

Evaluation of the effects of different mouthrinses on dental remineralization

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Conflict of interest

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Abstract

Background. Dental caries occurs with the release of organic acids from the fermentable carbohydrates metabolized by cariogenic microorganisms. Microbial, genetic, immunological, behavioral, and environmental factors play a role in the development and severity of dental caries.

Objectives. The aim of the present study was to investigate the possible effects of different mouthwash solutions on dental remineralization.

Material and methods. This in vitro study compared the remineralization capacity of different mouthwash solutions applied topically to the enamel surface. A total of 50 tooth specimens were prepared from the buccal and lingual halves, with 10 teeth in each group: G1 (control); G2 (Listerine®); G3 (Sensodyne®); G4 (Oral B® Pro-Expert); and G5 (DentaSave® Zinc). Remineralization capacity was evaluated in all groups. The one-way analysis of variance (ANOVA) and the paired samples *t* test were used for statistical analysis, with a *p*-value <0.05 considered significant.

Results. There were significant differences in the calcium (Ca)/phosphorus (P) atomic percentage (at%) ratio between the demineralized and remineralized dentin (*p* = 0.001), and between the demineralized and remineralized enamel (*p* = 0.006). Similarly, there were significant differences in the at% of P (*p* = 0.017) and zinc (Zn) (*p* = 0.010) between the demineralized and remineralized dentin. There was a significant difference in the at% of P (*p* = 0.030) between the demineralized and remineralized enamel. The Zn at% in enamel was significantly higher after remineralization in G5 as compared to the control group (*p* < 0.05). The images of the demineralized enamel showed the usual keyhole prism appearance, with intact prism sheaths and negligible inter-prism porosity.

Conclusions. The scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) findings seem to confirm the effectiveness of DentaSave Zinc for the remineralization of enamel lesions.

Keywords: tooth demineralization, X-ray emission, tooth remineralization, scanning electron microscopy

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Introduction

Dental caries begins with the release of organic acids from the fermentable carbohydrates metabolized by cariogenic microorganisms. During this process, along with the imbalance between the tooth tissues and the plaque fluid, dental caries lesions occur as a result of the demineralization of the inorganic components of the tooth.¹ Microbial, genetic, immunological, behavioral, and environmental factors play a role in the development and severity of dental caries. Dental caries remains an important health problem today, although its prevalence has declined in many developed countries in respect to previous years.² Owing to the understanding of the pathological process of caries and the identification of the factors affecting the demineralization process, it is well established that initial caries lesions can be remineralized.³

If the environmental balance can be adjusted to promote remineralization when initial caries lesions occur, remineralization can be achieved by depositing the calcium (Ca) and phosphate ions lost from the enamel tissue back onto the enamel surface. The process requires eliminating the factors reducing intraoral pH, buffering the acids produced by certain bacteria by the saliva over time so that pH can increase to a neutral level, and the saturation of plaque and the saliva with the minerals dissolved in enamel.⁴

Remineralization is defined as the redeposition on the enamel surface of Ca, phosphorus (P) and other ions lost from the enamel tissue due to caries or other reasons; it is a process of enamel tissue regeneration.⁵ The awareness of the importance of preventive oral and dental care is increasing day by day due to a better understanding of the multifactorial etiology underlying dental caries, the advanced technology of dental materials, and achievements in protective applications through early diagnosis methods and minimally invasive techniques.⁶

The present study aimed to investigate the possible dental remineralization effects of different mouthwash solutions available in Turkey.

Material and methods

This in vitro study was carried out at Artvin Oral and Dental Health Center and Artvin Çoruh University Central Research Laboratory, Turkey. Before commencing the study, the Non-Interventional Trials Ethics Committee at Artvin Çoruh University approved the protocol (No. 24/11/2017-E.20466).

Collection and preparation of dental specimens

In the 1st phase of the study, erupted upper and lower third molar samples were used; the teeth had no signs

of caries, but they were extracted for various reasons within the previous month. After extraction, the teeth were kept in distilled water at +4°C until testing.⁷ Soft tissue residues and additives were removed, and the teeth were cleansed using a fluorine-free pumice and a brush. To remove pumice remains, the teeth were washed for 15 s, left to dry, and then examined under a stereomicroscope. The ones determined to be free of caries, hypoplasia, fractures, and cracks were included in the study.

A total of 50 teeth were prepared from the buccal and lingual halves, with 10 teeth in each group: G1 (control; the remineralization solution); G2 (Listerine®; Lambert Pharmacal Company, St. Louis, USA); G3 (Sensodyne®; GlaxoSmithKline Consumer Healthcare, London, UK); G4 (Oral B® Pro-Expert; Procter & Gamble, Cincinnati, USA); and G5 (DentaSave® Zinc; Drog-san, Ankara, Turkey). Remineralization capacity was evaluated in all groups. To achieve this, the crowns of the teeth were removed from the roots with a 0.3-millimeter-thick, low-speed, high-precision, double-sided diamond separator. Then, the teeth were divided mesiodistally into 2 parts to obtain buccal and lingual surfaces, and ground using 600-grit, 800-grit, and 1,200-grit abrasive paper disks. The sanding direction was changed to remove any marks caused by the previous sanding application. The obtained enamel surfaces were examined under a stereomicroscope, and sanding was repeated in cases the surface was not smooth enough.⁸

The obtained samples were embedded in acrylic molds with an inner diameter of 15 mm and a height of 20 mm, with their upper and lower parts parallel to each other, and in such a way that the surfaces to be exposed to demineralization and remineralization were left open. Then, stickers were fixed in the middle of the samples to prepare 4 mm × 4 mm windows. The natural surfaces of the teeth outside the area concealed by the stickers were covered with acid-resistant varnish.⁹

Preparation of the artificial saliva solution

The artificial saliva solution was freshly prepared before the tests, using the composition described by Ten Cate et al.¹⁰ The solution contained 1.28568 g NaCl, 0.0320 g MgCl₂·6H₂O, 0.07945 g CaCl₂·2H₂O, 0.29857 g KCl, 0.897 g KOH, and 472 µl H₃PO₄.

Preparation of the remineralization solution

The remineralization solution was freshly prepared before the tests according to the composition described by Ten Cate et al., with 1.0 mM CaCl₂, 2 mM KH₂PO₄ and 150 mM KCl.¹⁰ It was preserved by adding 0.01% NaN₃, pH was adjusted to 7.0 by using 1M KOH, and it was kept at room temperature.

Preparation and application of the demineralization solution

The next phase involved inducing artificial caries lesions on the surfaces of 10 samples in each group. The samples were incubated for 7 days in capped containers, with 10 mL of the demineralization solution added to each sample. The demineralization solution was prepared by mixing 100 mmol/L NaOH and 100 mmol/L lactic acid (pH 5.0). To achieve a viscosity of 100 cp, 0.2 g/L carboxymethyl cellulose sodium salt was added to the solution.

Implementation of treatment procedures

The demineralized teeth were treated twice a day for 1 month, using different mouthwash solutions, as prescribed by each manufacturer.¹¹ Four different mouthwash solutions were applied to all samples at equal rates, with deionized water applied to the control group. The samples were stored in artificial saliva at 37°C for 7 days.

SEM imaging and EDS analysis

Scanning electron microscopy (SEM) imaging for assessing the mineral depositions caused by different materials applied to the enamel specimens with initial enamel lesions, as well as the energy-dispersive X-ray spectroscopy (EDS) analysis for determining changes in the mineral content, were performed using a SEM connected to an EDS detector (EVO LS 10; Carl Zeiss NTS, Oberkochen, Germany). For this purpose, the samples were coated with a 100-angstrom gold layer under a vacuum rate of 10^{-4} Tr, using a vacuum coating apparatus (EVO LS 10). After coating, the samples were placed on the SEM stage via aluminum tables, and SEM images were obtained at $\times 5,000$ and $\times 2,000$ magnifications at 20 kV and a working distance of 10 mm. After obtaining the images, the content of oxygen (O), P, Ca, and zinc (Zn) elements on the enamel surface were determined as percentages with the EDS detector.

Statistical analysis

All data is presented as mean (M) and standard deviation (SD). The Shapiro–Wilk test evaluated the normality of the data. The one-way analysis of variance (ANOVA) was applied to compare the mineral content among the 5 experimental groups. The mineral content of enamel and dentin before and after demineralization was compared with the paired samples t test. All analyses employed IBM SPSS Statistics for Windows, v. 19.0 (IBM Corp., Armonk, USA). A p -value < 0.05 was considered statistically significant.

Results

The mineral content of each specimen in the 5 groups was measured by means of EDS. The chemical analysis of the demineralized enamel showed that it predominantly contained O, P, Ca, and Zn. The Ca/P atomic percentage (at%) ratio was approx. 1.78 for the demineralized enamel and 1.87 for the remineralized enamel (Table 1). The Ca/P at% ratio for the demineralized and remineralized dentin was 1.71 and 1.83, respectively (Table 2).

Table 1 shows the mean values for the enamel mineral content of 50 specimens before and after remineralization. There were no significant differences in the O ($p = 0.292$), Ca ($p = 0.595$) and Zn ($p = 0.117$) at%. There was a significant difference in the Ca/P at% ratio between the demineralized and remineralized enamel ($p = 0.006$). Similarly, there was a significant difference in the P at% between the demineralized and remineralized enamel ($p = 0.030$).

Table 2 shows the mean values for the dentin mineral content of 50 specimens before and after remineralization. There were no significant differences in the

Table 1. Enamel mineral content before and after remineralization

Element	Enamel	<i>n</i>	Mineral content	<i>p</i> -value
Oxygen (O) [at%]	demineralized	50	64.21 \pm 7.04	0.292
	remineralized	50	65.79 \pm 8.61	
Phosphorus (P) [at%]	demineralized	50	12.71 \pm 2.03	0.030*
	remineralized	50	11.80 \pm 2.69	
Calcium (Ca) [at%]	demineralized	50	22.76 \pm 4.70	0.595
	remineralized	50	22.21 \pm 5.98	
Zinc (Zn) [at%]	demineralized	50	0.12 \pm 0.08	0.117
	remineralized	50	0.19 \pm 0.28	
Ca/P at% ratio	demineralized	50	1.78 \pm 0.14	0.006*
	remineralized	50	1.87 \pm 0.16	

Data presented as mean \pm standard deviation ($M \pm SD$). * statistically significant ($p < 0.05$).

Table 2. Dentin mineral content before and after remineralization

Element	Dentin	<i>n</i>	Mineral content	<i>p</i> -value
Oxygen (O) [at%]	demineralized	50	69.48 \pm 5.76	0.462
	remineralized	50	70.49 \pm 8.28	
Phosphorus (P) [at%]	demineralized	50	11.11 \pm 1.98	0.017*
	remineralized	50	10.14 \pm 2.38	
Calcium (Ca) [at%]	demineralized	50	19.01 \pm 3.43	0.771
	remineralized	50	18.75 \pm 5.77	
Zinc (Zn) [at%]	demineralized	50	0.22 \pm 0.13	0.010*
	remineralized	50	0.62 \pm 1.06	
Ca/P at% ratio	demineralized	50	1.71 \pm 0.07	0.001*
	remineralized	50	1.83 \pm 0.21	

Data presented as $M \pm SD$. * statistically significant ($p < 0.05$).

O ($p = 0.462$) and Ca ($p = 0.771$) at%. There was a significant difference in the Ca/P at% ratio between the demineralized and remineralized dentin ($p = 0.001$). Similarly, there were significant differences in the P ($p = 0.017$) and Zn ($p = 0.010$) at% between the demineralized and remineralized dentin. The Ca/P at% was slightly higher in enamel than in dentin.

The Zn at% in enamel was significantly higher after remineralization in G5 as compared to the control group ($p < 0.05$). However, there were no significant differences in the Zn levels between G2, G3 or G4 and the control group ($p > 0.05$). Also, there were no significant differences between the experimental groups and the control in terms of the O, P and Ca at%, and the Ca/P at% ratio ($p > 0.05$) (Table 3).

After remineralization, the Zn at% in dentin was significantly higher in G5 than in the control group ($p < 0.05$). In contrast, the Zn at% was significantly lower in G3 than in the control group ($p < 0.05$). The Zn at% was also lower in G2 and G4 than in the control group, but the differences were not statistically significant ($p > 0.05$). The O, P and Ca at% were not different

Table 3. Effect of mouthwash solutions on the enamel mineral content after remineralization

Element	G1	G2	G3	G4	G5
Oxygen (O) [at%]	71.74 ±6.63	64.51 ±9.40	64.43 ±10.77	65.88 ±7.04	62.38 ±7.02
Phosphorus (P) [at%]	9.99 ±2.39	11.86 ±2.96	12.39 ±3.11	12.08 ±2.28	12.67 ±2.28
Calcium (Ca) [at%]	18.19 ±4.25	23.51 ±6.61	23.11 ±7.78	21.91 ±4.74	24.34 ±4.90
Zinc (Zn) [at%]	0.08 ±0.09	0.04 ±0.05	0.08 ±0.51	0.13 ±0.12	0.61 ±0.39 ^a
Ca/P at% ratio	1.85 ±0.08	1.98 ±0.19	1.84 ±0.20	1.81 ±0.11	1.92 ±1.17

Data presented as $M \pm SD$. Groups: G1 – control; G2 – Listerine; G3 – Sensodyne; G4 – Oral B Pro-Expert; and G5 – DentaSave Zinc. ^a statistically significantly different when compared to the control group ($p < 0.05$).

Table 4. Effect of mouthwash solutions on the dentin mineral content after remineralization

Element	G1	G2	G3	G4	G5
Oxygen (O) [at%]	75.85 ±5.84	67.62 ±8.42	67.28 ±8.53	75.35 ±6.57	66.36 ±7.36
Phosphorus (P) [at%]	8.67 ±2.33	11.15 ±2.37	11.27 ±1.94	9.35 ±2.46	10.24 ±2.05
Calcium (Ca) [at%]	15.32 ±3.49	21.11 ±6.12	21.38 ±6.67	15.16 ±4.06	20.76 ±4.96
Zinc (Zn) [at%]	0.18 ±0.07	0.10 ±0.10	0.07 ±0.08 ^a	0.14 ±0.12	2.64 ±0.65 ^a
Ca/P at% ratio	1.78 ±0.12	1.88 ±0.15	1.87 ±0.28	1.62 ±0.10	2.01 ±0.17 ^a

Data presented as $M \pm SD$. Groups: G1 – control; G2 – Listerine; G3 – Sensodyne; G4 – Oral B Pro-Expert; and G5 – DentaSave Zinc. ^a statistically significantly different when compared to the control group ($p < 0.05$).

between the groups or when compared to the control group ($p > 0.05$). As for the Ca/P at% ratio, G5 had a significantly higher ratio as compared to the control group ($p < 0.05$). The Ca/P at% ratio was also higher in G2 and G3 as compared to the control, but without statistical significance ($p > 0.05$). Meanwhile, the Ca/P at% ratio in G4 was lower than in the control, but again without statistical significance ($p > 0.05$) (Table 4).

Figures 1–5 show representative SEM images ($\times 2,000$ magnification) of dentin after remineralization for different treatment groups. The specimens from the 5 treatment groups showed some dentin remineralization on the top surface, with a more homogeneous and denser mineral content. Also, the surfaces of the control group samples were densely covered with the mineral content. The specimens treated with Oral B Pro-Expert and DentaSave Zinc had a surface layer of irregular and porous minerals deposited. The groups treated with Listerine and Sensodyne had a remineralized layer with a more homogeneous and denser dentin mineral content that went deeper into the demineralized region.

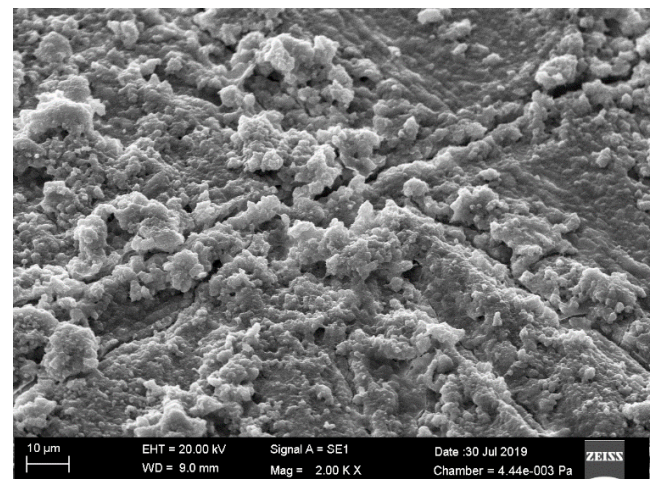


Fig. 1. Scanning electron microscopy (SEM) image of the remineralized dentin at $\times 2,000$ magnification in G1

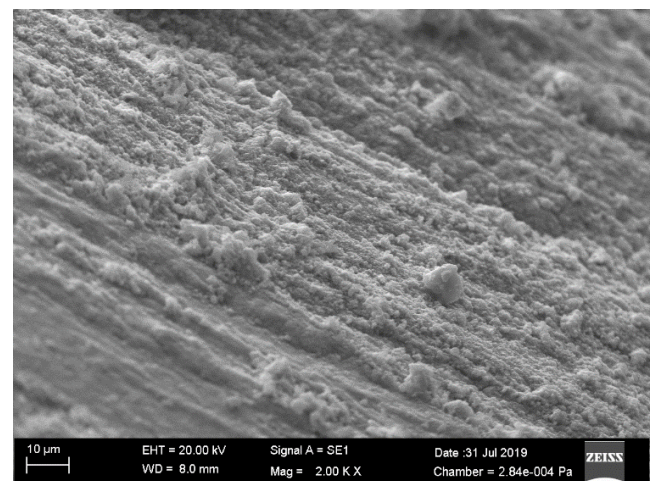


Fig. 2. Scanning electron microscopy (SEM) image of the remineralized dentin at $\times 2,000$ magnification in G2

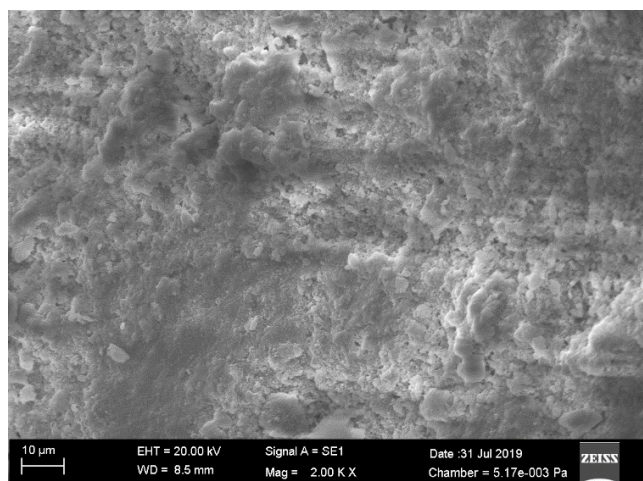


Fig. 3. Scanning electron microscopy (SEM) image of the remineralized dentin at $\times 2,000$ magnification in G3

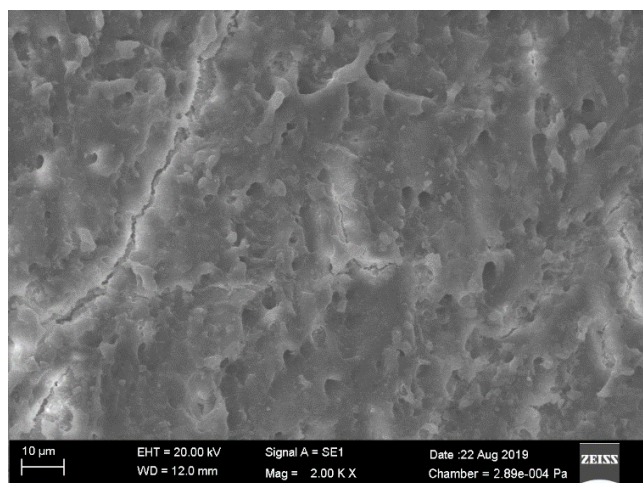


Fig. 4. Scanning electron microscopy (SEM) image of the remineralized dentin at $\times 2,000$ magnification in G4

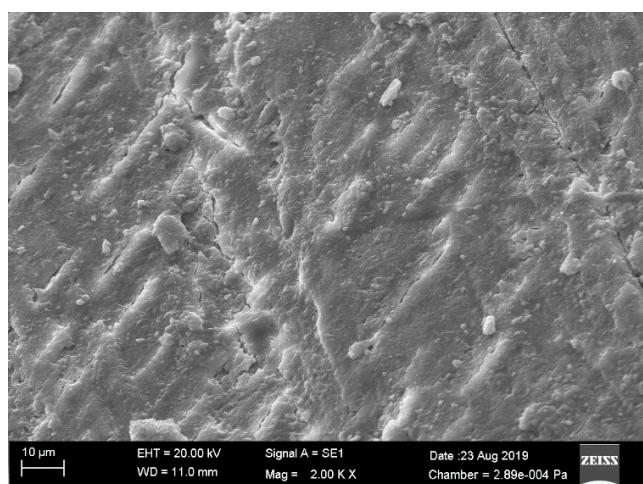


Fig. 5. Scanning electron microscopy (SEM) image of the remineralized dentin at $\times 2,000$ magnification in G5

Discussion

As a significant health problem, dental caries is a pathological process characterized by the localized destruction

of dental hard tissues by cariogenic microorganisms.¹² Dental hard tissues are constantly influenced by intraoral pH changes, and a process called demineralization occurs when pH is below 5.5. During demineralization, Ca and phosphate ions are dissolved and released from the enamel structure, causing enamel loss.^{13,14} The dissolved ions accumulate on the enamel surface with the raised pH levels, which initiates the remineralization process.¹⁵ Various products and foods with a low acid content cause the intraoral pH level to increase. In recent years, a wide variety of products preventing demineralization have become available. Therefore, the present study aimed to determine the remineralization capacity of 4 products of different content.

It is known that various processes and different materials applied to the surfaces of dental hard tissues can change their mineral composition and structure. Hydroxyapatite crystal is the main inorganic component of enamel, and the size, permeability and solubility of the crystal are significantly affected by changes in the amount of Ca and P with respect to other elements that constitute its structure.¹⁶ The Ca and P at%, as well as the Ca/P at% ratio, are higher in intact enamel than in the demineralized tooth tissue. For this reason, studies on the remineralization of dental hard tissues generally evaluate Ca and P elements and the Ca/P ratio.^{17,18} In the current study, we determined the Ca and P at% and the Ca/P at% ratio in both enamel and dentin. In enamel, the Ca/P at% ratio showed a significant increase after remineralization as compared to the condition after demineralization. Meanwhile, the P at% showed a significant decrease after remineralization as compared to the level during the demineralization process. The Ca level also decreased after remineralization, but the change was not statistically significant. We also evaluated the O and Zn at% in both enamel and dentin. There were no statistically significant differences in the O and Zn at% after enamel remineralization as compared to the values after enamel demineralization. As for the O, P, Ca, and Zn at% and the Ca/P at% ratio in dentin, the Zn level and the Ca/P at% ratio increased significantly after remineralization as compared to demineralization, whereas the P level decreased. The process of remineralization led to a reduction in the Ca and P losses occurring in both enamel and dentin.

Mouthwashes are non-sterile aqueous solutions with a fragrant, refreshing and antiseptic effect. Mouthwashes are used in dentistry as protective and therapeutic agents, and are helpful during some professional procedures. With different content and forms, as well as due to the ease of use, they constitute a vital aspect of preventive treatment for physicians and patients. They are designed to reduce the count of oral bacteria, remove residual food particles, temporarily eliminate bad breath, and leave a pleasant taste in the mouth.^{19,20} Dietary acids are the crucial external etiologic factors of dental erosion. Effective agents that can prevent and treat dental erosion

should be investigated. For this reason, the present study evaluated the fluorine (F)-containing Listerine, Sensodyne and Oral B Pro-Expert, and Zn-containing DentaSave Zinc products.

For caries prevention, the World Health Organization (WHO) recommends using 0.05% sodium fluoride mouthwash (230 ppm F) daily, or 0.2% sodium fluoride mouthwash (900 ppm F) once a week or every 15 days.²¹ In the literature, fluoride mouthwashes were reported to be effective in preventing caries at a rate of 26%.^{22,23} In the current study, DentaSave Zinc displayed a better performance in preventing enamel and dentin erosion, as well as in the remineralization of the enamel–dentin surface, as compared to the other mouthwash products and the control group. Indeed, the Ca/P at% ratio and the Zn at% were significantly higher in the DentaSave Zinc group than in the control group. Furthermore, there were no statistically significant differences between the Listerine, Sensodyne and Oral B Pro-Expert applications and the control group concerning the O, P and Ca at% and the Ca/P at% ratio.

Initial enamel lesions have the potential to progress very rapidly, and there is a balance between the demineralization and remineralization cycles in the oral environment. The main goal is shifting the mineral balance in the mouth in favor of tooth remineralization, with various products used for this process. Dental cavitation may occur if no preventive measures are taken against the progression of enamel lesions.²⁴ Erosion was determined on the enamel surfaces of all specimens in a pH-cycling model mimicking the oral environment. The SEM analysis showed the morphological changes caused by treating the induced enamel and dentin lesions with various products. The EDS analysis showed increased enamel and dentin Ca/P at% ratio following the treatment. The above findings seem to converge and agree on the effectiveness of DentaSave Zinc for the remineralization of enamel lesions.

Conclusions

This study was limited to the evaluated mouthwashes. Also, the remineralization effects of these mouthwashes were studied only in enamel and dentin. Although the chemistry of the demineralization–remineralization process is similar in dentin and root cement, the structure of each of these materials, and their mineral and organic tissue content are different, which causes differences in the formation and progression of the carious lesion. The applications of the Listerine, Sensodyne or Oral B Pro-Expert mouthwashes was not as effective in terms of tooth remineralization as DentaSave Zinc. Nonetheless, the remineralization capacity of these mouthwashes in dentin should also be investigated. Therefore, further clinical studies should be conducted.

Ethics approval and consent to participate

The study was approved by the Non-Interventional Trials Ethics Committee at Artvin Çoruh University, Artvin, Turkey (No. 24/11/2017-E.20466).


Data availability


The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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