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Micro-CT Evaluation of the Remineralization Effect of Toothpastes with Different Ingredients on Artificial Initial Enamel Lesions: An Ex-Vivo Study.

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Research Article

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ABSTRACT

Objectives: It was aimed to evaluate the change in mineral density after the application of toothpastes containing fluoride, CPP-ACP, nHAp and CaGP to artificial caries lesions in primary teeth using Micro-CT.
Materials and Methods: A total of 40 non-caries primary anterior teeth were randomly divided into 4 groups. The 3x1 mm healthy enamel area in the middle of the 3x3 mm area on the buccal enamel surfaces was covered with 2 layers of nail polish to be used as the control group. The samples were kept in a demineralization solution for 96 hours to create initial enamel lesions. The 3x1 area on the left was covered with nail polish after demineralization. A system was set up to mimic the pH changes occurring in the oral environment throughout the day and a demineralization-remineralization cycle was applied to the samples at regular intervals each day for 7 days. Along with the pH cycle, remineralization materials were applied to the 3x1 area on the right each day according to the manufacturer's instructions. The remineralization agents used in the treatment of initial caries lesions were sodium fluoride (NaF), casein phosphopeptide amorphous-calcium phosphate (CPP-ACP), nano-hydroxyapatite (nHAp) and calcium glycerophosphate (CaGP). The changes in mineral density caused by the remineralization agents applied to enamel samples with artificial caries lesions were evaluated by micro-computed tomography (Micro-CT). Data were evaluated with Friedman's Two Way ANOVA test.
Results: There was no statistically significant difference between the initial and post-demineralization mineral density values obtained from all groups. There was no statistically significant difference between the remineralization and control groups in terms of mineral density.
Conclusions: CPP-ACP, nHAp and CaGP can be used as an alternative to fluoride by supporting remineralization in initial caries lesions of primary teeth.

Keywords: Toothpaste, Remineralization, Microcomputed Tomography

Farklı İçerikli Diş Macunlarının Yapay Başlangıç Mine Lezyonları Üzerindeki Remineralizasyon Etkisinin Mikro-BT ile Değerlendirilmesi: Ex-vivo bir çalışma

Araştırma Makalesi

Süreç

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ÖZ

Amaç: Bu çalışmada süt dişlerinde yapay olarak oluşturulan çürük lezyonlarına florür, CPP-ACP, nHAP ve CaGP içerikli diş macunlarının uygulanması sonrası mineral yoğunluğu değişiminin Mikro-CT kullanılarak değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntem: Toplam 40 adet çürüksüz süt ön diş rastgele 4 gruba ayrılmıştır. Bukkal mine yüzeylerindeki 3x3 mm'lik alanın ortasındaki 3x1 mm'lik sağlıklı mine alanı, kontrol grubu olarak kullanılmak üzere 2 kat oje ile kaplanmıştır. Örnekler, başlangıç mine lezyonlarını oluşturmak için 96 saat boyunca bir demineralizasyon solüsyonunda bekletilmiştir. Soldaki 3x1 alan, demineralizasyondan sonra tırnak cilası ile kaplanmıştır. Gün boyunca ağız ortamında meydana gelen pH değişikliklerini taklit etmek için bir düzenek kurulmuştur ve örneklere her gün düzenli aralıklarla 7 gün boyunca bir demineralizasyon-remineralizasyon döngüsü uygulanmıştır. pH döngüsü ile birlikte, remineralizasyon materyalleri üreticinin talimatları doğrultusunda her gün sağdaki 3x1 alana uygulanmıştır. Başlangıç çürük lezyonlarının tedavisinde kullanılan remineralizasyon ajanları; sodyum Flouride (Signal Kids), kazein fosfopeptid-amorf kalsiyum fosfat (GC Tooth Mousse), nano-hidroksiapatit (Prevdent, Enamel Repairs, Re-whitening, Desensitizing) ve kalsiyum gliserofosfattır. (R.O.C.S. Kids Fruity Cone). Yapay çürük lezyonlu mine örneklerine uygulanan remineralizasyon ajanlarının mineral yoğunluğunda neden olduğu değişiklikler Micro-CT ile değerlendirilmiştir. Veriler Friedman's Two Way ANOVA testi ile değerlendirilmiştir.
Bulgular: Tüm gruplardan elde edilen başlangıç ve demineralizasyon sonrası mineral yoğunluğu değerleri arasında istatistiksel olarak anlamlı bir fark yoktur. Tüm gruplarda demineralizasyon sonrası mineral yoğunluk değerlerinin, başlangıç ve pH döngüsü sonrası mineral yoğunluk değerlerinden istatistiksel olarak anlamlı düzeyde düşük olduğu belirlenmiştir ($p<0,05$). Remineralizasyon ve kontrol grupları arasında mineral yoğunluğu açısından istatistiksel olarak anlamlı bir fark yoktur. Gruplar arasında remineralizasyon ve demineralizasyon sonrası mineral yoğunluğu değerleri arasında istatistiksel olarak anlamlı bir fark yoktur.
Sonuçlar: CPP-ACP, nHAP ve CaGP süt dişlerinin başlangıç mine lezyonlarında remineralizasyonu destekleyerek florüre alternatif olarak kullanılabilir.

Anahtar Kelimeler: Diş Macunu, Remineralizasyon, Mikrobilgisayarlı Tomografi.

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Introduction

Minimally invasive approaches that aims to preserve the healthy dental tissues have gained a great popularity in the recent dentistry practices.[1] These preventive approaches aim to prevent demineralization before it occurs or to remineralize demineralized areas before the presentation of cavitation.[2] With the development of minimally invasive dentistry, the search for effective methods for enamel remineralization has increased and several studies have been investigated to assess the effect of remineralization agents containing various amounts of calcium (Ca^{+2}), phosphate ($PO4^{-3}$) or fluoride (F^{-}) on initial caries lesions. Various remineralization strategies have been utilized since the date. However, in addition to the positive effects of each of these methods, it has been observed that many of them are not sufficient to prevent caries formation alone due to their ease of use, accessibility, cost, and possible side effects.[3-7] Therefore, the development of alternative agents continues to be necessary. In the recent approaches, as an alternative to fluoride, the use of products containing casein phosphopeptide amorphous-calcium phosphate (CPP-ACP), nano-hydroxyapatite (nHAp) and calcium glycerophosphate (CaGP) has come to the fore. Various direct and indirect methods have been used to assess the remineralization of initial caries lesions under in-vitro conditions. These methods can be listed as iodide permeability test, radioactive absorptometer method, microhardness test, wet chemical analysis, laser fluorescence, polarized light microscopy, scanning confocal electron microscope (SCEM), scanning electron microscope (SEM), transfusion electron microscope (TEM), micro radiography and micro computed tomography (Micro-CT).[7-10]

Micro-CT for the determination of the mineral concentration of bone and teeth is an innovative and non-invasive procedure that allows the examination of dental tissues and tissue samples without damaging them.[11-13] Micro-CT is more sensitive and easier to apply than direct (chemical analysis) and indirect (contact microradiography) methods in the evaluation of the mineral concentration of teeth. Micro-CT can measure the mineral density of bones and teeth at concentrations less than 1% and with a resolution of 5-30 µm. Nowadays, micro-CT devices can be used in dental research for qualitative analyses and three-dimensional (3D) analysis of materials.[14,15]

When the literature was reviewed, no study was detected in which the mineral density changes of CPP-ACP, nHAp, CaGP and fluoride containing remineralizing agents were evaluated all together in vitro on initial enamel lesions. In the light of the aforemention data, this study aimed to evaluate the mineral density change of artificial caries lesions in primary teeth following the application of CPP-ACP, nHAp and CaGP, by using Micro-CT.

Material and Methods

Ethical Approval

Ethics committee approval was obtained before the study was conducted by Ankara University Faculty of Dentistry Clinical Research Ethics Committee (Date: 10.08.2016 Number:15/1). All the parents of the participants signed the written informed consent forms.

Sample Size Determination

In order to calculate the sample size, a power analysis was done before starting the study. To compare the bond strengths between the groups, it was planned to work with a total of 40 samples to work with at least 10 subjects in each group with 95% confidence and 80% power by estimating the intra-group variation as 0.9.

Preparation of Samples

A total of 40 non-carious teeth were obtained from patients aged 6-7 years, whose upper primary anterior teeth were indicated for extraction due to physiologic root resorption. The enamel surfaces of the collected teeth were evaluated for caries, hypocalcification and extraction-related defects using a stereomicroscope (Leica MZ12, Meyer Instruments, Houston, TX, USA) at ×10 and ×25 magnification. Plaque and debris on the teeth were rinsed by brushing under running water. The roots of the teeth were removed from the crown-root junction with a diamond separe disc and the obtained teeth were stored in deionized water containing 0.1% thymol crystal at room temperature until the time of the experiment and used within 3 months. Each tooth was embedded in acrylic resin (Imicryl Acrylic Repair Material, Konya, Turkey) cast in cylindrical plastic molds with the labial surfaces parallel to the base and facing upwards. To create a flat surface on the enamel, the buccal surfaces of the specimens were polished on a Metkon GRIPO 2V grinder-polisher (Metkon Instruments Ltd, Bursa, Turkey) under water cooling using silicon carbide abrasives with grits of 600, 800, 1200 (Atlas Brand English Abrasives, UK) respectively and uniform, smooth and polished enamel surfaces were obtained.[4,16] A total of 40 enamel samples were randomly divided into 4 groups and each enamel sample was given a code according to the group it belonged to. To create standard enamel surfaces on which the study materials would be applied, a 3x3 mm area was left blank on the buccal enamel surfaces and the remaining areas were coated with 2 coats of acid-resistant nail polish (Revlon, New York, USA). An area of 3x1 mm of intact enamel in the center of the buccal surface was used as a control group, 2 coats of nail polish were applied. The area on the left of the two areas formed was coated with nail polish after demineralization. The area on the right was used for evaluation by applying remineralization materials after pH cycling. The demineralization solution recommended by Ten Cate and Duijsters [17] was used to create initial enamel lesions on the prepared enamel surfaces. All solutions used were freshly prepared before the study. The content of the demineralization solution used to create initial enamel lesions was as follows;

2.2 mM Calcium nitrate [$Ca(NO_3)_2$]
2.2 mM Monopotassium phosphate [KH_2PO_4]
0.1 ppm Sodium fluoride [NaF]
50 mM Acetic acid [$C_2H_4O_2$] and pH was adjusted to 4.5.

The prepared specimens were placed in a container in the demineralization solution so that the enamel surfaces were in contact. The specimens were placed in the demineralization solution for 96 hours. [18] The demineralization solution was freshly prepared again after 48 hours. After 96 hours, the specimens with initial enamel lesions were washed with deionized water and stored in special containers in deionized water at room temperature until the end of the study period (Figure 1).

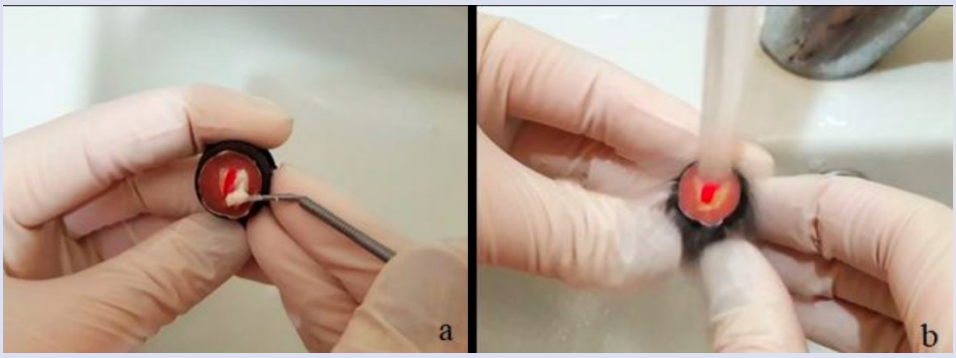

Figure 1 Application of remineralization agents to specimens (a) and washing (b)

Figure 2 Apparatus prepared to mimic the oral environment

The conditions in which the samples will remain during the study should mimic the conditions inside the mouth as much as possible. For this reason, an apparatus prepared to simulate the saliva flow in the mouth under laboratory conditions was used. During the experiments, the enamel samples placed in the apparatus were subjected to a demineralization-remineralization cycle at certain intervals every day for 7 days at 37 °C in order to mimic the pH changes that occur throughout the day in the oral environment (Figure 2). During this cycle, the samples were kept in the following solutions;

3 hours of demineralization solution,

2 hours remineralization solution,

3 hours demineralization solution,

16 hours in remineralization solution.[18] The tooth samples were washed with deionised water before being placed in a new solution. The solutions used in the pH cycle were as follows;

Demineralization solution:

2.2 mM $CaCl_2$

2.2. mM NaH_2PO_4

0.05mM acetic acid. The pH was adjusted to 4.4 with KOH.

Remineralization solution:

1.5 mM $CaCl_2$

0.9 mM NaH_2PO_4,

0.15 mM KCl The pH was adjusted to 7.

Remineralization Materials Used in the Study

The remineralization agents used for the treatment of experimentally prepared initial caries lesions are shown Figure 3.

Application of Experimental Materials to Enamel Samples

Remineralization materials were applied gently using a standard size applicator according to the manufacturer's instructions.

Group 1: Fluoride group (Signal Kids, SESIC, Alexandria, Eagypt) was applied to the tooth surface using micro brushes and left on the tooth surface for 2 minutes.

Group 2: CPP-ACP group (GC Tooth Mousse™, GC Corporation, Tokyo, Japan) was applied to the tooth surface using micro brushes and left on the tooth surface for 3 minutes.

Group 3: nHAp group (Prevdent / Enamel Repairs, Re-whitening, Desensitizing, Prevdent Toothpaste, Netherlands) was applied to the tooth surface using micro brushes and left on the tooth surface for 2 minutes.

Group 4: CaGP group (R.O.C.S. Kids Fruity Cone, R.O.C.S., Sweden) It was applied to the tooth surface using micro brushes and left on the tooth surface for 2 minutes.

Control Group: Healthy enamel surface.

The experimental materials were applied twice a day at 09.00 and 19.00 hours every day for 7 days. After the application of the experimental materials, the samples were washed under running water and kept in deionised water for the rest of the experiments.

Trade name of toothpaste	Trademark	Content
Signal Kids (Fluoride group)	SESIC, Alexandria, Eagypt	Sodium fluoride
GC Tooth Mousse™ (CPP-ACP group)	GC Corporation, Tokyo, Japan	Casein phosphopeptide-amorphous calcium phosphate
Prevdent / Enamel Repairs, Re-whitening, Desensitizing (nHAp group)	Prevdent Toothpaste, Netherlands	Nano-hydroxyapatite
R.O.C.S. Kids Fruity Cone (CaGP group)	R.O.C.S., Sweden	Calcium glycerophosphate

Figure 3 Remineralization materials used in the study

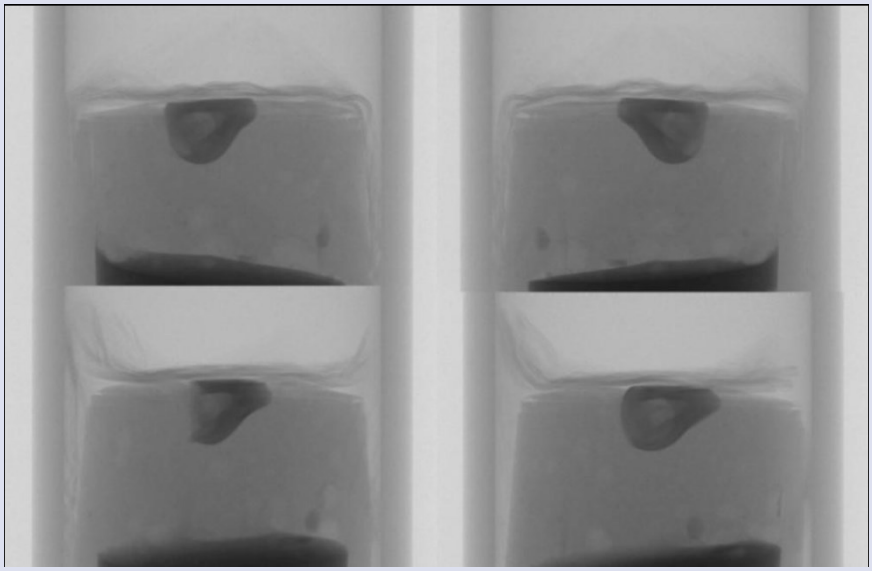

Figure 4 Two-dimensional axial projections of the specimens obtained after scanning

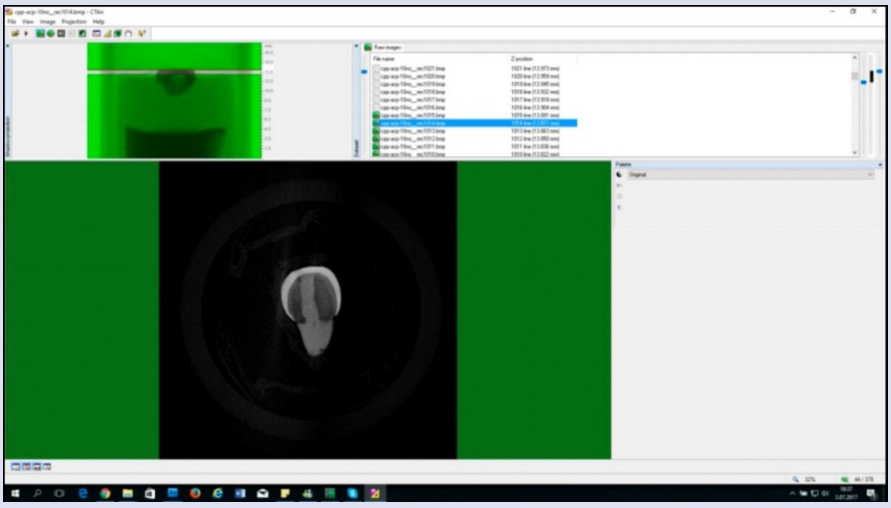

Figure 5 Preparation of sections of the specimens for analyses

Micro-CT Evaluation of Samples

Micro-CT evaluation of the samples was used after the application of remineralization agents. Micro tomography of the specimens was performed with a Skyscan (Bruker) model 1172 high-resolution micro tomography device using 100 kV, 104 mA and 0.5 mm aluminum and copper filters. Approximately 445 two-dimensional axial projections were obtained from each sample. The exposure time during the acquisition was set to 2600 ms. DICOM (Digital Imaging and Communications in Medicine) compliant images from the sections were converted to BMP (Bit Map Picture) format. They were then scanned using NRecon (NReconstruction) software and each tooth was individually reconstructed. In total, 341 2D axial projections were obtained for each image group. These two-dimensional axial projections were prepared for analysis with the software Computer Tomography Analyse (CTan) (version 1,14,4,1 Skycan, Kontich, Belgium) provided by the manufacturer (Figure 4). The reconstructions of the specimens after this scanning stage were made with the software NRecon (NRecon version 1.6.10, Skyscan, Kontich, Belgium) provided by the manufacturer. CTan software was used for volumetric analysis of the images of the samples reconstructed in NRecon software and mineral density measurements were made (Figure 5). The numerical data obtained were transferred to Microsoft Office Excel 13.0 software and made ready for statistical analysis.

Statistical Analysis

The data collected in this study were analyzed with IBM SPSS Statistics Version 20.0 package program. While examining the differences between the groups, Shapiro Wilk Test was used when the variables were normally distributed, and Kruskal Wallis-H Test was used when the variables were not normally distributed. In case of significant differences in the Kruskal Wallis-H Test, Post Hoc Multiple Comparison Test was used to determine the groups with differences. The data related to the difference in Micro-CT measurement values of control, demineralization and remineralization areas in all groups were evaluated by Friedman's Two Way ANOVA test. When interpreting the results, 0.05 was used as the significance level and it was stated that there was a significant difference if $p<0.05$ and there was no significant difference if $p>0.05$.

Results

The mineral density of the groups is shown in Table 1. When compared the mineral density difference between the remineralization and control groups of F, CPP-ACP, nHAp and CaGP groups; although there was no statistically significant difference between the groups; it was found that the remineralization values of the CaGP group had numerically higher mineral density than the control group values. However, it was determined that the mineral density value after demineralization in all groups was statistically significantly lower than the initial and post pH cycle mineral density values ($p<0.05$)

When the changes in the mineral density of the healthy enamel caused by the remineralization agents applied to the enamel samples with artificial caries lesions were evaluated for each group, the following findings were obtained.

Group 1 (Fluoride)

According to the results of the test performed to determine whether there is a difference between the control, demineralization and remineralization groups in terms of mineral density, the mineral density of the demineralization group values was found to be statistically significantly lower than the other two groups ($p<0.05$). Although it was the only group in which the mineral density values obtained after remineralization were higher than the control group values, no statistically significant difference was found (Table 2).

Group 2 (CPP-ACP)

The mineral density of the demineralization values was statistically significantly lower than the other two groups ($p<0.05$), and the mineral density values obtained after remineralization were similar to the control groups. No statistically significant difference was detected between them (Table 2).

Group 3 (nHAp)

When the values of the control, demineralization and remineralization groups were evaluated in terms of mineral density, it was found that the mineral density of the remineralization values was statistically significantly higher than the demineralization values ($p<0.05$), but there was no significant difference between the control group (Table 2).

Group 4 (CaGP)

When the control, demineralization and remineralization values were evaluated in terms of mineral density, no statistically significant difference was found between the mineral density values of the remineralization groups and the control groups. The mineral density values of the demineralization area were statistically significantly lower than the other two groups ($p<0.05$) (Table 2).

When the difference in mineral density of remineralization and demineralization values between the groups was evaluated, no statistically significant difference was found between the groups (Table 3).

Table 1 Mineral density averages of the groups on healthy enamel surface, after demineralization and after remineralization

	n	Mineral density averages of healthy enamel surface	Mineral density averages of after demineralization	Mineral density averages of after remineralization
F	10	2.83^{Aa}	2.36^{Ba}	2.86^{Aa}
CPP-ACP	10	2.87^{Aa}	2.36^{Ba}	2.86^{Aa}
nHAp	10	2.87^{Aa}	2.32^{Ba}	2.85^{Aa}
CaGP	10	2.89^{Aa}	2.34^{Ba}	2.88^{Aa}

*Different capital letters in each row indicate that the difference is statistically significant. Different lowercase letters in each column indicate that the difference is statistically significant ($p<0.05$).
Abbreviations used in this table: n:number of samples

Table 2 Micro-ct mineral density values of group F, CPP-ACP, CaGP and nHAP.

							Friedman's Two Way ANOVA Test	Multiple comparison
		n	**Mean**	**Min**	**Max**	**SD**	**p**	
Fluoride	**Control**	10	2.83	2.8	2.9	0.03	0.001*	c-r c-d
	Demineralization	10	2.36	2.33	2.39	0.02		
	Remineralization	10	2.86	2.8	2.94	0.05		
CPP-ACP	**Control**	10	2.87	2.81	2.98	0.04	0.001 *	
	Demineralization	10	2.36	2.31	2.43	0.04		
	Remineralization	10	2.86	2.74	2.96	0.06		
nHAP	**Control**	10	2.87	2.83	2.99	0.04	0.001*	
	Demineralization	10	2.32	2.24	2.4	0.04		
	Remineralization	10	2.85	2.75	2.98	0.08		
CaGP	**Control**	10	2.89	2.8	2.98	0.06	0.001*	
	Demineralization	10	2.34	2.23	2.44	0.06		
	Remineralization	10	2.88	2.69	3.82	0.34		

Abbreviations used in this table: n:number of samples; SD:standart deviation; min:minimum; max:maximum, c:control, d:demineralization, r:remineralization.

Table 3 Comparison of the difference in mineral density between demineralization-remineralization values between groups

	Group						Kruskal Wallis H Test	
	n	**Mean**	**Median**	**Min**	**Max**	**SD**	**Mean Rank**	**H**	**p**
Fluoride	10	-0.5	-0.5	-0.58	-0.43	0.05	20.3	3.818	0.282
CPP-ACP	10	-0.49	-0.5	-0.59	-0.38	0.06	20.3		
nHAp	10	-0.53	-0.5	-0.66	-0.46	0.07	15.6		
CaGp	10	-0.54	-0.44	-1.59	-0.26	0.38	25.8		

Abbreviations used in this table: n:number of samples; SD:standart deviation; min:minimum; max:maximum.

Discussion

Modern dentistry aims to stop or slow the progression of initial enamel lesions with preventive applications.[19] The goal of this study was to examine the effectiveness of alternative materials to F for remineralization, which is one of the most focused issues in preventive dentistry, which is becoming increasingly important today. Fluoride has contributed to decreased tooth caries, but evidence suggests that fluoride alone does not completely prevent tooth caries.[20] In vitro studies attempt to mimic the oral environment as much as possible, so that the effect of a single variable can be evaluated by minimizing the variables that may affect the result. In addition, it is preferred because the tests can be performed easily, quickly and inexpensively.[21] In the literature, it was observed that studies evaluating demineralization and remineralization activities were generally performed under in vitro conditions.[4,22-24] Therefore, this study was conducted in-vitro. Early childhood caries lesions initially affect the primary upper incisors.[25] Since the study aimed to evaluate the efficacy of the agents used in the study on the remineralization of initial enamel lesions in primary teeth, the samples were selected from primary incisors. Therefore, upper anterior primary incisors obtained from 7-8-year-old children with physiological root resorption indication were included in the study.

In the literature, there are many studies in which demineralization and remineralization were evaluated using Micro-CT method.[7,11,16,26] Huang *et al.* (2007) used Micro-CT method to examine opaque enamel lesions formed under invitro conditions and reported that the method was very effective in determining the characteristics of the lesion and detecting mineral loss.[9] In

this study, Micro-CT method was used to determine the mineral loss in artificial caries lesions created under in vitro conditions on the enamel surface and the mineral changes caused by remineralization agents applied to the caries lesions during the pH cycle. When the mineral density values of the control group, demineralization and remineralization groups were compared within groups according to the remineralization materials applied after the samples were evaluated by Micro-CT, it was observed that the mineral density of the demineralization groups was significantly lower than the control group in all groups ($p<0.05$). However, there was no statistically significant difference in the mineral density values obtained from the remineralization and control groups of all toothpastes. These data showed that all agents provided effective remineralization. Reynolds et al. compared the treatment efficacy of 500 ppm F solution with solutions containing CPP-ACP at different concentrations (0.1, 0.2, 0.5 and 1%) and reported that 500 ppm F and 1% CPP-ACP provided similar levels of remineralization as in this study. [27] Recent studies have reported that nHAp added to toothpastes and mouthwashes has the potential to remineralize artificial caries lesions.[28-30] King et al. compared the effectiveness of two different toothpastes containing nHAp and 1100 ppm NaF on the remineralization of artificial caries lesions in enamel. After 10 days of pH cycling, it was concluded that the level of remineralization was similar to that of the NaF-containing toothpaste. In this study, nHAp and fluoride exhibited significant remineralization of primary teeth but did not show statistically significant differences among themselves.[28] Koçyiğit et al. (2015), toothpastes containing 1000 ppm NaF, nHAp and nHAp+F were applied to artificially induced initial enamel lesions on milk incisors. The data obtained were examined using Micro-CT and SEM devices and when the mineral densities were evaluated in general, no statistically significant difference was found between nHAp and toothpaste with F and it was concluded that nHAp is at least as effective as F as a remineralization material.[31] Similarly, in this study, no significant difference was found between the mineral concentrations of toothpastes containing F and nHAp, which supports the view that nHAp can be used as an alternative to F. Freire et al. (2016), in a randomized controlled trial investigating the anticariogenic effects of low-dose F-containing phosphate toothpastes, added calcium glycerophosphate and sodium trimethaphosphate (TMP) to F-containing toothpastes and examined their effects on caries progression in the primary dentition. They concluded that toothpaste containing 1100 ppm F and toothpaste containing 500 ppm F and CaGP showed similar efficacy and that the anticariogenic effect of toothpaste containing 500 ppm F with added TMP was significantly lower than the other toothpastes. [32] In this study, similar results were obtained and support the view that CaGP-containing toothpastes have anticariogenic effect. In an in vitro study by Zaze *et al.* (2014), the addition of 0.25% CaGP to a toothpaste containing 500 ppm F showed caries protective effects similar to those of toothpastes containing 1100 ppm F.[33] In this study, it was concluded that calcium glycerophosphate formulas promoted remineralization. In a study aiming to investigate the remineralization efficacy of sodium flouride (NaF), CPP-ACP and herbal toothpaste on initial caries and erosion using micro-CT and SEM, no statistically significant difference was found between the three agents in terms of remineralization efficacy ($p>0.05$). A statistically significant difference was found between the groups in terms of the mineral density of the increased tissue after remineralization (NaF > CPP-ACP > He; $p < 0.05$).[34] In this study, the mineral increase in the initial enamel lesions of toothpastes containing NaF and CPP-ACP was found to be equal.

When the findings obtained with Micro-CT in this study were evaluated in general; it was seen that the mineral density differences between healthy enamel, initial enamel lesion and remineralised enamel could be clearly determined by micro-CT, the mineral increase in initial enamel lesions in the calcium glycerophosphate-containing toothpaste group was higher than the other groups, although not statistically significant, and the mineral increase of fluoride-containing toothpaste and CPP-ACP containing toothpaste was lower than nanohydroxyapatite. The application of fluoride-containing toothpaste throughout the pH cycle, although not statistically significant, increased the mineral density compared to the initial state of the tooth. In this study, CPP-ACP, nHAp, CaGP and fluoride exhibited significant remineralization of primary teeth but did not show statistically significant differences among themselves. The CaGP-containing toothpaste showed a numerically higher increase in mineral density, although not statistically significant. CaGP used in this study showed similar results to F, nHAp and CPP-ACP in primary teeth.

Many new alternative materials are being investigated for the prevention and remineralization of dental caries, and successful results have been obtained with the use of these agents, but these materials have some limitations such as possible toxic effects and cannot be used safely in all age groups.[35] For this reason, pediatric dentists should recommend toothpaste with different ingredients for each patient according to their specific and individual needs. The findings of the study support the use of current remineralizing agents as an alternative to fluoride. Further studies on this subject would be useful. In addition, this study conducted under in vitro conditions should be supported by in vivo studies.

Conclusions

1. In the CaGP containing toothpaste group, the mineral increase in the initial enamel lesions was more than the other groups, although not statistically significant.
2. Studies have shown that the combined use of CaGP and F produces a synergistic effect, and considering the positive effects of CaGP on mineral density in this study. It is expected that the combined use of CaGP and toothpaste containing lower doses of F may be more effective.

3. When CPP-ACP, nHAp and CaGP used on initial enamel lesions of primary teeth support remineralization in the treatment of early childhood caries (ECC) and could be used as an alternative to fluoride.

Conflict of Interest Statement

The authors report no conflict of interest.

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Author Contributions

S.U. and F.T.Ö conceived the study and analyzed the data. S.U. collected the data. Ö.B.S wrote the article. All the authors approved the final version of the article.

References

1. Çelik EU, Yazkan B, Katırcı G. Treatment of incipient carious lesions. J Dent Fac Atatürk Uni. 2011;1:48-56.
2. Longbottom C, Ekstrand K, Zero D, Kambara M. Novel preventive treatment options. Monogr Oral Sci. 2009;21:156-163.
3. Iijima Y, Takagi O, Ruben J, Arends J. In vitro remineralization of in vivo and in vitro formed enamel lesions. Caries Res. 1999;33(3):206-213.
4. Zhang Q, Zou J, Yang R, Zhou X. Remineralization effects of casein phosphopeptide-amorphous calcium phosphate creme on artificial early enamel lesions of primary teeth. Int J Paediatr Dent. 2011;21(5):374-381.
5. Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res. 2001;80(12):2066-2070.
6. Reynolds EC, Cai F, Cochrane NJ, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res. 2008;87(4):344-348.
7. Kucuk EB, Malkoc S, Demir A. Microcomputed tomography evaluation of white spot lesion remineralization with various procedures. Am J Orthod Dentofacial Orthop. 2016;150(3):483-490.
8. Arends J, ten Bosch JJ. Demineralization and remineralization evaluation techniques. J Dent Res. 1992;71 Spec No:924-928.
9. Huang TT, Jones AS, He LH, Darendeliler MA, Swain MV. Characterisation of enamel white spot lesions using X-ray micro-tomography. J Dent. 2007;35(9):737-743.
10. Lussi A, Megert B, Longbottom C, Reich E, Francescut P. Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. Eur J Oral Sci. 2001;109(1):14-19.
11. Swain MV, Xue J. State of the art of Micro-CT applications in dental research. Int J Oral Sci. Dec 2009;1(4):177-188.
12. Schwass DR, Swain MV, Purton DG, Leichter JW. A system of calibrating microtomography for use in caries research. Caries Res. 2009;43(4):314-321.
13. Davis GR, Wong FS. X-ray microtomography of bones and teeth. Physiol Meas. 1996;17(3):121-146.
14. Daghrery A, Yaman P, Lynch M, Dennison J. Evaluation of micro-CT in the assessment of microleakage under bulk fill composite restorations. Am J Dent. 2022;35(2):128-132.
15. Bijle MN, Mallineni SK, Tsoi J. Qualitative and Quantitative Micro-CT Analysis of Natal and Neonatal Teeth. Children (Basel). 14 2022;9(4).
16. Cheng L, ten Cate JM. Effect of Galla chinensis on the in vitro remineralization of advanced enamel lesions. Int J Oral Sci. 2010;2(1):15-20.
17. ten Cate JM, Duijsters PP. Alternating demineralization and remineralization of artificial enamel lesions. Caries Res. 1982;16(3):201-210.
18. Itthagarun A, Wei SH, Wefel JS. The effect of different commercial dentifrices on enamel lesion progression: an in vitro pH-cycling study. Int Dent J. 2000;50(1):21-28.
19. Seredin P, Goloshchapov D, Prutskij T, Ippolitov Y. Phase transformations in a human tooth tissue at the initial stage of caries. PLoS One. 2015;10(4):e0124008.
20. Rao SK, Bhat GS, Aradhya S, Devi A, Bhat M. Study of the Efficacy of Toothpaste Containing Casein Phosphopeptide in the Prevention of Dental Caries: a Randomized Controlled Trial in 12- to 15-Year-Old High Caries Risk Children in Bangalore, India. Caries Research. 2009;43(6):430-435.
21. Hatibović-Kofman Š, El-Kassem M, Inocencio F, Selimovic M, Raimundo L. Evidence Based Effectiveness of Pit and Fissure Sealants Applied by Students and Paediatric Dentists After Five Years. Acta Stomatol. Croat. 2008;42(3)
22. Huang S, Gao S, Cheng L, Yu H. Combined effects of nano-hydroxyapatite and Galla chinensis on remineralisation of initial enamel lesion in vitro. J Dent. 2010;38(10):811-819.
23. Srinivasan N, Kavitha M, Loganathan SC. Comparison of the remineralization potential of CPP-ACP and CPP-ACP with 900 ppm fluoride on eroded human enamel: An in situ study. Arch Oral Biol. 2010;55(7):541-544.
24. Mehta AB, Kumari V, Jose R, Izadikhah V. Remineralization potential of bioactive glass and casein phosphopeptide-amorphous calcium phosphate on initial carious lesion: An in-vitro pH-cycling study. J Conserv Dent. Jan 2014;17(1):3-7.
25. Santos AP, Soviero VM. Caries prevalence and risk factors among children aged 0 to 36 months. Pesqui Odontol Bras. 2002;16(3):203-208.
26. Cochrane NJ, Anderson P, Davis GR, Adams GG, Stacey MA, Reynolds EC. An X-ray microtomographic study of natural white-spot enamel lesions. J Dent Res. 2012;91(2):185-191.
27. Reynolds EC, Cain CJ, Webber FL, et al. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. J Dent Res. 1995;74(6):1272-1279.
28. King N, Itthagarun A, Cheung M. Remineralization by Nanohydroxyapatite Containing Dentifrice: a pH Cycling Study Using Slurry. J Dental Res. 2006;85.
29. Lv KL, Zhang JX, Meng XC, Li XY. Remineralization effect of the nano-HA toothpaste on artificial caries. Key Engineering Materials. 2007;330:267-270.
30. Yamagishi K, Onuma K, Suzuki T, et al. Materials chemistry: a synthetic enamel for rapid tooth repair. Nature. 2005;433(7028):819.
31. C K, BN Y, N Ö. Effects of nano-hydroxyapatite dentifrices with and without fluoride on primary teeth enamel: a Micro-ct and a SEM study. Cumhuriyet Dent J. 2020;23(3):191-199.
32. Freire IR, Pessan JP, Amaral JG, Martinhon CC, Cunha RF, Delbem AC. Anticaries effect of low-fluoride dentifrices with phosphates in children: A randomized, controlled trial. J Dent. 2016;50:37-42.
33. Zaze AC, Dias AP, Sassaki KT, Delbem AC. The effects of low-fluoride toothpaste supplemented with calcium

glycerophosphate on enamel demineralization. Clin Oral Investig. 2014;18(6):1619-1624.
34. Akkuc S, Duruk G, Keles A. Remineralization effect of three different agents on initial caries and erosive lesions: a micro-computed tomography and scanning electron microscopy analysis. BMC Oral Health. 16 2023;23(1):106.
35. Kırzıoğlu Z, Özkul Önol S. Alternative materials used to prevent tooth decay. J Dent Fac Atatürk Uni. 2015;24(-3):104-112.