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Research Paper

Evaluation of remineralizing potential of hydroxyapatite, phosphopeptide-amorphous calcium phospahate and fluoride dentifrices using SEM/EDX analysis: A randomized controlled in-vitro study

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ABSTRACT

Aim: The aim of this study is to evaluate remineralization efficiency of hydroxyapatite (HA) containing dentifrices comparing to phosphopeptide-amorphous calcium phosphate (CPP-ACP) and fluoride (F) containing dentifrices using scanning electron microscope (SEM) and energy dispersive spectrometry (SEM/EDX).

Material and methods: 3×3 mm^2 areas on the buccal surfaces of extracted primary second molars were divided vertically into 3 sections as demineralization, control and remineralization surfaces. Control surfaces (1/3 middle) coated without any application. 1/3 left and right surfaces were subjected to demineralization solution. After this application 1/3 right surfaces were coated as demineralization surfaces. Then teeth were randomly separated in 3 groups ($n = 10$): group HA (Natural toothpaste, Splat®, Russia); Group CPP-ACP (MI Paste ONE®, GC, U.S.); group F (Oral-B® Kids, Oral-B, Germany). 1/3 right surfaces were brushed with dentifrices twice a day for one week with pH cycle. Coatings were removed. Surface evaluations were conducted by SEM and EDX. Mann Whitney U and Kruskal Wallis tests were used for statistical analysis.

Results: Both atomic (at%) and weight (wt%) percentages of P values were statistically significant for F group in remineralization surfaces comparing to demineralization surfaces. Ca at%, P at% and P wt% values were statistically significant for CPP-ACP group in remineralization surfaces. P at% and P wt% values for F group were higher in remineralization surfaces compared to all.

Conclusion: Remineralization was observed in all groups. Most homogeneous surface features were seen in group CPP-ACP.

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1. Introduction

Dental caries is accepted as a multifactorial disease that affects whole mankind regardless of age, gender or ethnicity. The first sign of demineralization is white spot lesions as enamel loses its translucency which is correlated with remineralization. Brushing is considered one of the most reliable mechanical plaque control methods. In addition to this, an anticariogenic agent or a combination of them are required to be added for prevention of tooth demineralization [1]. Primary teeth contain more organic components hence they are more susceptible for caries. The structure of artificial caries obtained on primary teeth would be also more porous [2].

In lights of researches, it is known that non-cavitated initial enamel caries (IEC) can be stopped or reversed by non-invasive treatments [1]. Thanks to biomimetic and regenerative biology, materials have been developed that provide the remineralization and repair of IEC. Fluoride is used for treatment of IEC as an active agent in dentifrices for decades [3].

Dentifrices that contains hydroxyapatite (HA) has started to be used as anti-sensitivity and anti-cariogenic agents that maintains natural brightness and color of teeth [3]. It is shown that HA has a remineralization potential on IECs [4]. According to researchers, remineralization develops as a result of HA precipitation in the porous areas of the enamel [5]. It is reported that HA containing gels are as effective as fluoride containing gels in remineralizing processes for IECs [6]. On the other hand another idea is like casein phosphopeptid-amorphous calcium phosphate (CPP-ACP), it acts as a reservoir, allowing calcium ions to be released into the environment when necessary [7].

Known as Recaldent™ (GC Corporation, USA) technology, CPP-ACP is thought to assist in the formation of HA by acting as a calcium and phosphate reservoir in cases where pH values decrease by providing mineral reposition in subsurface lesions. It is reported that when CPP-ACP unites with enough fluoride ions, remarkable remineralization occurs on IECs [8].

The energy dispersion spectrometry (EDX) technique provides elemental microanalysis on the surface of the materials. With the excitation of electrons, it is possible to identify and measure the amounts of all elements (except H, He and Li) in the periodic table with the changes in x-rays [9]. The basic principle of the system is the separation of each element from other elements with its own characteristic features when facing an X-ray. To induce X-ray absorption, high-energy beam particles are directed at the sample surface. With the electron beam hitting the inner shell the energy level is changed, allowing the atom to pass into the inner orbit, and a vacancy remains in the place of the positively charged atom. X-ray, which replaces the vacancy, creates a difference between the energy levels and this difference is detected by the spectrometer. With the characterization of these differences occurring in different energy levels in each element, information about the element composition on the surface of the sample is obtained. Studies report that scanning electron microscopy (SEM) examination is accepted as the gold standard for imaging of IECs [9,10]. Qualitative and quantitative investigations of enamel microstructures can be made with the combination of SEM and energy dispersion spectrometry (EDX) detector. In most of the studies Ca, P, F and Ca/P values were used in statistical analyzes to examine in the evaluation of remineralization [11,12].

This study is designed to evaluate the hypothesis that there is no significant differences between fluoridated dentifrices, HA and CPP-ACP containing dentifrices in the remineralizing potential on IEC. The aim of this study is to evaluate remineralization efficacy of HA, CPP-ACP and F containing dentifrices for children by using SEM/EDX microscopes which enables to understand elemental changes on enamel surfaces. It is believed that results of the study would be considered as a helpful guide for recommendation of effective dentifrices for the children patients.

2. Materials and methods

2.1. *Selection of teeth*

The ethics committee approval of this study was accepted by the Istanbul University Faculty of Dentistry Clinical Research Ethics Committee with the number 2019/39 dated 24/04/2019 (Ethics Committee Decision).

Extracted primary molars which previously decided for extraction because of orthodontic, restorative and etc. reasons were obtained from Istanbul University Faculty of Dentistry, Oral and Maxillofacial Surgery Department. Parents/guardians of paticipants permission has obtained by signing an informed consent form.

Totally 30 primary second molars were used and 10 teeth were selected randomly for each group. The study had been conducted in *in-vitro* circumstances. Inclusion criterias were not having any caries, cracks or structural abnormalities on tooth surfaces. Selected teeth were evaluated under $\times 2.5$ magnification with a dental loupe (Keeler Ltd., Windsor, Berkshire, England). Cracked, decayed or hypomineralized teeth were excluded out of the study.

2.2. *Preparation of teeth*

Plaque has removed from surfaces by a dental scale and teeth were cleaned with micromotor and polishing brush (Stoddard Manufac. Co. Ltd., Hertfordshire, England) under water. Teeth were kept in 0.1% tymol solution at 4 °C no longer than 2 months [13]. Before any application the teeth were embedded in acrylic (Imicryl Dental Supplies Industry & Trade Co., Konya, Turkey) to the level of cemento-enamel junction. Circle molds with a diameter of 2 cm were used for evenly specimens.

With the help of equal adhesive tapes, 3×3 mm^2 window areas were created on the buccal surface of teeth and the rest of the teeth surfaces were covered with two layers of nail polish (Euromonitor, Reinventing Global Color Cosmetics, London, England). For each subject, window areas were divived vertically into 3 subgroups as remineralization, demineralization and control areas. The main reason of this method was to evaluate all changings in one surface at the same time. The window areas and subgroups created on the buccal surface of the subjects are schematized in Fig. 1.

2.3. Creating initial enamel caries

Firstly, middle third of each specimens were covered with two layers of nail polish as control surfaces so we could be able to compare each tooth with their own starting elemental levels. Remaining open right and left surfaces of window areas were subjected to demineralization solution which was prepared at Istanbul University, Department of Biochemistry.

Systematic reviews show that simple chemical caries models are preferred in 62% of the studies. Chemical caries methods shorter the time and decrease the steps of the study [14]. In this study, initial caries were created with a demineralization solution. The solution was obtained using 75 mM of potassium acetate buffer containing PO_4, 2 mM Ca from $Ca(NO_3)_2$, 2 mM PO_4 from KH_2PO_4 with a pH of 4.3 [15]. The specimens subjected to demineralization solution for 72 h at 37 °C in an etuve for creating IECs. Then demineralization surfaces covered with nail polish. Only one part left open for remineralization.

To stimulate oral conditions, pH cycling method was used. Specimens were subjected 16 h of remineralization solution and 6 h of demineralization solution [16]. Each specimen brushed twice a day before the demineralization solution, for 2 min with a microbrush (TPC Advanced Technology, Inc., Los Angeles, USA). Remineralization solution for pH cycle obtained 1 μM $CaCl_2$, 50 μM KCl, 2 μM KH_2PO_4, 0,01% NaN_3 and 1 M KOH with 7.0 pH and demineralization solution obtained 2,2 μM $CaCl_2$, 2,2 μM NaH_2PO_4, KOH added 0,05 μM acetic acid with pH 4.4. Solutions had been changed every other day and controlled with a pH meter. pH cycle was continued for 7 days. Study schema is shown in Fig. 2.

2.4. Dentifrices

As HA containing dentifrice (Natural toothpaste, Splat®, Russia), a commercially available toothpaste has been used (HA group). As CPP-ACP containing dentifrice (MI Paste ONE®, GC, USA), a commercially available toothpaste has been used (CPP-ACP group). For control group, F containing dentifrice (Oral-B® Kids, Oral-B, Germany) with 1100 ppm flouride has been used (F group). Ingredients of dentifrices has been given in Table 1.

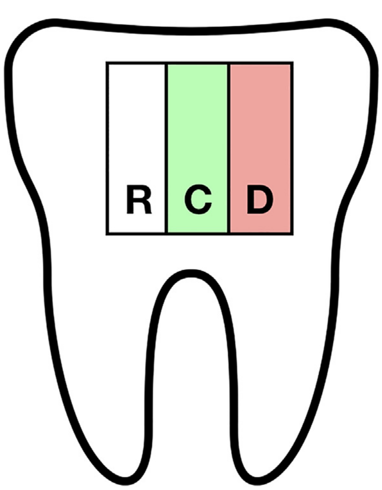

Fig. 1 – Schematized window areas and subgroups that created on the buccal surface of the subjects, R (remineralization), C (control) and D (demineralization) surfaces.

2.5. Examination of samples with scanning electron microscopy (SEM)

After the pH cycle, the samples were washed with distilled water and left to dry for one day for scanning electron microscopy (Quanta FEG 250, Hillsboro, Oregon, America) examinations. The examinations were carried out in Tekirdağ University Central Laboratory (NABILTEM). In order for the window surfaces of the samples to be positioned correctly in the device, the samples were fixed with a shaft. A total of 7 images were taken from three different vertical subgroups, on each sample at ×1000 and ×4000 magnification.

2.6. Examination of samples with scanning electron microscopy/energy dispersion spectrometry (SEM/EDX)

In the study, imaging of the element distributions by energy distribution spectroscopy of the samples was carried out in Tekirdağ University Central Laboratory (NABILTEM). Each of the different demineralization, remineralization and control surfaces on the samples were examined with an SDD Apollo X detector in SEM (Quanta Feg 250, Hillsboro, Oregon, America) at ×4000 magnification at 30 kV twice. The element distributions of the samples, which were scanned by EDX analysis, were determined according to the atomic percentage (at%) and the mass percentage (wt%) of the elements. Elements of Ca, F, Na, P, C and O were evaluated to be examined. 6 surface scan graphs were obtained for each tooth specimen.

2.7. Statistical analyzes

Statistical analyzes were carried out with SPSS version 17.0 program. The suitability of the variables to normal distribution was examined by histogram graphics and Kolmogorov-Smirnov test. Mean, standard deviation, and median values were used while presenting descriptive analyzes. The Mann-Whitney U Test was used when evaluating nonparametric variables between two groups, and the Kruskal-Wallis Test was used when evaluating between more than two groups. The statistical significance was set at $p \leq 0.05$.

3. Results

Initial surface values of control and demineralization surfaces are given in Table 2. The efficacy of the dentifrices was evaluated using the Mann Whitney U test, with pairwise comparisons between control/remineralization and demineralization/remineralization surfaces. Surface analysis of group F showed that at% (10.30 ± 1.91) and wt% (17.55 ± 2.49) of P elements were higher on the remineralization surfaces compared to demineralization surface and this difference was statistically significant ($p \leq 0.05$). Comparison of control and remineralization surfaces of group F were found to be statistically insignificant for all values (Table 3). Surface

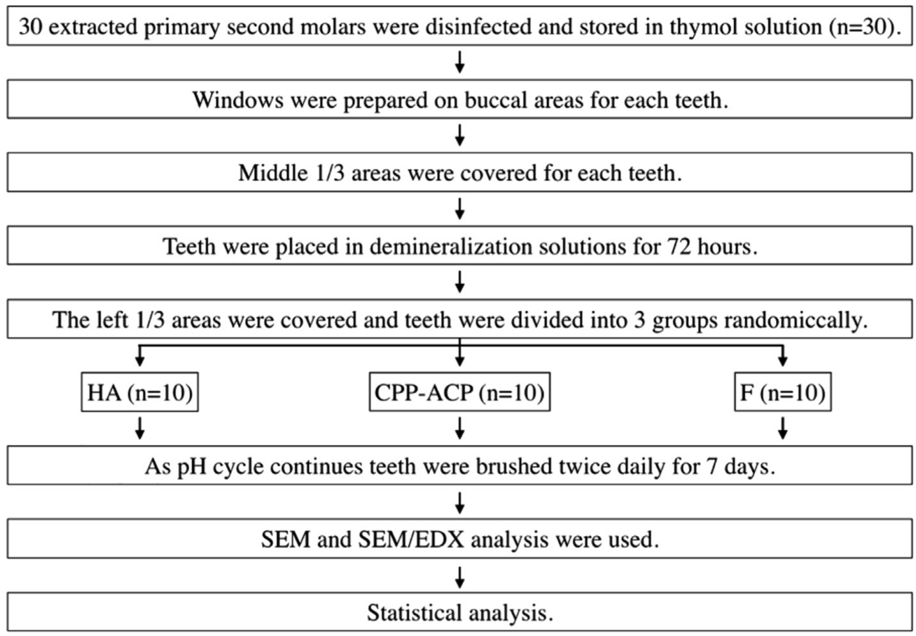

Fig. 2 – Consort diagram of the study method.

Table 1 – The ingredients of dentifrices used in the study.

Name	Brand	Ingredients
Natural toothpaste	Splat Global, Russia	Calcium hydroxyapatite, Hydrogenated starch hydrolysate, Glycerin, Hydrated silica, cellulose gum, Lactoferrin, Lactoperoxidase, Glucose oxidase, Aroma, Aloe barbadensis leaf extract, Xanthan gum, Cocamidopropyl betaine, Lonicera caprifolium flower extract, Lonicera Japonica flower extract, Sodium benzoate, Potassium sorbate, Arginine, Capsanthin, Cl 75470, Mallitol, Citric acid, Pentylene glycol
MI Paste ONE	GC, USA	RECALDENT™ (CPP-ACP) Casein phosphopeptide-amorphous calsium phosphate, Sorbitol, CMC-Na, Propylene glycol, Silicon dioxide, Titanium dioxide, Xylitol, Phosphoric acid, Methyl saliclate, Sodium saccharin, Ethyl p-hydroxybenzoate, Butyl p- hydroxybenzoate, Sodium-N-lauroyl sarcosinate, Sodium fluoride (1100 ppm)
Oral-B Kids	Oral B, Germany	Sodium fluoride (1100 ppm), Sorbitol, Hydrated silica, Sodium lauryl sulfate, Trisoidum phosphate, Cellulose gum, Sodium phosphate, Sodium saccharin, Carbomer, Limonene, Polysorbate 80, Sodium Hydroxide, Cl 42090

analysis of group HA showed that there was no statistically significant differences between demineralization and remineralization surfaces for all values. Only at% (8.90 ± 1.39) of P element was found to be statistically significantly higher at remineralization surfaces compared to control surfaces ($p \leq 0.05$, Table 4). Values of at% of Ca (5.55 ± 1.79), wt% of P (14.70 ± 2.08) and at% of P (8.29 ± 1.53) elements were found to be higher on the remineralization surfaces of group CPP-ACP and this difference were statistically significant (Table 5, $p \leq 0.05$). No difference were considered statistically significant in the comparison of control and remineralization surfaces of group CPP-ACP (Table 5). When the pairwise comparisons between the groups were evaluated, at% and wt% of P values on the remineralization surfaces of the group F were found to be statistically significantly higher than both HA and CPP-ACP groups. When the values on the remineralization surface of the HA and CPP-ACP groups were compared, no statistically significant difference was observed. Comparisons between groups are given in Table 6.

The SEM images after 72 h of demineralization solution showed many micro porosities, smooth surfaces of enamel (Fig. 3) were found to be less and paterns were suggestive of demineralization (Fig. 4). Amorphous deposits were seen on all groups when the specimens were remineralized. On evaluations of remineralized surfaces, specimens of group CPP-ACP (Fig. 5) were considered to have more homogeneous surface features compared to group F (Fig. 6) and HA (Fig. 7).

4. Discussion

This study evaluated the remineralization capacity of HA containing dentifrice compared to that of F and CPP-ACP. Dentifrices are accepted as agents that enable us to provide

Table 2 – Initial elemental analyses of control and demineralization surfaces.

		CPP-ACP		HA		F	
		Mean ± S.D.	Median	Mean ± S.D.	Median	Mean ± S.D.	Median
Control	Ca wt%	11.09 ± 3.80	10.43	10.39 ± 2.10	11.11	12.87 ± 2.77	13.52
	Ca at%	4.66 ± 1.75	4.27	4.37 ± 0.96	4.70	5.71 ± 1.41	6.16
	P wt%	13.04 ± 2.68	13.80	14.14 ± 1.68	14.71	16.19 ± 1.98	16.72
	P at%	7.07 ± 1.71	7.51	7.71 ± 1.11	8.08	9.13 ± 1.42	9.39
	F wt%	0.48 ± 0.39	0.40	0.41 ± 0.28	0.37	0.30 ± 0.17	0.29
	F at%	0.41 ± 0.32	0.36	0.36 ± 0.24	0.33	0.27 ± 0.16	0.26
	Ca/P wt%	0.84 ± 0.17	0.79	0.73 ± 0.09	0.73	0.79 ± 0.11	0.78
	Ca/P at%	0.65 ± 0.13	0.61	0.56 ± 0.07	0.57	0.62 ± 0.08	0.63
Demineralization	Ca wt%	10.09 ± 3.56	9.48	10.29 ± 3.33	11.22	12.14 ± 2.82	12.55
	Ca at%	4.17 ± 1.59	3.87	4.33 ± 1.56	4.80	5.18 ± 1.35	5.40
	P wt%	12.30 ± 2.16	12.50	13.61 ± 2.97	14.33	15.00 ± 2.32	15.53
	P at%	6.57 ± 1.32	6.75	7.38 ± 1.96	7.78	8.26 ± 1.58	8.52
	F wt%	0.48 ± 0.46	0.41	0.33 ± 0.21	0.28	0.24 ± 0.12	0.24
	F at%	0.41 ± 0.40	0.36	0.29 ± 0.17	0.24	0.21 ± 0.11	0.21
	Ca/P wt%	0.80 ± 0.17	0.76	0.74 ± 0.12	0.73	0.80 ± 0.09	0.82
	Ca/P at%	0.62 ± 0.13	0.59	0.57 ± 0.09	0.56	0.62 ± 0.07	0.64

Table 3 – Surface analysis of group F. Control, remineralization and demineralization values of EDX spectrum.

F	Control		Remineralization		Demineralization	
	Mean ± S.D.	Median	Mean ± S.D.	Median	Mean ± S.D.	Median
Ca wt%	12.87 ± 2.77	13.52	14.25 ± 3.68	14.32	12.14 ± 2.82	12.55
Ca at%	5.71 ± 1.41	6.16	6.50 ± 1.94	6.52	5.18 ± 1.35	5.40
P wt%	16.19 ± 1.98^{A}	16.72	17.55 ± 2.49^{A}	18.06	15.00 ± 2.32^{B}	15.53
P at%	9.13 ± 1.42^{A}	9.39	10.30 ± 1.91^{A}	10.61	8.26 ± 1.58^{B}	8.52
F wt%	0.30 ± 0.17	0.29	0.48 ± 0.45	0.31	0.24 ± 0.12	0.24
F at%	0.27 ± 0.16	0.26	0.45 ± 0.39	0.30	0.21 ± 0.11	0.21
Ca/P wt%	0.79 ± 0.1	0.78	0.80 ± 0.11	0.79	0.80 ± 0.09	0.82
Ca/P at%	0.62 ± 0.08	0.63	0.62 ± 0.08	0.61	0.62 ± 0.07	0.64

Means with different uppercase superscript letters in rows represent statistically significant differences (Mann Whitney U test, $p \leq 0.05$).

Table 4 – Surface analysis of group HA. Control, remineralization and demineralization values of EDX spectrum.

HA	Control		Remineralization		Demineralization	
	Mean ± S.D.	Median	Mean ± S.D.	Median	Mean ± S.D.	Median
Ca wt%	10.39 ± 2.10	11.11	11.90 ± 2.95	11.53	10.29 ± 3.33	11.22
Ca at%	4.37 ± 0.96	4.70	5.22 ± 1.49	5.04	4.33 ± 1.56	4.80
P wt%	14.14 ± 1.68	14.71	15.74 ± 1.93	15.93	13.61 ± 2.97	13.33
P at%	7.71 ± 1.11^{A}	8.08	8.90 ± 1.39^{B}	8.95	7.38 ± 1.96^{B}	7.78
F wt%	0.41 ± 0.28	0.37	0.37 ± 0.36	0.29	0.33 ± 0.21	0.28
F at%	0.36 ± 0.24	0.33	0.34 ± 0.32	0.26	0.29 ± 0.17	0.24
Ca/P wt%	0.73 ± 0.09	0.73	0.75 ± 0.09	0.72	0.74 ± 0.12	0.73
Ca/P at%	0.56 ± 0.07	0.57	0.58 ± 0.07	0.56	0.57 ± 0.09	0.56

Means with different uppercase superscript letters in rows represent statistically significant differences (Mann Whitney U test, $p \leq 0.05$).

oral care in the cheapest way, and their effects on the country's economy have been shown in meta-analyzes [17]. The remineralization efficiency of these agents which can be used daily is also significant in terms of caries prevention. Given the rising concerns about fluoride [18], there is a search for different materials that can be used in dentifrices. CPP-ACP and HA are highly preferred remineralization materials in current studies [6,18].

It has been reported that calcium and phosphate deposits in deciduous and permanent teeth are different from each other, and ion accumulation in primary teeth is slightly less than in permanent teeth. Gavrila et al., suggested that ion exchanges in the samples were less in primary teeth [11]. Considering these differences, it was important to conduct the study on primary teeth in order to understand the effectiveness of the dentifrices which are planned to be used in primary teeth.

Remineralization is affected by the action of salivary enzymes, proteins, pellicle and dental plaque structure, additional fluoride in the oral environment, and *in-vitro* studies

Table 5 – Surface analysis of group CPP-ACP. Control, remineralization and demineralization values of EDX spectrum.

CPP-ACP	Control		Remineralization		Demineralization	
	Mean ± S.D.	Median	Mean ± S.D.	Median	Mean ± S.D.	Median
Ca wt%	11.09 ± 3.80	10.43	12.69 ± 3.46	11.72	10.09 ± 3.56	9.48
Ca at%	4.66 ± 1.75^{A}	4.27	5.55 ± 1.79^{A}	4.92	4.17 ± 1.59^{B}	3.87
P wt%	13.04 ± 2.68^{A}	13.80	14.70 ± 2.08^{A}	14.76	12.30 ± 2.16^{B}	12.50
P at%	7.07 ± 1.71^{A}	7.51	8.29 ± 1.53^{A}	8.37	6.57 ± 1.32^{B}	6.75
F wt%	0.48 ± 0.39	0.40	0.42 ± 0.35	0.31	0.48 ± 0.46	0.41
F at%	0.41 ± 0.32	0.36	0.38 ± 0.32	0.27	0.41 ± 0.40	0.36
Ca/P wt%	0.84 ± 0.17	0.79	0.86 ± 0.16	0.81	0.80 ± 0.17	0.76
Ca/P at%	0.65 ± 0.13	0.61	0.67 ± 0.13	0.63	0.62 ± 0.13	0.59

Means with different uppercase superscript letters in rows represent statistically significant differences (Mann Whitney U test, $p \leq 0.05$).

Table 6 – Comparison of all groups remineralization surface values.

Remineralization	CPP-ACP		HA		F		p[a]
	Mean ± S.D.	Median	Mean ± S.D.	Median	Mean ± S.D.	Median	
Ca wt%	12.69 ± 3.46	11.72	11.90 ± 2.95	11.53	14.25 ± 3.68	14.32	0.200
Ca at%	5.55 ± 1.79	4.92	5.22 ± 1.49	5.04	6.50 ± 1.94	6.52	0.177
P wt%	14.70 ± 2.08^{A}	14.76	15.74 ± 1.93^{A}	15.93	17.55 ± 2.49^{B}	18.06	**0.028**
P at%	8.29 ± 1.53^{A}	8.37	8.90 ± 1.39^{A}	8.95	10.30 ± 1.91^{B}	10.61	**0.039**
F wt%	0.42 ± 0.35	0.31	0.37 ± 0.36	0.29	0.48 ± 0.45	0.31	0.809
F at%	0.38 ± 0.32	0.27	0.34 ± 0.32	0.26	0.45 ± 0.39	0.30	0.686
Ca/P wt%	0.86 ± 0.16	0.81	0.75 ± 0.09	0.72	0.80 ± 0.11	0.79	0.213
Ca/P at%	0.67 ± 0.13	0.63	0.58 ± 0.07	0.56	0.62 ± 0.08	0.61	0.213

Means with different uppercase superscript letters in rows represent statistically significant differences (Mann Whitney U test, $p < 0.05$).
[a] Kruskal-Wallis test.

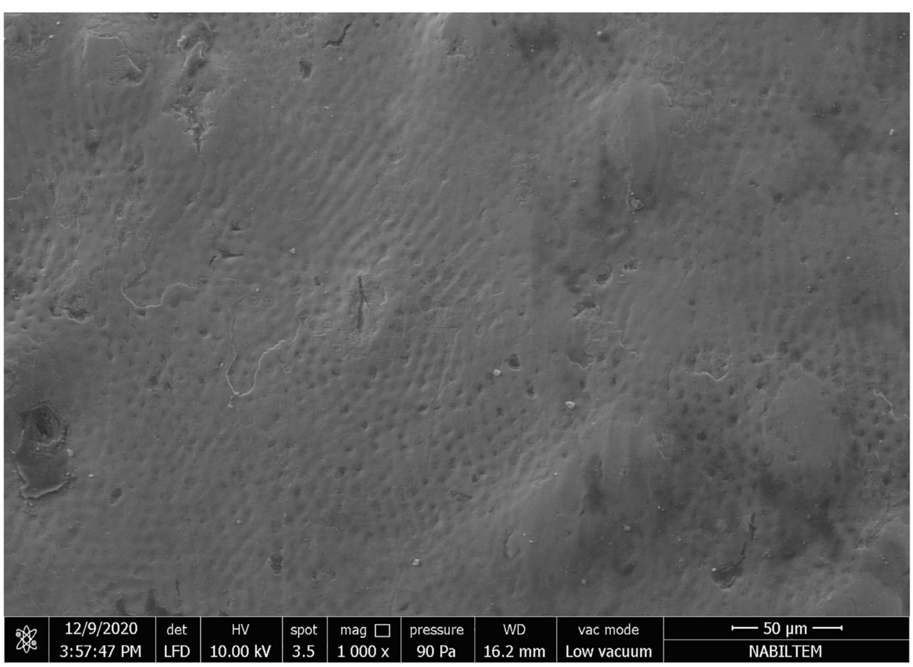

Fig. 3 – SEM images, ×1000 magnification. Control surface.

cannot fully reflect these effects. *In-vitro* studies allow for better control of each factor, resulting in more stable and acceptable results [19]. Study models in the investigation of the effectiveness of remineralization materials and in caries studies focus on the pH cycle as they are starting points for *in-vivo* studies [6]. One of the limitations of this study is the fact that the saliva and oral status are not reflected in this way, even though the pH cycle is used.

pH cycles is recommended for 10 days for permanent teeth [20]. It is reported that considering the mineral content of

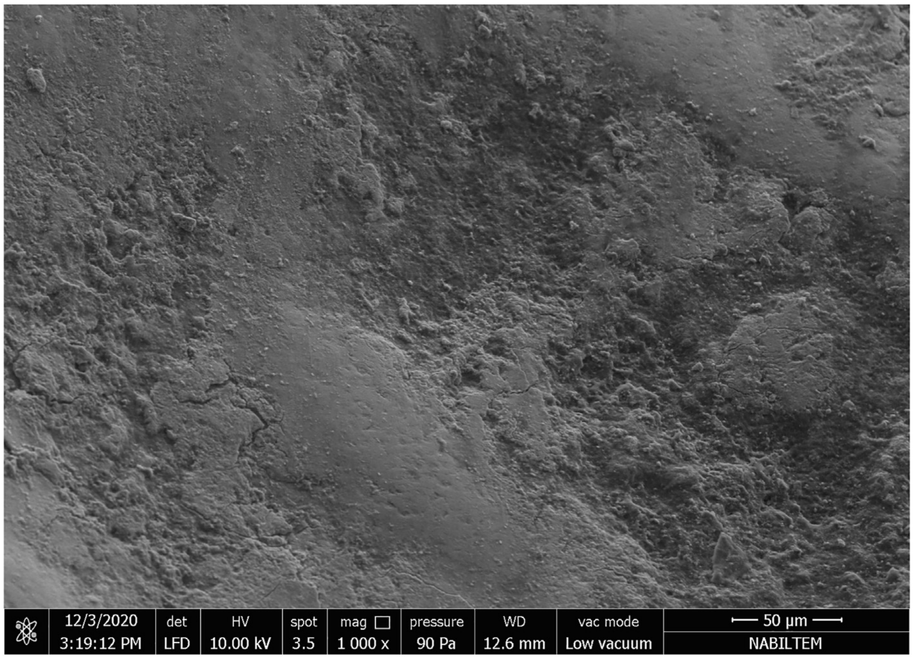

Fig. 4 – SEM images ×1000 magnification. Demineralization surface.

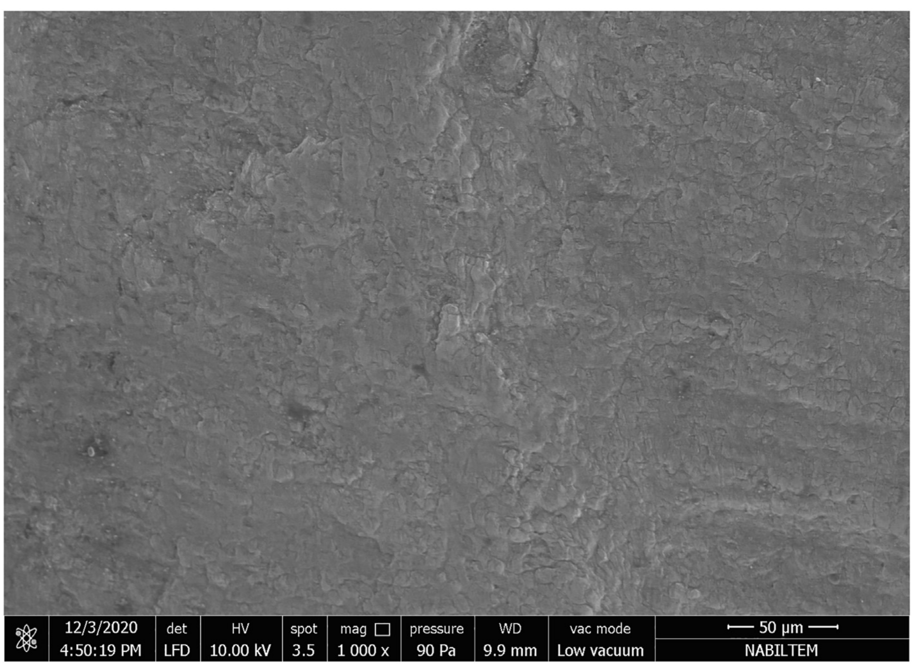

Fig. 5 – Group CPP-ACP, remineralization surface's SEM images under ×1000 magnification.

primary teeth, pH cycle has negative effects on remineralization after 8 days [21]. In many studies, it is reported that this period should not be exceed 7 days in order to avoid misleading results for the effectiveness of the materials [22,23].

When Arends et al. compiled the emerging techniques for the evaluation of demineralization and remineralization, they classified the examination techniques as measuring mineral density, calculating the change in mineral density, requiring sample preparation, and harming the sample. Most used ones are microradiography techniques, surface hardness evaluations, examinations with light and examinations with iodine. Many require surface etching and sample preparation [24]. Researchers have reported that samples become unusable at the stage of surface preparation for evaluation by microradiography [25]. Since primary teeth were used in this study, SEM analysis is considered to be suitable for sensitive visualization of initial enamel lesions without damaging the upper layer of enamel [26].

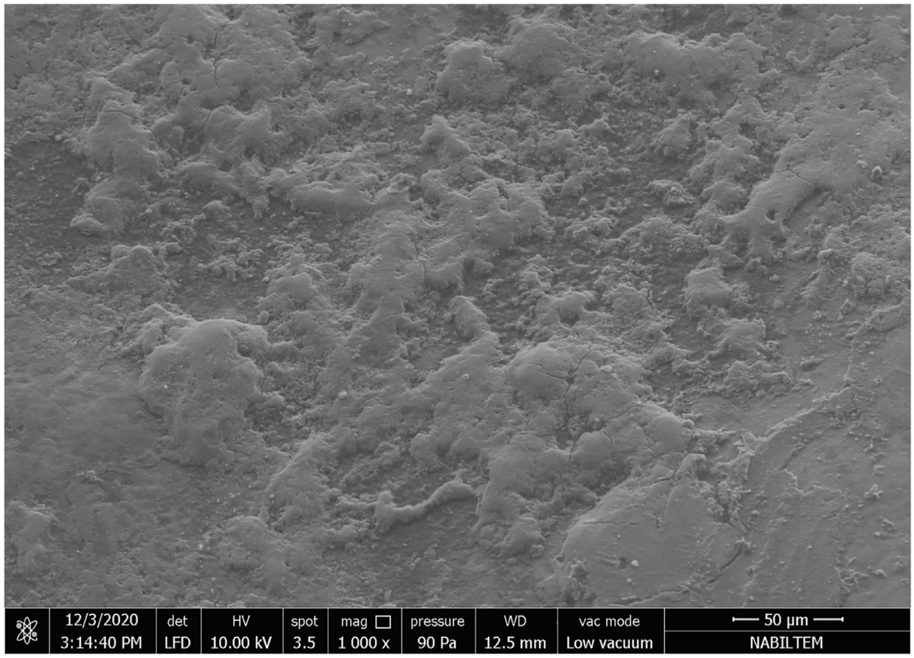

Fig. 6 – Group F, remineralization surface's SEM images under ×1000 magnification.

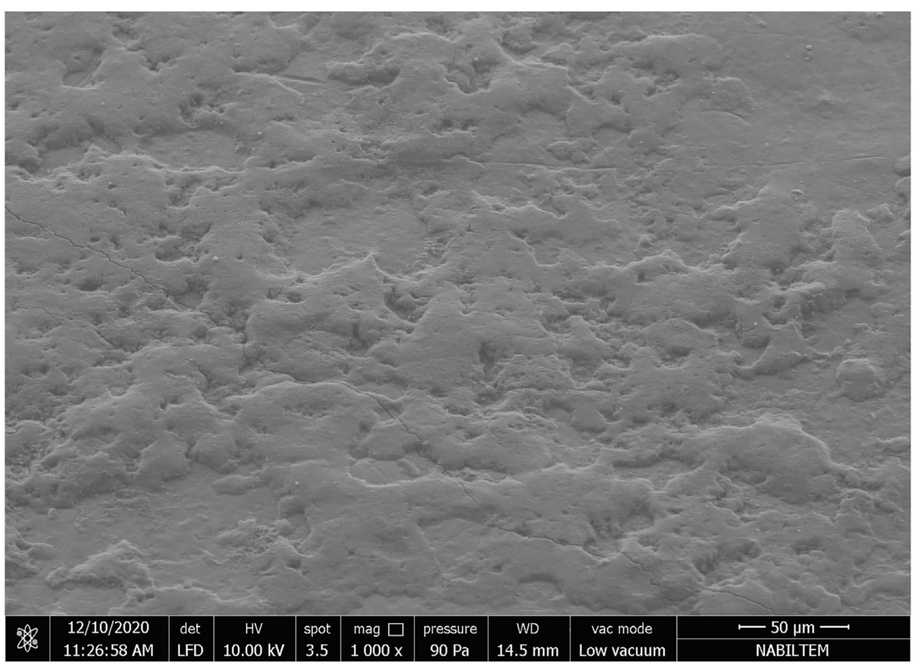

Fig. 7 – Group HA, remineralization surface's SEM images under ×1000 magnification.

The increase in the EDX spectrum of Ca and P (both at% and wt%) elements on the remineralization surfaces is as expected and the results are found to be similar to the results of the study of Vijayasankari et al. [27] for wt% values of Ca and P elements. As in the study of Kamath et al. [26], no significant difference was observed between the materials in the comparison of the remineralization efficiencies of HA, CPP-ACP and F groups. The absence of another study using the CPP-ACP-containing dentifrice form which used in this study makes it difficult to accurately compare the elemental percentages on the enamel surface with other studies.

The Ca/P ratio allows us to identify and compare the similarity between natural enamel and synthetic materials. Therefore, evaluating the Ca/P ratio on the enamel surface can be used as a remineralization criteria [28]. Thimmaiah et al. [12] reported in their study that the Ca/P ratio increased statistically significantly in the samples using casein phosphopeptide and amorphous calcium phosphate with fluoride

(CPP-ACPF). However, in this study, the increase in Ca/P value between demineralization and remineralization surfaces was not evaluated statistically significant in any group.

SEM examination is widely preferred in surface topographic examinations of remineralization studies because it provides the opportunity to examine without any preparation on enamel surfaces [29]. The globular CaF_2 deposits examined in the SEM images of group HA are thought to be similar to the study of Huang et al. [4] and those in the group F to the study of Swarup and Rao [28]. However, contrary to Altan et al.'s study, it is thought that the remineralization surfaces obtained with group CPP-ACP are more homogeneous than the structure obtained with group F [16]. The SEM images of the remineralization surfaces in all groups show more homogeneous structure when compared with the SEM images of the IECs surfaces, initial demineralization.

The results of the study partially support the initial hypothesis. Because, a significant difference was observed in the increase of P element on the surface in the remineralization provided with fluoride containing dentifrice. However, SEM images prove that remineralization was achieved in all groups. These results may have been affected by the short period of brushing of the dentifrices. In studies with longer brushing period, the results obtained in materials containing CPP-ACP contradict with the results of this study [12]. In order to evaluate the effectiveness of HA-containing toothpastes better, it is thought that it would be beneficial to include different percentages of HA-containing dentifrices in the future studies.

5. Conclusion

- The present study confirmed that a HA containing fluoride free dentifrice is effective in remineralizing IECs but still inferior to the F containing dentifrices (1100 ppm fluoride).
- EDX values of Ca and P elements on the remineralization surfaces of all dentifrices used in the study were found to be significantly higher than the demineralization surfaces. Between control and remineralization surfaces, only in group HA at% of P element was statistically significantly higher.
- SEM images prove that remineralization occurs with all materials that examined in this study. Still, more homogeneous images were achieved in group CPP-ACP.

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Author contributions

The study had been conducted on Istanbul University Dentistry Faculty, Paediatric Dentistry Department. Dr. Elis Mutlu—Corresponding author, responsible for planning the experiments and their conducting, collection of materials, writing of the manuscript. Dr. Mikail Ozdemir—Co-author, determining the number of samples, performing statistical analysis and evaluating the results. Prof. Dr. Koray Gençay—Co-author, revising the work critically for important intellectual content, final approval of the manuscript.

Data availability

All data generated or analysed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

Declaration of competing interest

The authors declare no conflict of interest.

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