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ORIGINAL ARTICLE

The effect of Er,Cr:YSGG laser, fluoride, and CPP-ACP on caries resistance of primary enamel

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Abstract

The aim of this in vitro study was to determine the effect of different remineralization agents and laser on caries resistance of primary enamel. In the study, 150 sound primary molars were used. The initial microhardness values were measured and the teeth were randomly assigned to ten treatment groups ($n = 15$): no treatment/negative control (C), NaF, APF, fluoride varnish (FV), CPP-ACP, laser (L), L + NaF, L + APF, L + FV, L + CPP-ACP. The microhardness values were measured after the treatments and the pH cycle. The obtained data were analyzed statistically. One sample from each group was examined before treatment, after treatment, and after the pH cycle with a scanning electron microscope. While microhardness values after treatment compared to baseline increased, microhardness after the pH cycle decreased compared to after treatment values in all experimental groups ($p < 0.05$). In regard to the difference in microhardness after the pH cycle and baseline, there were no statistically significant differences between groups C and NaF and between C and CPP-ACP ($p > 0.05$). There was a significant difference between groups L and L + FV ($p < 0.05$), while no significant difference was noted between groups L and L + NaF, L + APF, L + CPP-ACP ($p > 0.05$). As a conclusion, FV is more effective when used in combination with laser than laser alone. NaF, CPP-ACP, and laser may be insufficient in protecting the primary teeth against acid attacks compared to FV used with laser.

Keywords Dental caries · Tooth remineralization · Tooth demineralization · Fluoride · Laser therapy · CPP-ACP

Introduction

Dental caries continues to be the most common chronic childhood disease in many countries [1–3]. Different methods including fissure sealant, fluoride, and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) have been used in order to prevent dental caries, but none of them has been proven to be completely effective yet [4, 5]. Fluoride acts by enhancing remineralization of enamel and formation of fluorapatite (FAP), which is more resistant to caries than hydroxyapatite [6–8]. Sodium fluoride (NaF), in both neutral and acidulated forms (acidulated phosphate fluoride—APF), is the most commonly used fluoride compound in preventive dentistry [9]. NaF has been professionally applied in gel, foam, and varnish forms. The fluoride concentrations of commonly used neutral NaF gel, APF gel, and NaF varnish are 9050 ppm, 12,300 ppm and 22,600 ppm fluoride ion, respectively [10]. Despite their higher fluoride concentrations, fluoride varnishes have been regarded as safe for use in small children as they are applied to the teeth with small amounts and have 48–72 h adhesion times on the tooth surfaces [11]. Therefore, there has been a growing demand on the use of fluoride varnishes not only for individual applications but also for community-based caries prevention programs [12]. In addition to fluoride, CPP-ACP has been used against dental caries. The mechanism is based on the saturation of the enamel with calcium and phosphate to prevent demineralization while improving remineralization of the enamel [13–15].

Laser irradiation of the enamel has also been suggested for prevention of caries. Several investigations have demonstrated that enamel matrix is partially denaturated after laser irradiation, which decreases enamel permeability, inhibits acid

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diffusion, and thereby reduces enamel demineralization [15–17]. The erbium lasers used for this purpose are erbium: yttrium–aluminum–garnet (Er:YAG) laser with the wave length of 2940 and erbium and chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser with the wavelength of 2780 nm. Both types have been approved as effective and safe in hard and soft tissue procedures in dentistry. They can ablate enamel, dentin, and soft tissues with the use of varying power settings [18].

There have been a number of studies on the combined use of fluoride and laser applications for prevention of dental caries. As majority of the studies were conducted on permanent teeth [5, 16, 19, 20], there is only limited information regarding the effect of combined fluoride and laser application on primary teeth [9, 21, 22]. In the aforementioned studies, APF in gel form has been commonly used as the fluoride agent. To the best of our knowledge, no study has compared the three different forms of NaF, namely neutral NaF gel, APF gel, and NaF varnish. In addition, there are only few studies on the combined use of CPP-ACP and laser [15, 23, 24], but in none of them the efficacy of CPP-ACP compared to fluoride. The aim of this in vitro study was to evaluate the efficacy of three different forms of fluoride (neutral NaF gel, APF gel, and NaF varnish) and CPP-ACP associated with or without laser irradiation in primary teeth in terms of caries prevention.

The null hypotheses were as follows:

1. There would be no significant difference among four different remineralization agents and laser.
2. There would be no significant difference between the use of remineralization agents with or without laser.

Materials and methods

This in vitro study was approved by the local ethics committee of Hacettepe University, Ankara, Turkey (Project no: TDK-2016-12277). Experimental parts of our work were carried out in the AR-GE Laboratory and the Dental Medical Laser Application and Research Center in the Hacettepe University School of Dentistry.

Experimental design

It is found that at least 11 samples were needed for each group to detect a medium effect size ($d = 0.50$) between groups at the level 5% type I error rate and 95% power. Sample size in each group was set as 15. In the first step of the study, microhardness measurements were performed. Microhardness measurements at the beginning (baseline), after the treatments, and after the pH cycle were the primary response variables. In the second step, an additional sample from each group was prepared and analyzed by scanning electron microscope (SEM).

Preparation of the samples

One hundred fifty extracted sound, non-carious human primary molar teeth were used for the study. The tissue remnants were removed and the teeth were polished using a slow-speed rotating rubber cup under water cooling. Teeth were stored in saline until the beginning of the experiments. The roots of the teeth were separated from the crowns with a water-cooled high-speed diamond burr. All teeth were examined under magnification (× 40) to detect surface defects and cracks. The buccal tooth surfaces were ground under constant water cooling with 800, 1200, and 2000-grit silicon carbide paper on a rotating polishing machine (Presi Mecapol P230, 38320 Briet Angonnes, Grenoble, France) to obtain flat enamel surfaces. For each sample, an adhesive tape (2 × 2 mm) was placed onto the buccal surface, and the remaining surface was covered with two layers of an acid-resistant nail polish. The teeth were embedded in a self-curing resin (İntegra Incorporated Dental Group, Ankara, Turkey). After the resin had set, the adhesive tape was removed from the teeth surface. This procedure allowed a standardized enamel window surface of 2 × 2 mm to be obtained on each sample. To prevent dehydration, the samples were kept in artificial saliva [25].

Experimental groups

The obtained samples were randomly divided into ten groups as follows ($n = 15$). The materials and procedures are shown in Table 1.

pH cycling

The samples in each group were subjected to 7-day in vitro pH cycling validated protocol [25]. The samples were kept in demineralization solution (2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M CH_3COOH, 1 M KOH pH: 4.5) for 6 h once a day and remineralization solution (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCL, pH: 7.0) for the remaining 18 h of the day. After 5 days of the pH cycle, the samples were left in remineralization solution for 2 days, and the cycle was completed. The solutions were renewed every 3 days and changed daily.

Laser irradiation

The enamel surface of the specimens was lased with a Er,Cr:YSGG laser at a wavelength of 2.780 nm (Waterlase MD, San Clemente, CA, USA). The laser was applied for 10 s with a repetition rate of 20 Hz, pulse duration of 140 μs, and 0.25 W output power with 11% air and 0% water,

Table 1 The materials and procedures in the study

Groups	Manufacturer	Mode of application
C (control)	–	- No treatment
		- Kept in artificial saliva for 24 h
		- pH cycling (7 days)
NaF (9050 ppm Neutral Sodium Fluoride Gel)	Sultan Topex Neutral pH Gel, Sultan Dental Products, USA	- Applied to the enamel surface for 4 min with a cotton swab and removed with gauze
		- Kept in artificial saliva for 24 h
		- pH cycling (7 days)
APF (12,300 ppm acidulated phosphate fluoride gel)	APF, Gelato, APF Fluoride Gel, Keystone Industries, USA	- Applied to the enamel surface for 4 min with a cotton swab and removed with gauze
		- Kept in artificial saliva for 24 h
		- pH cycling (7 days)
FV (22,600 ppm sodium fluoride varnish)	Duraphat, Woelm & Pharma Co., Eschwege, Germany	- Applied to the enamel surfaces with a micro-brush
		- Kept in artificial saliva for 24 h and removed by scalpel blade
		- pH cycling (7 days)
CPP-ACP (casein phosphopeptide-amorphous calcium phosphate paste)	GC Tooth Mousse, GC Corp., Tokyo, Japan	- Applied to the enamel surface for 3 min and removed with gauze
		- Kept in artificial saliva for 24 h.
		- pH cycling (7 days) (daily application of CPP-ACP during the pH cycling)
L (2.780 nm erbiyum chromium yttirium scandium galium garnet laser)	Waterlase MD, San Clemente, CA, USA)	- The laser was kept at a distance of 1–2 mm from the surface with a repetition rate of 20 Hz, pulse duration of 140 μs, and 0.25 W output power with 11% air and 0% water. MG6-6 mm sapphire tip was used
		- Kept in artificial saliva for 24 h
		- pH cycling (7 days)
L + NaF		- Laser irradiation followed by NaF application
		- Kept in artificial saliva for 24 h
		- pH cycling (7 days)
L + APF		- Laser irradiation followed by APF application
		- Kept in artificial saliva for 24 h
		- pH cycling (7 days)
L + FV		- Laser irradiation followed by FV application
		- Kept in artificial saliva for 24 h
		- pH cycling (7 days)
L + CPP-ACP		- Laser irradiation followed by CPP-ACP application
		- Kept in artificial saliva for 24 h.
		- pH cycling (7 days) (daily application of CPP-ACP during the pH cycling)

at a distance of 1–2 mm from the surface. MG6-6 mm sapphire tip was used.

Microhardness test

Vickers microhardness was measured using a microhardness tester (Shimadzu HMV-2, Tokyo, Japan). During the measurement, a load of 200 g (1,961 N) was applied for 10 s at three different points of each sample [26, 27]. The mean of three measurements was accepted as the microhardness value of each sample.

Scanning electron microscope (SEM) analysis

The crown of an additional tooth from each group was cut into four pieces with a diamond blade. One of the four specimens

served as the control, while the other two samples underwent the previously described procedures. Of the treated samples, one was subjected to pH cycling. All samples were sputter coated with gold-palladium and analyzed by SEM (Jeol/JSM 6400, Tokyo, Japan).

Statistical analysis

In the study; Δ1, Δ2, and Δ3 were set as the follows:

Δ1: Microhardness values after treatment—baseline microhardness values
Δ2: Microhardness values after pH cycling—microhardness values after treatment
Δ3: Microhardness values after pH cycling—baseline microhardness values

The data were analyzed using IBM SPSS 21 software (IBM; Armonk, NY, USA). In the statistical analysis, for the within group changes, absolute microhardness values were used. Between groups comparisons were made with respect to (Δ1), (Δ2), and (Δ3). The data were tested for normal distribution using Shapiro Wilks test; however, it was found that the data did not follow the normal distribution. The repeated measures of microhardness with respect to baseline, after treatment, and after pH cycling were compared using the Friedman test. The Kruskal-Wallis test was used for independent group comparisons of the differences and the Conover-Dunn test was used for pairwise comparisons. The level of significance was set at $p < 0.05$.

Results

The mean microhardness values of each group are shown in Table 2. In all experimental groups, there was a statistically significant difference in microhardness between the baseline and after treatment values (Δ1), ($p < 0.05$). For the control group, there was no statistically significant difference in (Δ1). For all groups, there was a statistically significant difference in (Δ2), which indicates the microhardness difference after the treatment and after the pH cycle ($p < 0.05$, Table 2). In all groups, mean microhardness values after the pH cycle decreased compared to those of the baseline (Δ3) (Fig. 1), but there was no statistically significant difference for group 3 (APF), ($p = 0.053$, Table 2).

Regarding Δ1, the control group had the lowest [− 3.26 (± 4.27)], whereas Group 9 (L + FV) had the highest mean values [76.53 (± 13.87)]. For Δ2, the lowest mean value was in the control group [− 171.66 (± 29.10)] and the highest mean value was in group 10 (L + CPP-ACP) [− 95.37 (± 18.94)]. With respect to Δ3, the control group showed the lowest [− 168.40 (± 27.94)], whereas Group 9 (L + FV) showed the highest mean values [− 35.57 (± 13.44)] (Table 2).

Pairwise comparisons of all groups in terms of Δ1, Δ2, and Δ3 are shown in Table 2. There was no statistically significant difference between all remineralization agents and laser irradiation followed by remineralization agent groups ($p > 0.05$). Nevertheless, there was a statistically significant difference between group 6 (L) and Group 9 (L + FV) ($p < 0.05$; Table 2).

Scanning electron microscopy findings

Smooth enamel surface was observed in the non-treated enamel parts in all groups. There were CaF_2-like globules on the enamel surfaces treated with three different fluoride forms regardless of laser irradiation. In the FV group, the accumulation of the CaF_2-like globules was more prominent (Fig. 2).

In the sample treated with CPP-ACP, numerous granular particles and amorphous crystals were noticed on the enamel surface (Fig. 2).

The surface morphology of the enamel irradiated with Er,Cr:YSGG laser presented an increased roughness along with melting areas (Fig. 3).

After the pH cycle, a decrease in the CaF_2-like globules and deterioration of the surface structure and increase in porosity were observed. Nevertheless, the decrease in CaF_2-like globules was less in the L + FV group than the other groups. In the L + CPP-ACP sample, a relatively smooth and more homogeneous surface was noticed than that of the CPP-ACP group (Fig. 3).

Discussion

The purpose of this in vitro study was to compare the caries-preventive effect of different remineralization agents used alone or in combination with laser. In the dental literature, no consensus has been reached regarding the efficacy of the combined use of laser and fluoride in increasing the hardness of enamel [5, 16, 19, 28]. This result may be attributed to the methodological differences among the studies. The primary difference might be related with the type of laser used, including CO_2, Nd: YAG, Er:YAG, Er,Cr:YSGG, and Argon lasers. Only a limited number of studies used Er:YAG or Er,Cr:YSGG lasers [5, 16, 19, 23, 28–30]. Of those, only two studies compared the effectiveness of Er,Cr:YSGG laser to another type of laser [19, 29]. In one of them, Anaraki et al. [19] found the combined use of fluoride with both CO_2 and Er,Cr:YSGG lasers was effective in decreasing the enamel demineralization. On the other hand, Santos et al. [29] found no significant difference between the untreated samples and the samples treated with fluoride and lased either with Er,Cr:YSGG or Nd: YAG laser.

Table 2 Mean microhardness values, intergroup and intragroup comparisons

Group	Baseline ± SD	After treatment ± SD	After pH cycle ± SD	Δ1 ± SD*	*P*** (Δ1)	Δ2 ± SD*	*P*** (Δ2)	Δ3 ± SD*	*P*** (Δ3)
Group 1 (control)	363.57 ± 26.02	360.31 ± 24.59	191.91 ± 31.40	− 3.26 ± 4.27^{A}	.432	− 171.66 ± 29.10^{A}	.002	− 168.40 ± 27.94^{A}	.000
Group 2 (NaF)	323.04 ± 20.64	370.57 ± 16.07	252.66 ± 27.31	47.53 ± 9.42^{BC}	.019	− 117. 91 ± 26.21^{AB}	.000	− 70.37 ± 23.80^{AB}	.019
Group 3 (APF)	357.04 ± 16.88	408.04 ± 9.49	309.62 ± 22.22	50.99 ± 12.52 ^{CD}	.010	− 98.42 ± 17.58^{B}	.000	− 47.42 ± 18.71^{BC}	.053
Group 4 (FV)	327.86 ± 12.65	401.13 ± 17.17	277.62 ± 23.91	73.26 ± 8.32^{D}	.019	− 123. 51 ± 26.35^{AB}	.000	− 50.24 ± 23.31^{BC}	.019
Group 5 (CPP-ACP)	320.97 ± 14.13	366.66 ± 15.36	250.28 ± 30.06	45.68 ± 4.84^{BC}	.019	− 116. 37 ± 25.87^{AB}	.000	− 70.68 ± 25.10^{AB}	.019
Group 6 (L)	340.73 ± 26.03	373.82 ± 24.77	273.97 ± 27.84	33.08 ± 3.63^{AB}	.019	− 99.84 ± 23.97^{B}	.000	− 66.75 ± 24.04^{B}	.019
Group 7 (L + NaF)	329.02 ± 15.83	376.33 ± 18.09	275.95 ± 17.25	47.31 ± 8.83^{BC}	.019	− 100. 37 ± 10.50^{B}	.000	− 53.06 ± 7.84^{BC}	.019
Group 8 (L + APF)	340.57 ± 30.24	393.02 ± 24.39	290.82 ± 35.78	52.44 ± 10.22^{CD}	.019	− 102.20 ± 20.47^{B}	.000	− 49.75 ± 17.99^{BC}	.019
Group 9 (L + FV)	329.84 ± 23.99	406.37 ± 15.71	294.26 ± 19.88	76.53 ± 13.87^{D}	.019	− 112.11 ± 13.91^{B}	.000	− 35.57 ± 13.44^{C}	.019
Group 10 (L + CPP-ACP)	324.42 ± 22.05	368.66 ± 21.89	273.28 ± 18.74	44.24 ± 4.98^{BC}	.019	− 95.37 ± 18.94^{B}	.000	− 51.13 ± 18.96^{BC}	.019

SD standard deviation, *NaF* neutral sodium fluoride, *APF* acidulated phosphate fluoride, *FV* fluoride varnish, *CPP-ACP* casein phosphopeptide-amorphous calcium phosphate, *L* laser, *Δ1* difference in microhardness after treatment and baseline, *Δ2* difference in microhardness after pH cycle and after treatment, *Δ3* difference in microhardness after pH cycle and baseline

*Within each column different capital letters show statistically significant difference between groups ($p < 0.05$)

***P* (Δ1), *P* (Δ2), and *P* (Δ3) show intragroup difference ($p < 0.05$)

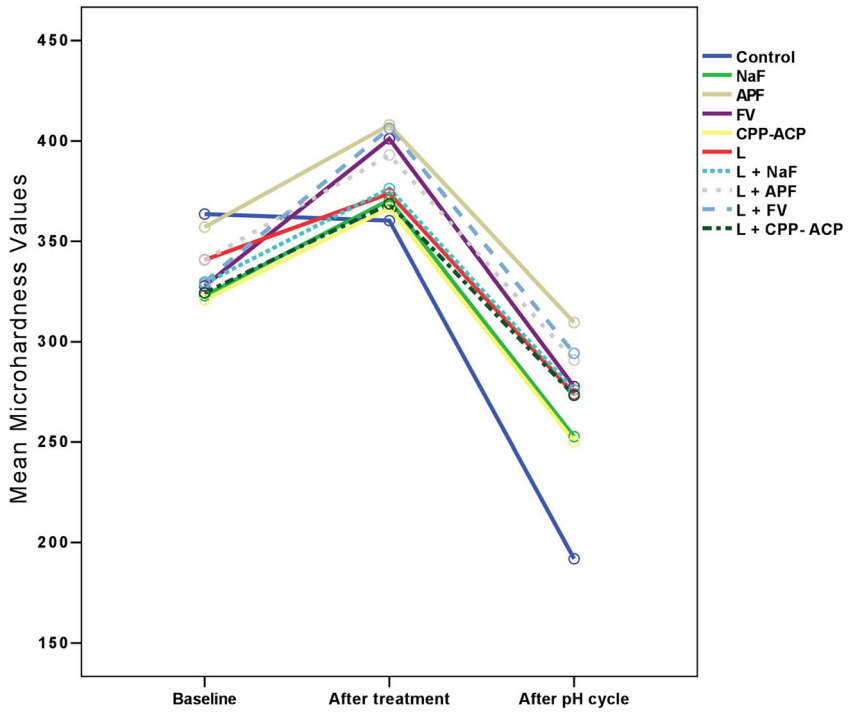

Fig. 1 Microhardness change of all groups at baseline, after treatment and after the pH cycle

The second methodological difference might be related to the irradiation of lasers before or after fluoride application. Increased fluoride uptake had been reported due to the laser-modified enamel surface [31, 32]. On the contrary, Meurman et al. [33] showed rapid transformation of hydroxyapatite (HAP) to fluorapatite (FAP) by laser irradiation preceded by fluoride. Moslemi et al. [5] applied Er,Cr:YSGG laser both before and after fluoride. The authors reported no significant differences between both methods. In the literature, in most of the studies, the lasers were applied before fluorides [16, 24, 28, 31, 34, 35]. Therefore, in the present study, it was preferred to irradiate the surface with laser before remineralization agent applications.

In most of the studies, APF gel was used as the representative of the fluoride compound [5, 16, 19, 22, 28]. In a few studies, other forms including neutral NaF gel, sodium fluoride varnish, and amine fluoride gel as well as fluoridated toothpaste were used [22, 30]. In the studies, only one agent was used without comparison of the different forms. In addition, there were a limited number of studies on the use of CPP-ACP in combination with laser [23, 24]. As none of these studies compared the efficacy of the CPP-ACP agent to that of fluoride, the results could not be compared to those of ours.

According to the results of this study, there was an increase in microhardness after all treatment types (Δ1) indicating that fluoride, CPP-ACP, and laser caused changes on the enamel surface [9, 15, 21–23, 27, 30]. However, no significant difference was found between the control and the laser group. Among the studies regarding the effect of laser and fluoride on microhardness, only one of them concerned Δ1 [36]. Concur with the results of our study, Esteves-Oliveria et al. [36] also found no significant difference between the control group and the laser-irradiated group.

In our study, not only the remineralizing agents but also their combined use with laser resulted in higher microhardness than that of the control group. The difference might have been related to the different fluoride preparations used, aminefluoride versus sodiumfluoride. Moreover, the authors used permanent teeth, whereas we used primary teeth in our study. It is well-known that the preventive effect of lasers on dental tissues is based on making changes in the chemical, physical, and crystal structure by bringing heat changes in the tissues [23]. The difference in the organic and inorganic contents and crystal structures of primary and permanent teeth might have contributed to the different results obtained. In the studies concerning the effect of laser on enamel microhardness, commonly permanent teeth were used [16, 30, 36]. There is only limited information and conflicting results on the effect of laser on primary enamel [22, 23]. Azavedo et al. [22] reported no difference in microhardness of primary enamel irradiated by Nd: YAG. In contrast, Subnamariam et al. [23] reported increased microhardness of primary enamel irradiated by Er, Cr: YSGG laser. The difference between the two studies might have been related to the different laser types and the parameters used. It is known that the mineral content of permanent enamel is higher than that of primary enamel. More laser absorption occurs due to the higher water content of primary enamel. Therefore, there may be a need for adjustment of the laser parameters according to the substitute used. Within this respect, in the present study laser parameters of 0.25 W and 20 Hz were used. The used parameters were in accordance with the previously reported studies [5, 16, 19, 30]. Oliveria et al. [37] compared different

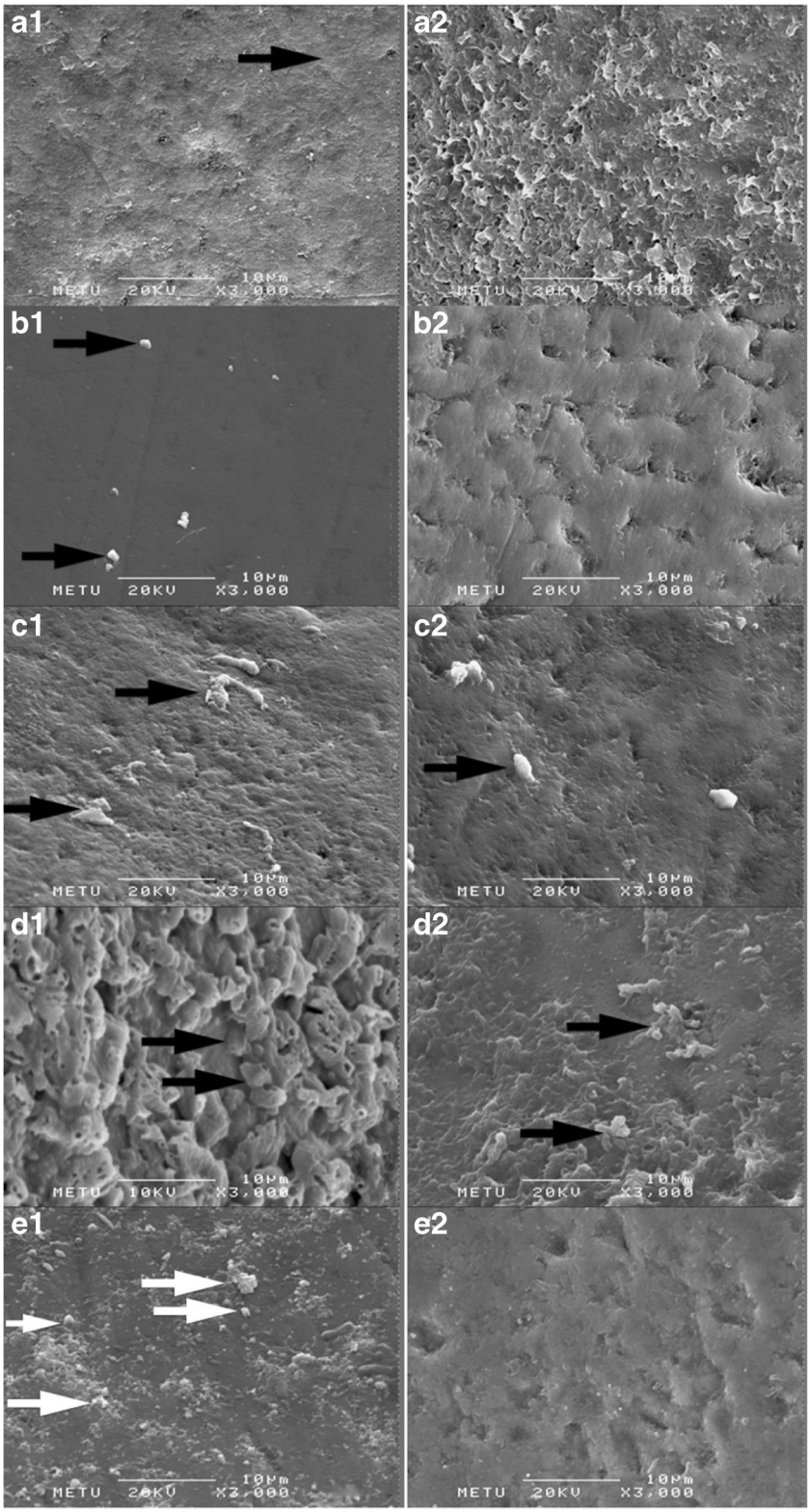

Fig. 2 A1 baseline (control); A2: after the pH cycle (control); B1,C1,D1: CaF_2-like globules (black arrows) on the enamel surfaces treated with B1: NaF, C1: APF and D1: FV; B2,C2,D2: decreased CaF_2-like globules and increased porosity after the pH cycle on the samples treated with B2: NaF, C2: APF and D2: FV; E1: granular particles and amorphous crystals (white arrows) on the enamel surfaces treated with CPP-ACP; E2: decreased granular particles and increased pores after the pH cycle on the sample treated with CPP-ACP. Original magnification: × 3000

parameters of Er, Cr: YSGG laser. The authors reported decreased microhardness with 0.75 W but increased microhardness with 0.25 W, 0.50 W, and with the frequency between, 20–50 Hz. In a literature review concerning the effect of laser parameters on the demineralization of enamel, it was concluded that there had been no consensus on the laser parameters [38]. The lack of comparison of different parameters may be regarded as a limitation of the present study. Therefore, more

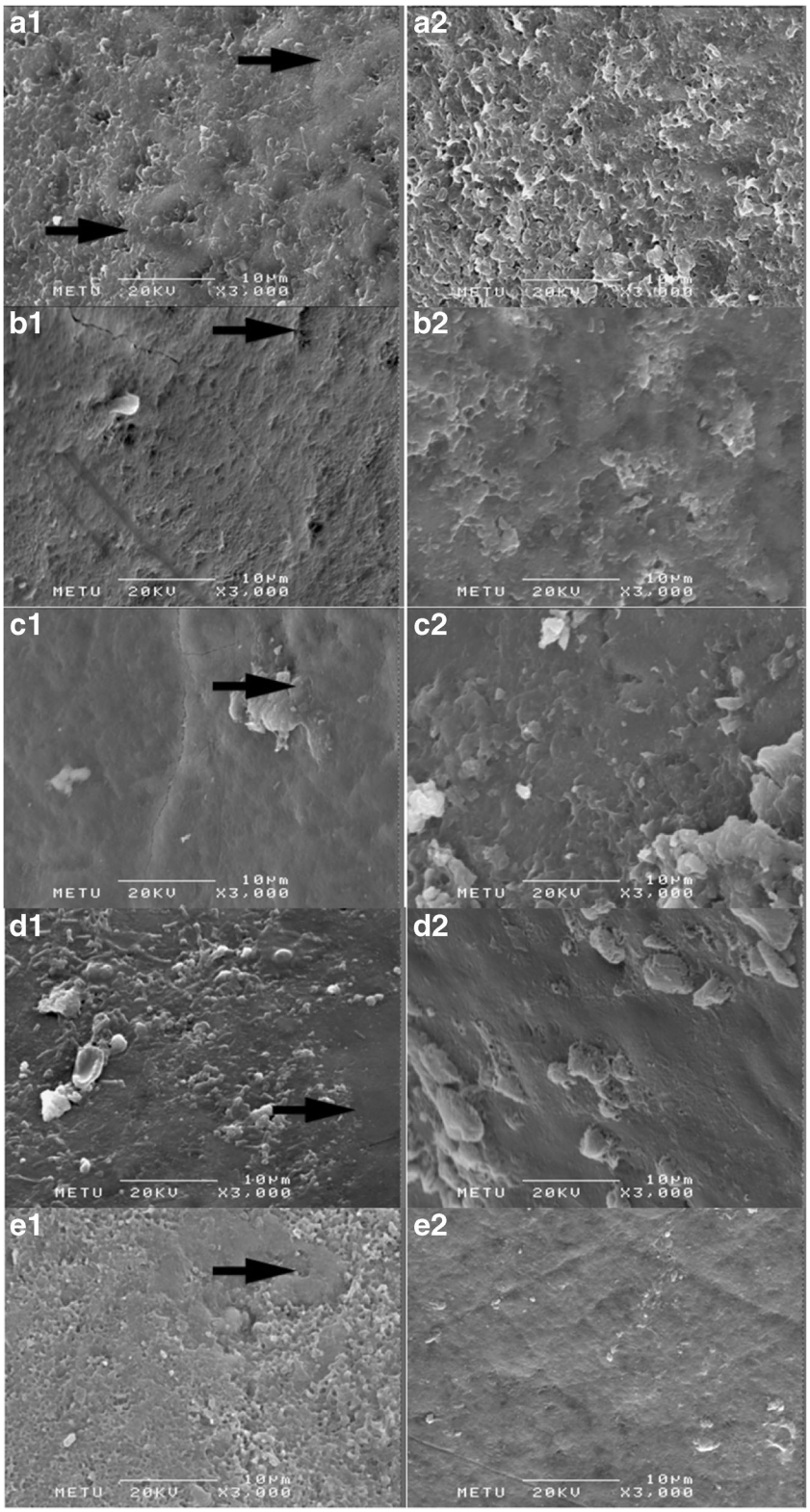

Fig. 3 A1,B1,C1,D1,E1: increased roughness along with melting areas (black arrows) on laser-irradiated samples A1: L, B1: L + NaF, C1: L + APF, D1: L + FV, E1: L + CPP-ACP; A2,B2,C2,D2,E2: after the pH cycle A2: L, B2: L + NaF, C2: L + APF, D2: L + FV, E2: L + CPP-ACP. Original magnification: × 3000

research is needed on this topic to clarify the parameters needed for both primary and permanent teeth.

Regarding laser, two conflicting results were obtained in our study. As mentioned previously, there was no difference between the laser and the control group in increasing the microhardness after irradiation (Δ1). However, in regard to difference in microhardness after the pH cycle (Δ2), there was a difference between laser (alone or in combination with other

agents) and the control group. This result was regarded as the efficiency of laser in resisting demineralization rather than increasing microhardness. In parallel to the microhardness results, in the SEM analysis, less porosity and deterioration were observed in the lased samples indicating the effect of melting areas against demineralization. Moslemi et al. [5] and Anaraki et al. [19] also reported that laser reduces enamel permeability and demineralization by introducing a partial denaturation in the enamel matrix.

In the most of the studies, Δ3 are predominantly emphasized rather than Δ1 and Δ2 [22, 23, 29, 30]. Δ3 represents the positive impact of fluoride or laser along with the negative effect of the pH cycle. According to Δ3 results of our study, there was no statistically significant difference between the control group and NaF and CPP-ACP groups. This result may reflect the necessity of laser irradiation before applying NaF or CPP-ACP for protection of enamel against acid attacks. This result was also supported by other studies [23, 36]. Subramaniam and Pandey [23] evaluated the effect of Er,Cr:YSGG laser and CCP-ACP on primary enamel's hardness. While no difference was detected between an untreated control group and a CCP-ACP group, laser irradiation before CCP-ACP application significantly increased the surface hardness. The hardness was higher in laser irradiation prior CCP-ACP application in comparison to even CCP-ACP applied and control groups.

Esteves-Oliveria et al. [36] compared the effect of CO_2 laser and amine fluoride on enamel microhardness, and they found no significant difference between the control and the fluoride groups.

In the present study, with respect to Δ3, as no statistically significant difference was found between the laser and four different remineralization agents, the first null hypothesis was accepted. In comparison of the remineralization agents with or without laser, there was also no statistically significant difference. Therefore, the second null hypothesis has to be accepted. This finding is similar to the results of studies of Azevedo et al. [22] and Esteves-Oliveira et al. [36]. The authors reported that fluoride varnish, APF, and amine fluoride gels could be used effectively without laser irradiation. In the literature, however, some studies reported the increased effect of fluoride and CPP-ACP when used with laser [5, 23, 24].

When the unaccompanied use of laser was compared to its combined use with remineralization agents, significant difference was only observed between L and L + FV. Similar to the results of our study, Azevedo et al. [22] reported lower demineralization depth with L + FV than L. In the SEM findings of our study, after the pH cycle, less porosity and impairment were observed on the samples treated with remineralization agents and laser. This result was attributed to the melting zones created by the laser irradiation. The enamel sample in L + FV group showed that even after pH cycling, the surface smoothness was maintained and the number of CaF_2-like globular structures did not decrease much. On the L + CPP-ACP and L + FV-treated samples, a relatively smooth surface was observed after the pH cycle.

In the present study, CPP-ACP paste was applied daily throughout the 7-day pH cycle to simulate the daily use. However, long-term usage of the paste may lead to different results. In a study, increased microhardness was reported at the end of 1 month than that of the 4-day usage [39]. The lack of longer application time may be regarded as another limitation of the present study.

In the dental literature most of the studies involving laser, fluoride and CPP-ACP in terms of caries-preventive efficacy, have been performed in permanent teeth and there are few studies in primary teeth [22–24]. However, the incidence of caries in the permanent dentition is relative to the caries prevalence in the primary dentition [40–43]. Therefore, the importance of protecting primary teeth from caries is important for providing healthy permanent teeth for all individuals. The lack of an adequate number of studies in primary teeth necessitates the future research on this topic. Long-term clinical studies should be conducted to confirm the obtained results.

Conclusions

The following conclusions can be drawn in terms of caries prevention:

- Laser was found to be as effective as tested remineralization agents (neutral NaF gel, APF gel, NaF varnish, and CPP-ACP).
- No significant difference in microhardness was detected among the remineralization agents used with laser or not (NaF = L + NaF, APF = L + APF, FV = L + FV, CPP-ACP = L + CPP-ACP).
- Caries-preventive effect of laser may be dependent on the formulation of the subsequently used remineralization agent. In this regard, fluoride varnish can be preferred as it had more preventive effect when used in combination with laser than laser alone (L + FV > L).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This in vitro study was approved by the local ethics committee of Hacettepe University, Ankara, Turkey (Project no: TDK-2016-12277).

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