentistry,
Mashhad University of Medical Sciences, Mashhad, Iran

H. Najjaran
Mashhad, Iran

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wavelengths), however, should be considered the mainstay in caries prevention, because the absorption bands of phosphate, carbonate, and hydroxyl groups of enamel and dentin structures are within 9.0 to 11.0-μ region, which coincide well with the wavelengths of the CO_2 laser [10, 11]. Following absorption, there would be a high temperature increase at the surface and near the surface layers, which results in structural and chemical alterations of the enamel including decomposition of organic matrix, reduction of carbonate content, and fusion and recrystallization of hydroxyapatite crystals [10, 12–15], making the tooth more resistant to acidic challenge. It has been demonstrated that laser irradiation combined with topical fluoride treatment produced a synergistic effect, which increased fluoride uptake and decreased the dissolution rate of the enamel significantly as compared with laser or fluoride application alone [16–22]. Despite the abundance of research on caries inhibition effects of lasers, little data are available regarding their effects on the treatment of demineralized enamel.

Most studies to date utilized the conventional CO_2 laser for caries inhibition and remineralization, and to the best of the authors' knowledge, no one employed a fractional CO_2 laser for remineralization of carious enamel. The concept of fractional photothermolysis was introduced in 2003 by Huzaira and coworkers [23] to counteract the main drawbacks of skin resurfacing with the conventional CO_2 laser such as prolonged downtime, persistent erythema, edema, burning, and scarring. Instead of generating layers of thermal heating, fractional photothermolysis produces multiple columns of microthermal injury called microscopic treatment zones, with predefined space between them. Since the surrounding tissues of the irradiated zones remain healthy and untreated, the wound healing process is enhanced, and less side effects would be expected [24, 25]. Fractional photothermolysis may have potential advantages in dentistry, as it is possible to predetermine the exact area of irradiation, and the laser itself irradiates multiple spots within the target area without a need for manual scanning of the surface [26]. The aim of this study was to investigate the effects of fractional CO_2 laser irradiation, either before or through an APF gel, on improving color and microhardness of demineralized enamel.

Materials and methods

Sixty extracted premolar teeth without visible caries or structural defects on enamel surface were collected, cleaned, and stored in a 0.1 % thymol solution at room temperature. The surface of each tooth was covered with an acid-resistant nail varnish, leaving a window of approximately 4×4 mm at the center of the buccal surface exposed. The teeth were randomly allocated into four groups. A colorimeter (ColorEye XTH, X-rite, Grand Rapids, MI, USA) was used to record the color of natural enamel surfaces (baseline examination, T1) according to the Commission International de l'Eclairage (CIE) lab system in which the *L* coordinate corresponds to the value or degree of lightness, whereas the *a* and *b* values indicate positions on red/green (+a = red, −a = green) and yellow/blue (+b = yellow, −b = blue) axes, respectively.

Each tooth was then individually immersed in 10 ml of a demineralizing solution for 12 weeks to create artificial white spot lesions. The demineralizing solution at pH4.8 consisted of 50 mM acetic acid, 2.2 mM $CaCl_2$, and 2.2 mM NaH_2PO_4 [27]. The solution was changed every 5 days. After demineralization, color evaluation was performed again (demineralization stage, T2).

The experimental groups were then subjected to different treatments. Group 1 was left untreated at this stage and served as the control group. Group 2 underwent treatment with an acidulated phosphate fluoride gel (APF, Sultan Healthcare, Inc., Englewood, NJ, USA; containing 1.23 % fluoride ion, pH3.5), which was applied for 4 min on the enamel surface (APF alone group). In group 3, enamel windows were first exposed to irradiation from a fractional CO_2 laser (wavelength 10.6 μm; Lutronic Inc., Princeton Junction, NJ, USA) and then were immediately treated with the APF gel for 4 min (Laser before APF group). The laser was operated in the dynamic mode with frequency of 200 Hz, 10 mJ of energy, and power of 10 W, and the beam was adjusted to cover a square area of 4×4 mm^2 for 10 s per tooth. The laser's handpiece was held manually at an approximate distance of 25 mm from the sample surface by one investigator (F.A.). The specimens in group 4 were subjected to simultaneous fluoride and laser irradiation (laser through APF group). In this group, enamel windows were first treated with the APF gel for 2 min; then, the laser was applied for 10 s through the APF, and the fluoride was allowed to remain on the surface until 4 min. The laser parameters were the same as those in group 3.

The teeth in groups 2, 3, and 4 were rinsed thoroughly with tap water, and color determination was repeated (intense remineralization stage, T3). Then, a 90-day remineralization period simulating at-home remineralization of white spot lesions was started in which the teeth were kept in Fusayama Meyer artificial saliva at 37 °C and subjected to daily fluoride rinsing and weekly brushing. The artificial saliva consisted of KCl (0.4 g/l), NaCl (0.4 g/l), $CaCl_2$ $2H_2O$ (0.906 g/l), NaH_2PO_4, $2H_2O$ (0.690 g/l), Na_2S, $9H_2O$ (0.005 g/l), and urea (1 g/l) with pH adjusted to 7.03. This solution was replaced with new one every day. During the 90-day period, the teeth were immersed in a 0.05 % NaF mouthrinse (Oral-B Advantage; Oral-B Laboratories, Newbridge, UK) for 2 min per day, then rinsed, and reverted back to the artificial saliva solution. To mimic the tooth brushing procedure, an Oral-B CrossAction Power toothbrush was employed under a

standardized load with Sensodyne toothpaste containing 0.31 % *w*/*w* sodium fluoride (1,400 ppm fluoride). The device brushed each tooth for 7 min per week. After the 90-day interval, color measurement was carried out, and *L*, *a*, and *b* values were recorded again (final measurement, T4).

The color change (ΔE) between different treatment stages were measured using the following formula:

$$\Delta E = \left[(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2\right]^{0.5}$$

Cross-sectional microhardness assessment

After completing color measurements, the crowns were separated from the roots, and the teeth were sectioned occlusogingivally through the center of the buccal window using a diamond disk. The tooth sections were embedded in epoxy resin, and their surfaces were polished with sandpaper disks. One section was prepared from each tooth. A micro-Vickers hardness tester (Matsuzawa, model MHT2, Japan) was utilized under a load of 100 g and a dwell time of 5 s for taking microhardness measurement (Fig. 1), placing its indenter at the enamel surface, and at 30 and 60 μ depths from the surface.

Statistical analysis

Normal distribution of the data and homogeneity of variances were confirmed by the Kolmogorov–Smirnov and Levene's tests, respectively. A one-way ANOVA was performed to compare the color change (ΔE) obtained from the two measurements at T1 to T4 time points among the experimental groups, followed by Tukey multiple range test for pairwise comparisons.

The between-group differences in microhardness were also determined by ANOVA and Tukey test at the enamel surface and at 30 and 60 μ from the surface. The statistical analysis was performed using Statistical Package for the Social Sciences (version 16.0; SPSS, Inc., Chicago, IL) at the 95 % confidence interval of the difference.

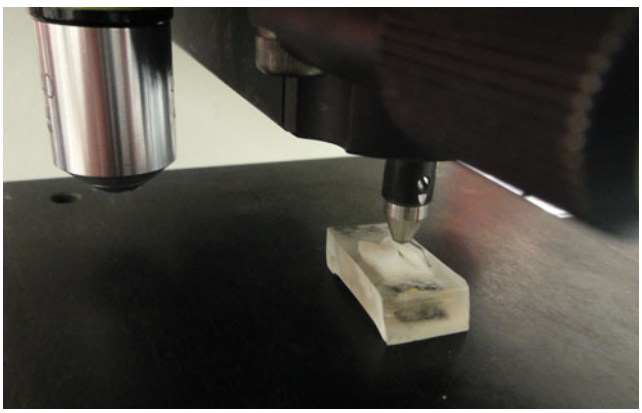

Fig. 1 A tooth section under the microhardness tester

Results

Color examination

The mean and SD of *L*, *a*, and *b* values in the experimental groups are summarized in Table 1. Following demineralization, the *L* value decreased (shifted to the dark domain) in all groups. There was also a general decrease in *a* and *b* values after demineralization, which means color shift to green and blue directions, respectively. During the 90-day remineralization period, the *L*, *a*, and *b* values in all groups came close to the pretreatment state to variable extents (Table 1).

Table 2 demonstrates descriptive statistics and the results of one-way ANOVA for comparison of color change (ΔE) between different treatment stages among the study groups. No significant difference was found either in the color difference between T1 (baseline) and T2 (demineralization) stages ($\Delta E_{T1–T2}$) or in the color change between T3 (intense remineralization) and T4 (90-day remineralization) time points ($\Delta E_{T3–T4}$) (Table 2). The experimental groups, however, exhibited statistically different extents of color change between demineralization and intense remineralization stages ($\Delta E_{T2–T3}$) and also between the baseline and final measurements ($\Delta E_{T1–T4}$) (Table 2).

Pairwise comparisons by Tukey test (Table 2) revealed that $\Delta E_{T2–T3}$ was significantly higher in group 4 (laser through APF) compared with those in groups 2 (APF alone) and 3 (laser before APF) ($p<0.05$). Tukey test also revealed that the specimens irradiated either before or through the APF gel experienced a significantly lower color change between the baseline and final measurements ($\Delta E_{T1–T4}$ = 3.1 in group 3 and $\Delta E_{T1–T4}$ = 2.6 in group 4) as compared to those of the fluoride-treated and control groups ($\Delta E_{T1–T4}$ = 5.5 in group 2 and $\Delta E_{T1–T4}$ = 6.6 in group 1) ($p<0.05$) (Table 2).

Microhardness assessment

The results of Vickers microhardness (VMH) measurements are provided in Table 3. The one-way ANOVA displayed significant differences in microhardness at the enamel surface and also at 30 and 60 μ from the surface among the experimental groups (Table 3). Further analysis with Tukey test (Table 3) revealed that surface microhardness was significantly greater in group 3 (laser before APF) as compared with the control and fluoride-treated groups ($p<0.05$). No significant difference was found in surface microhardness of the specimens irradiated before (group 3) or through (group

Table 1 Mean and standard deviation (SD) of *L*, *a*, and *b* values in the study groups during the experiment (T1=baseline examination, T2=demineralization stage, T3=intense remineralization stage, T4=final measurement)

Groups	CIE	T1		T2		T3		T4	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	*L*	80.17	3.61	76.04	2.28	–	–	75.06	2.23
	a	0.85	0.67	0.75	0.58	–	–	0.61	0.57
	b	12.98	2.50	10.09	2.33	–	–	11.02	2.90
APF alone	*L*	80.11	3.11	77.07	2.35	75.90	2.71	81.24	2.89
	a	0.92	0.50	0.99	0.56	1.05	0.511	1.08	0.45
	b	14.10	1.87	11.43	1.50	10.53	2.18	12.03	1.71
Laser before APF	*L*	80.33	2.15	77.10	2.35	76.00	2.84	81.50	2.16
	a	0.84	0.77	0.71	0.38	0.64	0.42	0.92	0.39
	b	12.87	1.74	10.88	2.06	9.81	2.16	11.95	1.37
Laser through APF	*L*	79.45	1.92	76.42	2.49	72.68	3.75	79.60	2.80
	a	0.68	0.51	0.65	0.39	1.26	0.44	0.90	0.60
	b	12.44	2.33	10.62	1.59	13.08	2.27	12.46	1.91

4) the APF gel ($p>0.05$). Laser irradiation followed by fluoride application caused a significantly greater microhardness measurement at both 30 and 60 μ depths compared to all other groups ($p<0.05$) (Table 3).

Discussion

This study investigated the efficacy of a fractional CO_2 laser in combination with APF on the treatment of artificially demineralized enamel, using color measurement and cross-sectional microhardness test as indicators of enamel remineralization. The laser parameters were selected to be as close as possible to those suggested by Esteves-Oliveira et al. [28], who reported as much as 81 % caries inhibition by using CO_2 laser without damage to the enamel structure. The application of fractional CO_2 laser in dentistry was proposed in a recent study [26] for conditioning feldspathic porcelain. It is believed that a ΔE (discrepancy between two hues) exceeding 3.3 units indicates color mismatching [29], as it would be clinically visible in any site by independent observers.

After demineralization (T2), the degree of lightness decreased in all groups, and there was a general decrease in *a* and *b* values, too. The color difference between the baseline and demineralization stages ($\Delta E_{T1–T2}$) was greater than 3.3 units in the four groups, indicating the creation of clinically visible white spot lesions. There was no statistical difference in $\Delta E_{T1–T2}$ among the experimental groups, which was a necessary condition for proper comparison of treatment effects on carious enamel.

Following the intense remineralization regimen (T3), which included fluoride treatment with/without laser application, a more decrease in degree of lightness was observed. The color change between the demineralization and intense remineralization stages ($\Delta E_{T2–T3}$) was significantly greater in group 4 (laser through APF) compared to those in groups 2 (APF alone) and 3 (laser before APF). This indicates that laser application through the APF gel may cause more precipitation of fluoride on the enamel surface and induce a negative effect on the color of demineralized enamel. It

Table 2 Mean, standard deviation (SD) and the results of statistical analysis for comparison of color differences (ΔE) between different treatment stages among the experimental groups

	$\Delta E_{T1–T2}$		$\Delta E_{T2–T3}$			$\Delta E_{T3–T4}$		$\Delta E_{T1–T4}$		
	Mean	SD	Mean	SD	Pairwise comparisons	Mean	SD	Mean	SD	Pairwise comparisons
Control	5.96	1.25	–	–	–	–	–	6.58	2.90	a
APF alone	4.74	1.98	3.20	2.11	a	7.28	3.73	5.50	2.63	a
Laser before APF	4.53	2.34	3.53	1.83	a	5.75	2.21	3.13	1.30	b
Laser through APF	4.55	1.79	5.25	2.32	b	7.20	2.62	2.64	1.28	b
p value	0.266		0.023*			0.283		<0.001*		

Different letters indicate significant differences at $p<0.05$

*$p<0.05$; statistically significant difference

Table 3 Mean, standard deviation (SD) and the results of statistical analysis for comparison of microhardness at the enamel surface and at 30 and 60 μ from the surface among the experimental groups

	Enamel surface			30 μ depth			60 μ depth		
	Mean	SD	Pairwise comparisons	Mean	SD	Pairwise comparisons	Mean	SD	Pairwise comparisons
Control	224.5	21.6	a	226.2	11.6	a	232.2	15.6	a
APF alone	234.5	32.3	a	252.4	40.3	a	264.5	40.4	a
Laser before APF	270.7	45.7	b	299.5	47	b	322.3	51.7	b
Laser through APF	249.1	44.4	a, b	259.6	42.4	a	262.8	42.1	a
p value	0.008*			<0.001*			<0.001*		

Different letters indicate significant differences at $p<0.05$

*$p<0.05$; statistically significant difference

should be mentioned that the control group was not colorimetrically evaluated at T3, because it was not exposed to intense remineralization treatment.

The three test groups (groups 2, 3, and 4) showed comparable color improvement between T3 (intense remineralization) and T4 (90-day remineralization) stages ($\Delta E_{T3–T4}$). The color improvement of demineralized enamel can be attributed to the remineralizing effect of the artificial saliva, which was enhanced by daily rinsing with the 0.05 % NaF solution, and the use of the fluoridated toothpaste. Another explanation for white spot recovery is the effect of brushing that can wear away the demineralized enamel. Backer [30] observed gradual wearing of the outermost enamel crystals of the carious lesion by functional wear and tooth brushing. Artun and Thylstrup [31] assumed that surface abrasion in association with mineral redeposition is responsible for regression of white spot lesions after debonding of orthodontic brackets.

When the color change between the baseline (T1) and final (T4) examinations was compared among the study groups, it was found that the two laser groups exhibited significantly lower color differences compared to APF alone and control groups. The $\Delta E_{T1–T4}$ was 3.1 in group 3 (laser before APF) and 2.6 in group 4 (laser through APF), which were statistically comparable to each other, and both were significantly lower than those of the control ($\Delta E_{T1–T4} = 6.6$) and fluoride-treated ($\Delta E_{T1–T4} = 5.5$) groups. This was possibly due to the creation of microscopic pores within the structure of laser-irradiated enamel, which enhanced fluoride and mineral entrapment during the 90-day remineralization period. Although the color change between T3 and T4 stages failed to achieve statistical difference between the experimental groups, the *L*, *a*, and *b* values came remarkably close to the pretreatment level in the two laser groups during the 90-day remineralization period, as evidenced in Table 1. It should be noted that the color change between the baseline (T1) and final (T4) examinations was lower than 3.3 units in the two groups involving laser irradiation, implying that the enamel appearance at the end of the remineralization therapy was not different from the intact enamel surface when evaluated by independent observers. In contrast, the corresponding color change was greater than 3.3 units in the fluoride-treated and control groups.

Zachrisson and Buyukyilmaz [32] believed that concentrated fluoride agents should not be employed immediately after the completion of orthodontic treatment in patients with visible white spots, because they may cause fluoride precipitation to occur in the surface-softened enamel with less effect on the subsurface lesions, and thus, the optical appearance of the white spot lesions would not be reduced. Although the results of this study indicated that the application of APF with/without laser could exacerbate the milky color of the demineralized enamel immediately after treatment, it did not affect the capacity of the tooth structure for future remineralization, because $\Delta E_{T1–T4}$ was not significantly different between the control and fluoride-treated groups. The current study is the first attempt to prove that laser irradiation either before or through APF is successful in restoring the color of white spot lesions in a long-lasting remineralization model. This may have important clinical implications because the altered color of demineralized enamel may produce an esthetic concern even more than 5 years after removal of orthodontic appliances [33].

Surface microhardness of the specimens irradiated prior to fluoride application (VMH = 271) was significantly greater than those of the fluoride-treated (VMH = 234) and control (VMH = 224) groups, but comparable to the microhardness of the specimens irradiated through the APF gel (VMH = 249). At 30 and 60 μ from the surface, microhardness was significantly greater in group 3 compared to those in all other groups, indicating that the rehardening effect of the fractional CO_2 laser irradiation penetrates down to deeper enamel layers, if it is applied before fluoride application. Previous studies demonstrated similar findings. Chen et al. [34] reported that the application of lasers (CO_2 and Nd:YAG) and fluoride increased the acid resistance of decalcified enamel, and the effects of

lasers were better than those of fluoride. Esteves-Oliveira et al. [35] indicated that CO_2 laser irradiation was capable to reharden previously softened enamel in vitro. Schmidlin et al. [36] found that laser application through an amine fluoride solution caused a significant increase in fluoride uptake and thus made the demineralized enamel more acid resistant. It is believed that the increased microhardness following laser irradiation is related to the ultrastructural changes including crystal size growth and recrystallization of porous enamel as a result of high temperature rise at the surface [35, 37]. There was no significant increase in microhardness when irradiation was performed through the APF either at 30 or 60 μ depths. It can be drawn that the presence of APF prevents from temperature increase at the surface to some extent, thus limiting the rehardening effect of the CO_2 laser.

There is controversy regarding whether laser irradiation should be performed before, through, or after fluoride application. Some authors showed significantly higher acid resistance when enamel was irradiated before fluoride application [38], whereas others believed that laser application should be performed following fluoride treatment [35]. Tepper et al. [39] and Schmidlin et al. [36] recommended laser irradiation through an amine fluoride solution in order to promote acid resistance while reducing adverse structural effects on the enamel surface. The results of the present study indicated that laser irradiation either before or through the APF improved the color of demineralized enamel after a 90-day remineralization period to a clinically acceptable level, but irradiation prior to fluoride application was more effective in increasing the microhardness of the surface and near the surface enamel layers. However, we did not observe possible detrimental influences on the enamel morphology such as surface cracking and melted areas, which may be greater when laser is applied before fluoride application. Furthermore, the study design did not include a group that was only irradiated by laser, and this could be considered a limitation of the present investigation. The laser irradiation alone may produce chemical and structural alterations that facilitate mineral deposition in long term, thus improving the color and mechanical properties of demineralized enamel.

The fractional CO_2 laser is not commercially designed for dental applications. Further improvement in the design and accessories is required to make it more comfortable for dental applications. Future studies should be designed on the potential benefits of the fractional CO_2 laser in other fields of dentistry and its effects on temperature increase in underlying tissues and surface morphology of sound and demineralized enamel.

Conclusions

1. Application of a fractional CO_2 laser either before or through the APF was more effective in restoring the appearance of carious enamel after a 90-day remineralization period compared to APF alone and control groups.
2. Laser irradiation before fluoride treatment caused a significant increase in the surface microhardness of the enamel, which continued down to depths of 30 and 60 μ.
3. Exposure to a high-concentrated fluoride gel did not reduce the susceptibility of demineralized enamel to future remineralization in a long-term remineralization model.

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