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NEW VARNISHES ALTERNATIVE TO FLUORIDE FOR PREVENTION OF DENTAL EROSION

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ABSTRACT: The present study aims to evaluate the effects of fluoride varnish (FV), bioactive glass varnish (BV), sodium-trimetaphosphate treated eggshell, and eggshell membrane containing varnish (EV) against dental erosion. In this study, 27 teeth were cut using a low-speed diamond separator. Two 2×3 mm windows were created on buccal surfaces, and the remaining area was covered with acid-resistant nail polish. Fluoride varnish, Bioactive glass varnish, Sodium-trimetaphosphate treated eggshell, and eggshell membrane containing varnish were applied to one of the windows, and the other window was used as a control. Using separate containers, samples were immersed in cola drink for two minutes and shaken gently. Then, the samples were kept in 0.4 ml artificial saliva for two minutes. This pH cycle was repeated four times. The surface properties of the samples were examined in a scanning electron microscope, the Ca, P, F, O, C, Mg and Na ratios were determined, and the Ca/P ratio was calculated. The Ca/P ratio in the area where FV is applied is 1.56, lower than 1.61, the stoichiometric ratio in human enamel. This ratio was 1.50 in the FV control group. The Ca/P ratio was 1.63 in the BV group and 1.51 in the BV control group, 1.63 in the EV group, and 1.52 in the EV control group. No statistical difference was found among the groups. On the enamel surface treated with BV and EV, a protective layer was observed. Against dental erosion, BV and EV can be used as an alternative to fluoride varnishes.

Keywords: Dental erosion; Fluoride varnish; Eggshell; Eggshell membrane; STMP; Bioactive glass.

1. INTRODUCTION

The loss of surface tooth structure by chemical action in the continued presence of demineralizing agents with low pH is defined as erosion[1]. Dental erosion occurs due to external reasons, such as consumption of acidic foods and beverages, use of acidic drugs, acidic hygiene products, or internal reasons due to disorders, such as gastroesophageal reflux, regurgitation, vomiting, anorexia and bulimia nervosa that bring gastric acid to the oral cavity[2]. The exposure of teeth to acid has increased due to modern lifestyle, especially with a high prevalence among children and adolescents[3].

The caries preventive effects of fluoride (F) have been known since researchers questioned why mottled enamel (dental fluorosis), which they thought to be defective, was resistant to caries[4]. Likewise, to its anti-cariogenic properties, F might also assist in strengthening hard tooth tissue against dental erosion[5]. Drinking water is often the main source of F intake[6-8], also gel and varnish forms available for topical use due to their dental benefits[9]. Topical use of F is safe[10] but high concentrations of F are associated with skeletal fluorosis, low testosterone level, low cognitive ability alongside dental fluorosis[11, 12], and there is increasing opposition to the use of F, and as a result, interest in products labeled as "natural," "organic," "herbal" is increasing[13].

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Bioactive glass releases sodium, calcium, and phosphorus ions used for remineralization and forming hydroxycarbonate apatite similar to hydroxyapatite when encounters saliva[14]. For the strong potential for remineralization, bioactive glass containing varnishes was introduced to prevent dental erosion[15].

Eggshell, in addition to the antibacterial agents it carries, contains bioactive substances that initiate and control mineralization, as well as other ions, mainly calcium, used in remineralization. The presence of ovotransferrin, osteopontin, ovalbumin, ovocalyxin, clusterin, and lysozyme, which play an important role in bone formation, has been demonstrated in chicken eggshell extracts. These are bioactive molecules that regulate the initiation of calcite crystal formation, morphology, and precipitation rate during shell formation[16]. Eggshell membranes settle under the shell in two layers, one thick and outer, the other thin and inner, and surround the egg white all around. Shell membranes also have bioactive substances that initiate and control mineralization. In addition, it is rich in type I collagen and thus contributes to remineralization[17]. STMP (sodium trimetaphosphate) is another compound potentially used in biomimetic remineralization, which provides phosphorylation of type I collagen by forming covalent bonds between phosphate groups and hydroxyl or amino groups in alkaline conditions, and it cannot be easily removed from proteins for that covalent bonds[18]. Thus, it has been suggested that STMP treated eggshell and eggshell membrane containing varnish effectively prevent dental erosion.

Our hypothesis is that novel STMP treated eggshell and eggshell membrane varnish is not as effective as F, and bioactive glass varnish at preventing dental erosion produced by the soft drink.

2. MATERIALS AND METHODS

Twenty-seven caries-free third molars extracted for medical reasons and with consent of donors between the ages of 20–30 were preserved in 0.1% thymol solution. The teeth were cut with a water-cooled low-speed diamond separator 1 mm below the enamel-cement border, then vertically in the buccolingual direction, and the buccal parts of the teeth were used for the experiments. Two 2×3 mm windows were created in the middle three of the buccal surfaces of the specimens, and the remaining parts were covered with acid-resistant nail polish. Before varnish application, the samples were thoroughly rinsed with deionized water and gently dried using a paper towel. Three experimental groups were formed (n = 9). The workflow is summarized in Figure 1.

Varnish to be tested (Table 1) was applied to one of the two windows in each tooth sample; the other window was used as a control. Samples were kept in artificial saliva for 24 hours after varnish was applied. Varnishes were gently removed without damaging the enamel surface, and no chemicals were used in this procedure to avoid changing the surface properties and then all samples were taken into the pH cycle. Using separate containers, samples were immersed in cola drink (6 ml/sample, p H 2.6, Coca-Cola, Ankara, Turkey) gentle shaking (Duomax 1030, Heidolph) at room temperature for two minutes. Then, the samples were kept in 0.4 ml artificial saliva for two minutes. This pH cycle was repeated four times. Cola drink and artificial saliva solution were changed each cycle, and also, during demineralization cycles,

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samples were kept in hermetically sealed containers because degassing the beverage can increase its pH uncontrollably. Then, the samples were kept in artificial saliva (containing 4.2 g/l sodium bicarbonate, 0.5 g/l sodium chloride, 0.2 g/l potassium chloride prepared with distilled water at pH 7.3) until scanning electron microscopy-energy dispersive X-ray (SEM-EDX) spectroscopic analysis was performed.

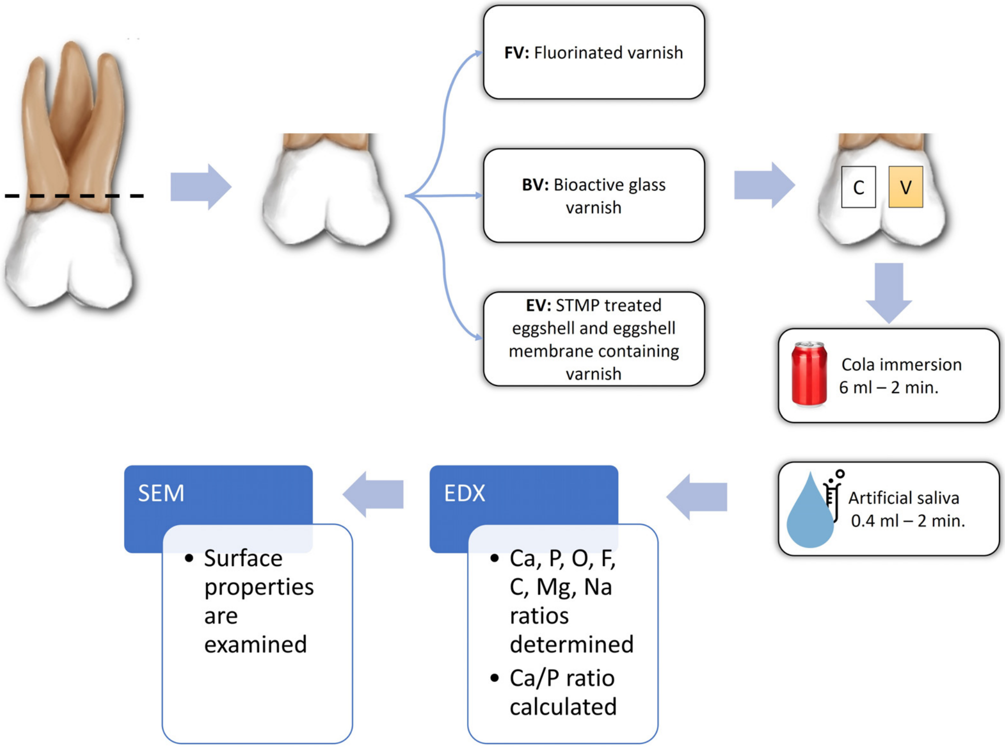

Figure 1. Schematization of the experimental groups and the procedures.

Table 1. Test groups of the study.

Groups	Ingredients	Manufacturer and Lot number
FV Fluoride varnish	sodium fluoride, solvent, organic resin, xylitol, flavor	IMICRYL (Konya, Turkey) 18222
BV Bioactive glass varnish	organic resin, ethyl alcohol, xylitol, flavor, bioactive glass	IMICRYL (Konya, Turkey) 19043
EV STMP treated eggshell and eggshell membrane containing varnish	organic resin, ethyl alcohol, xylitol, flavor, tri-metaphosphate treated eggshell membrane protein, eggshell powder	IMICRYL (Konya, Turkey) 19116

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Ca/P ratio was calculated by the formula below:

$$Ca/P\ ratio\ (molar) = \frac{Ca\ (wt\%)/40.08}{P\ (wt\%)/30.97}$$

Statistical analysis was performed by SPSS (v.25; IBM, New York, USA). Shapiro-Wilk test was used to evaluate the homogeneity of the variance. One-way ANOVA and post-hoc Tukey tests were used to analyze EDX data. The level of significance was set at 0.05.

3. RESULTS

According to EDX elemental analysis, the average Ca, P, F, O, C, Mg, and Na levels of the control and varnish applied areas are given in Table 2. In general, the experimental groups had a higher Ca/P ratio and F element than the controls but were not statistically different ($p > 0.05$). The comparison of the mean Ca/P ratios of the groups calculated from the data is given in Figure 2.

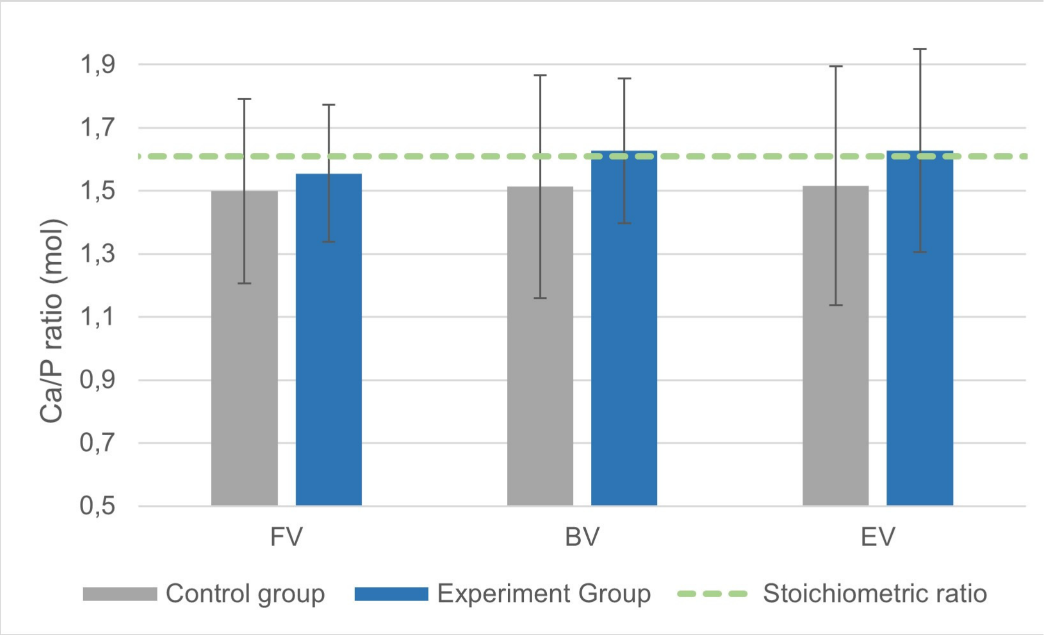

Figure 2. Average Ca/P ratio (mol) of dental varnishes; the dotted line shows the stoichiometric ratio (1.61) of human enamel.

The Ca/P ratio of the BV group and BV control group were 1.63 and 1.51, respectively. The Ca/P ratio of the EV group and EV control group were 1.63, 1.52, respectively. It was observed that BV and EV applied surfaces had Ca/P ratio closer to human enamel.

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Table 2. Mean distributions and standard deviations of all selected elements for dental varnishes and control groups as a percentage (wt%)

		FV		BV		EV	
		Mean	SD	Mean	SD	Mean	SD
Ca	Control	36.33	4.01	38.02	4.89	34.61	3.77
	Experiment	29.72	3.38	33.11	5.56	32.18	4.31
P	Control	19.17	3.94	20.17	4.84	18.03	2.33
	Experiment	14.80	1.45	16.18	5.16	15.52	2.28
O	Control	30.37	3.33	26.02	4.65	31.73	3.50
	Experiment	33.99	3.47	33.64	6.69	35.14	4.33
F	Control	8.86	2.89	9.52	2.91	9.73	1.72
	Experiment	10.40	1.27	10.73	2.66	10.30	2.53
C	Control	3.82	0.80	5.10	1.33	4.21	3.18
	Experiment	9.54	5.67	4.95	1.24	5.27	2.66
Mg	Control	0.39	0.21	0.31	0.17	0.49	0.21
	Experiment	0.37	0.21	0.31	0.22	0.34	0.26
Na	Control	1.05	0.29	0.86	0.41	1.21	0.26
	Experiment	1.17	0.43	1.07	0.42	1.11	0.44

After the acid attacks, the enamel surface was mildly and irregularly etched (Figures 3A and 3B). Eroded enamel prism cores were apparent on the surface (Figure 3C). The FV treated enamel surface was generally smooth and appeared to be slightly etched (Figure 3D). A lamellar, porous precipitate was visible on the surface of the BV-treated enamel (Figure 3E). On the enamel surface where EV was applied, a more compact sediment layer consisting of particles of different sizes relative to the BV was observed (Figure 3F).

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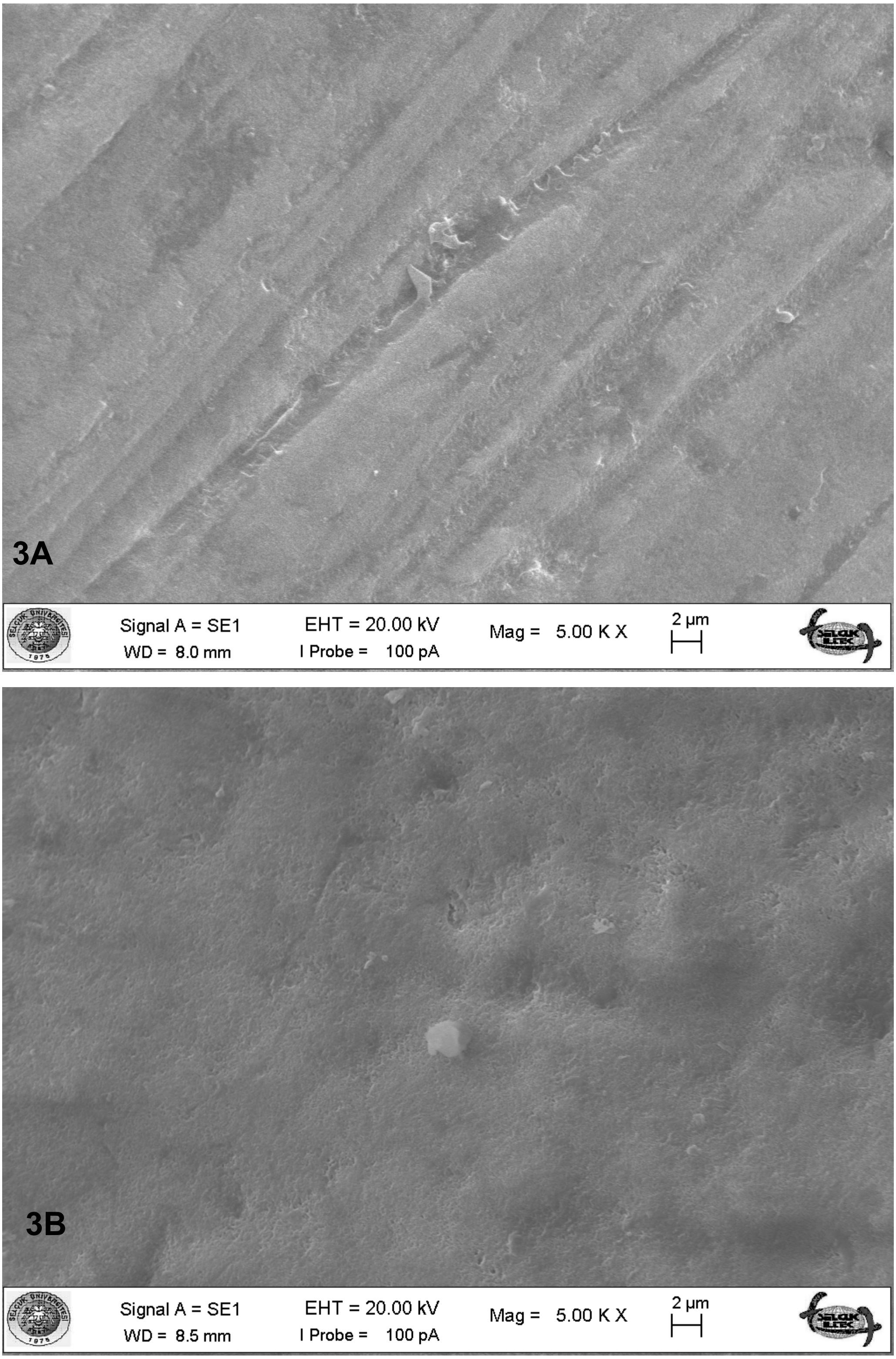
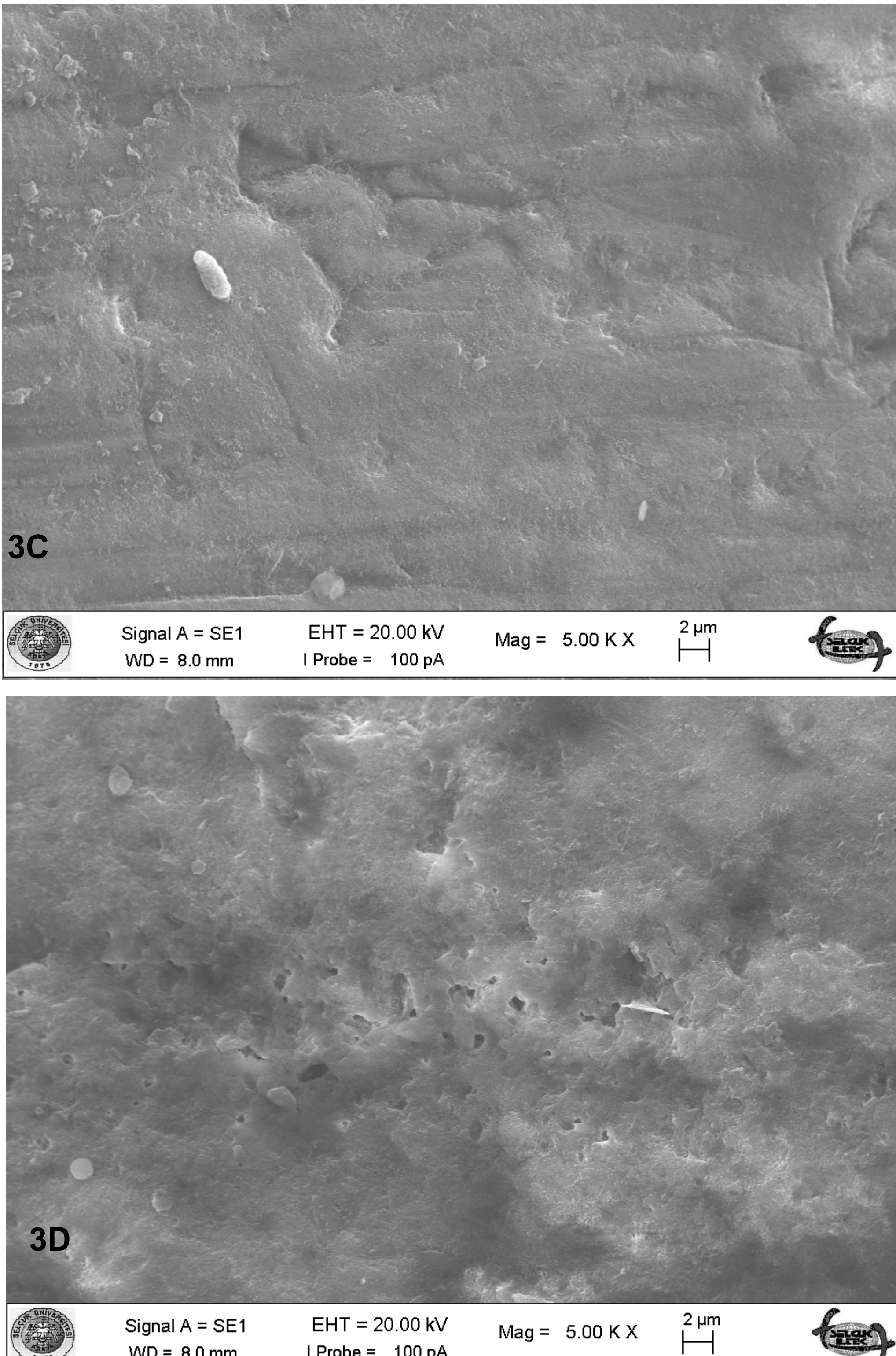


Figures 3A and 3B. 3A: SEM image of a tooth surface from the control area of the FV group; 3B: SEM image of the FV applied tooth surface. FV = fluoride varnish.



Figures 3C and 3D. 3C: SEM image of a tooth surface from the control area of the BV group; 3D: SEM image of the BV applied tooth surface. BV = bioactive glass varnish.

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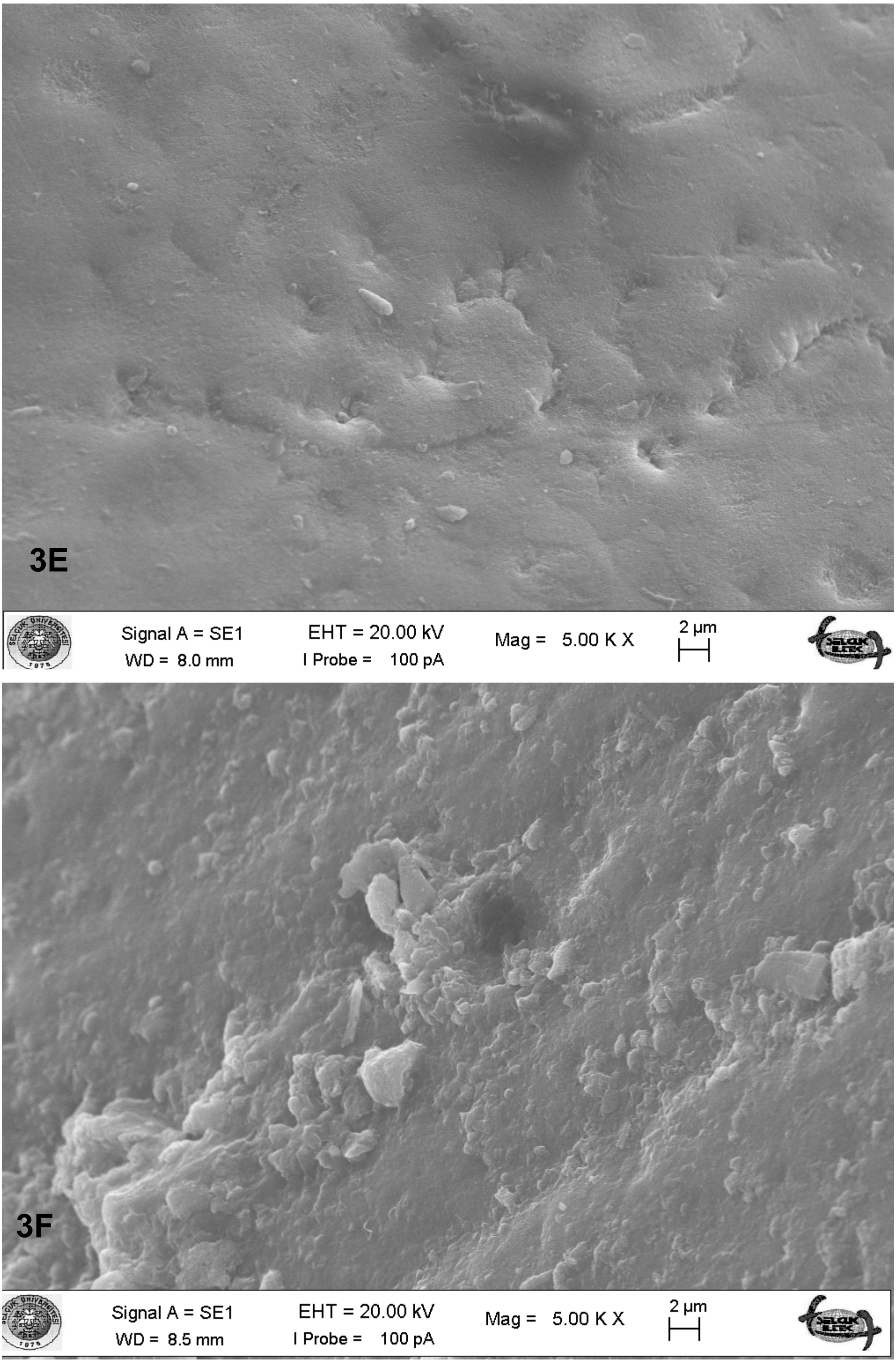

Figures 3E and 3F. 3E: SEM image of a tooth surface from the control area of the EV group; 3F: SEM image of the EV applied tooth surface. EV = sodium trimetaphosphate (STMP) treated eggshell and eggshell membrane containing varnish.

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4. DISCUSSION

As shown by the EDX analysis and SEM evaluation, our hypothesis that “novel STMP treated eggshell and eggshell membrane varnish is not as effective as F and bioactive glass varnish at preventing dental erosion produced by the soft drink” was rejected within the limits of this *in vitro* study. The findings obtained in this study suggest that STMP-treated eggshell and membrane powder containing varnish has a protective effect against acid attacks and has the potential to be used against dental erosion.

Excessive consumption of acidic food and beverages is one of the most important etiological factors of dental erosion[19]. Therefore, we found it appropriate to use cola, a very acidic drink, while planning this study. The demineralization formed by the cola treatment on enamel surfaces in the control group can be seen in Figures 3A–3F. However, enamel softened with acidic drinks can regain its hardness after exposure to saliva or artificial saliva[20].

Bioactive glasses can effectively enhance enamel remineralization as an alternative F, even more effectively[21], and there are studies showing that can be used as a dental varnish[22, 23]. In the literature, studies with different forms (solution, slurry, gel) of eggshell or eggshell membrane have shown that it increases remineralization[24-28] and it can be used against dental erosion[29]. In addition, in the STMP-treated egg membrane, biomimetic mineralization has been observed[30]. Therefore, the varnish in which eggshell powder, eggshell membrane, and STMP are combined may have a greater remineralization potential.

The most abundant chemicals elements in enamel are calcium and phosphorus. The Ca/P molar ratio is approximately 1.67 for pure hydroxyapatite, but most biological apatites appear to be “calcium deficient.” The Ca/P molar ratio is higher in enamel than dentine, as expected and in human enamel, it is 1.61[31]. The cariogenic challenge may change the Ca/P ratio; this ratio decreases in progressive lesions and is higher in stagnant lesions known to be more resistant to acid attacks[32]. In our study, the Ca/P ratio decreased due to demineralization that arises from acid attacks in control groups, which was between 1.50–1.52. A higher ratio was observed in the experimental groups. Although a higher Ca/P ratio was observed in the enamel surfaces treated with BV (1.63) and EV (1.63), which is closer to human enamel (1.61) and higher than the FV (1.56) group; however, no statistically significant difference was found between these groups in our study.

Bioactive glass is known to form mineral deposits that act as a protective layer on the enamel surface rich in calcium and phosphate[21]. Due to the SEM examination in the BV group, we also saw a layer in which lamellar, slightly porous precipitate is covering the enamel surface (Figures 3C and 3D). Similarly, in the EV group, there was a layer covering the enamel surface. This layer was even thicker than the BV group but more irregular (Figures 3E and 3F).

Establishing an acid-protective layer on the tooth surface, such as through the use of sealants or polymers, enhancing mechanisms of mineral precipitation by the use of different sources of calcium phosphate, such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and hydroxyapatite particles or polyvalent metal compounds, and the preservation of the organic matrix of dentin by collagenolytic

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enzyme inhibitors like epigallocatechin gallate (EGCG) and a chlorhexidine (CHX) are possible alternatives to the control of dental erosion[33]. However, it should be kept in mind that the results of this *in vitro* study, in which we tested fluoride varnish, bioactive glass varnish, STMP treated eggshell and eggshell membrane containing varnish, may not be generalizable.

5. CONCLUSIONS

In developed countries with the successful prevention of dental caries, dental erosion has become a priority oral health problem. Increasing global opposition to F, which is highly respected by the dental community, has brought alternative erosion prevention methods to the fore. BV and EV are applied to the tooth surface, it has been observed that even if it is exposed to erosive acid attacks, it forms a protective layer on the enamel surface and shows similar calcium phosphate ratios with the enamel structure. Against dental erosion, bioactive glass varnish and sodium-trimetaphosphate treated eggshell and eggshell membrane containing varnish can be used as an alternative to fluoride varnishes.

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E.D. and H.E.Ü. conceived the ideas, T.K. collected the data, H.E.Ü. and İ.Ç. analysed the data, T.K. led the writing, E.D. and H.E.Ü. gave final approval. The authors declare that there is no conflict of interest. This work was supported by the Selçuk University BAP Coordinatorship (grant number: 19401150 and 19401151) and approved by Faculty of Dentistry Ethics Committee at the Selçuk University (grant number: 2019/03-03). The data that support the findings of this study are openly available in Mendeley Data at http://doi.org/10.17632/9z3dhtywxn.1 reference number 34.

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