DOI: 10.5937/bjdm2401038K

BALKAN JOURNAL OF DENTAL MEDICINE

ISSN 2335-0245

Comparative Analysis of the Remineralization Potential of Different Active Ingredients Based Toothpastes using SEM/EDX- An *in vitro* study

Pelin Kukey[1], Cafer Turkmen[1], Zuhre Hale Cimilli[2]

[1] Department of Restorative Dentistry, Faculty of Dentistry, Marmara University, Istanbul, Turkey
[2] Department of Endodontics, Faculty of Dentistry, Marmara University, Istanbul, Turkey

SUMMARY

Background/Aim: *The principal purpose of this study was to investigate the efficacy of different active ingredients to inhibit demineralization around the margins of cervical cavities in natural teeth by scanning electron microscopy and energy dispersive X-Ray elemantal analysis (SEM-EDX).* **Material and Methods:** *Thirty-two sound human molars were used. Box-shaped cavities were prepared along the cemento enamel junction (CEJ). The samples were were immersed in a demineralization solution (pH=1) maintained for 72 hours and randomly divided into 4 groups. Surfaces according to the groups were treated with potassium nitrate, arginine and calciumsodiumphosphosilicate containing remineralization agents for 14 days, respectively and the samples in control group were submitted to toothbrushing with deionized water. The samples were analyzed by using SEM-EDX analysis. Data was statistically analyzed using by one way ANOVA (analysis of variance) test and LSD (least significant difference) test for comparison between means at a significance level of 0.05.* ***Results:*** *SEM-EDX elemental mapping was used to evaluate the degradation from depth profiles of fluoride (F), Calcium (Ca), and phosphate (P) leaching. Micromorphological and elemental analyses were done using SEM and EDX. SEM EDX Analysis on enamel showed a significant difference between the groups except the control group (p<0.05). The dentine results showed significant differences between the control group and all other groups (p<0.05). Elemental analysis showed significant differences in Ca weight percentage among the first and second observation levels in all groups (p<0.05).* ***Conclusions:*** *In conclusion, all tested toothpastes showed some ability to resist demineralization at the margins. The groups except control group showed better outcomes compared with the other tested samples.*

Key words: Remineralization, in vitro, elemental mapping, SEM/ EDX.

ORIGINAL PAPER (OP)
Balk J Dent Med, 2024;38-49

Introduction

The caries process does not involve a one-way demineralization process cycle. This process is based on errors in the succession of demineralization and remineralization cycles[1]. Primarily, demineralization occurs when there is loss of minerals from the dental hard tissues before cavitation occurs. However, remineralization of the lesion occurs when the direction of calcium and phosphate minerals is reversed and they move inwards rather than outwards[2].

The treatment method, which includes a remineralization protocol for early caries lesions, has a great potential for advancement in restorative clinical treatments[3]. It has been claimed that products containing calcium, phosphate and fluoride in their biologically available forms increase remineralization compared to products containing only fluoride, and even that bioactive materials containing fluoride-free calcium phosphate provide results equivalent to the remineralization potential of fluoride-containing materials[4].

Calcium-based toothpaste systems are prepared by reacting minerals using a mechanochemical ball milling process to create a functionalized remineralization agent[5-6]. With the latest developments in nanotechnology, the size of the particles has generally decreased to 0.1 to 100 nm, and some changes in their shape allow to obtain high bioactive calcium, phosphate compounds with a higher potential to penetrate the pores of the demineralized area with the potential for remineralization[7-8]. Scanning Electron Microscopy has been a useful tool for research in dentistry, allowing images to be viewed at high magnification (50X-10,000X and above). It is used in conjunction with Energy dispersive X-ray analysis, which is a microanalytical technique for the quantitative estimation of calcium phosphate ratio[9].

The aim of this study was to evaluate the remineralization capacity of different active ingredients containing toothpastes on artificial subsurface lesions.

Material and Methods

Our study is an in vitro experimental study conducted at Marmara University Faculty of Dentistry. The research project was approved by Marmara University Faculty of Dentistry Ethics Committee, Istanbul, Turkey. Informed consent was obtained before starting the research.

To make our research more reliable and reproducible, a pilot study was conducted with 16 enamel samples to evaluate the remineralization capacity by SEM/EDX analysis. The values obtained from the enamel samples were evaluated by researchers specialized in SEM/EDX Analysis. Forty enamel samples were prepared from extracted human molar teeth using a low speed diamond disc. Teeth with fractures on crowns or roots, carious lesions including white spot lesions and initial caries, determined hypoplastic lesions by visually, the teeth with developmental damage or any deformity, and teeth with any restoration regardless of size were excluded from the study.Prepared enamel samples were analyzed for Ca mineral content (% weight) using SEM-EDX analysis.

The samples were soaked in a demineralizing solution containing 20 ml of acid buffer containing 2 mmol/L Ca^{+2}, 2 mmol/L PO_4^{-3} and 0.075 mol/L acetate at pH 1 for 48 h at 37°C. All samples were analyzed about weight percentage using SEM-EDX on the third day to control of mechanism of mineral content loss. The samples were classified as two groups: Group 1 contained 10 samples (control group) and group 2 contained 30 specimens (study group). The study group was subdivided into three groups of 10 specimens per group. After demineralization procedure, the samples in control group were brushed with distilled water two times a day for two minutes as the optimum time of toothbrushing. The samples in study group were also applied by three different toothpastes twice a day for two minutes during 28 days. The samples in group 2 were brushed with the %8 arginine conmtaining toothpaste (Colgate), the samples in group 3 were brushed with potassium nitrate containing toothpaste (Concentrate Smile), the samples in group 4 were brushed with calciumsodiumphosphosilicate containing toothpaste (Sensosdyne) by the following the same procedure with the control group. The SEM EDX measurements were done twice as after demineralization procedure and after remineralization procedure.

Statistical Analysis

The suitability of numerical variables for normal distribution was tested with the Shaphiro Wilk test. Paired t test was used to compare two dependent measurements of normally distributed variables, and Wilcoxon test was used to compare non-normally distributed variables in two groups. (Table 2 and 3) ANOVA and LSD tests were used to compare normally distributed variables in four groups, and Kruskal Wallis and Dunn tests were used to compare non-normally distributed variables in four groups. (Table 1) *(p<0.05)* SPSS 22.0 <0.05 was considered significant.

Table 1. The one way analysis of variance test evaluation with four groups

	Ca weight 1	Ca weight 2	P
Group 1	8.97 ± 2.12	9.95 ± 0.78	0.289
Group 2	32.68 ± 4.72	35.69 ± 4.55	0.015
Group 3	16.02 ± 3.26	29.39 ± 4.55	0.018
Group 4	16.02 ± 3.26	32.92 ± 2.40	0.043

Table 2. The wilcoxon test evaluation in two groups (Ca weight detected for the first measurement)

	P
Control group-Concentrate smile	0.165
Control group-Sensodyne Protection	0.012*
Control group-Colgate Pro	0.001*
Concentrate smile-Sensodyne Protection	0.262
Concentrate smile-Colgate Pro	0.012*
Sensodyne Protection-Colgate Pro	0.165

Table 3. The wilcoxon test evaluation in two groups (Ca weight detected for the second measurement)

	P
Control group-Concentrate smile	0.001*
Control group-Sensodyne Protection	0.001*
Control group-Colgate Pro	0.001*
Concentrate smile-Sensodyne Protection	0.126
Concentrate smile-Colgate Pro	0.011*
Sensodyne Protection-Colgate Pro	0.223

Results

Our study evaluated the remineralization potential of three different toothpastes based on different remineralizing materials in artificially created carious lesions visually and qualitatively in enamel by SEM-EDX analysis. Energy-dispersive X-ray analysis was used to determine calcium content as a percentage of the weight of demineralized, and remineralized enamel in each group. It shows the remineralization capacities of the pastes and the elemental analysis based on the measurements made after the demineralization and remineralization processes. It also shows the comparison of the alteration in average Ca weight ratios of sound enamel, demineralized and remineralized enamel samples (Figures 1-8).

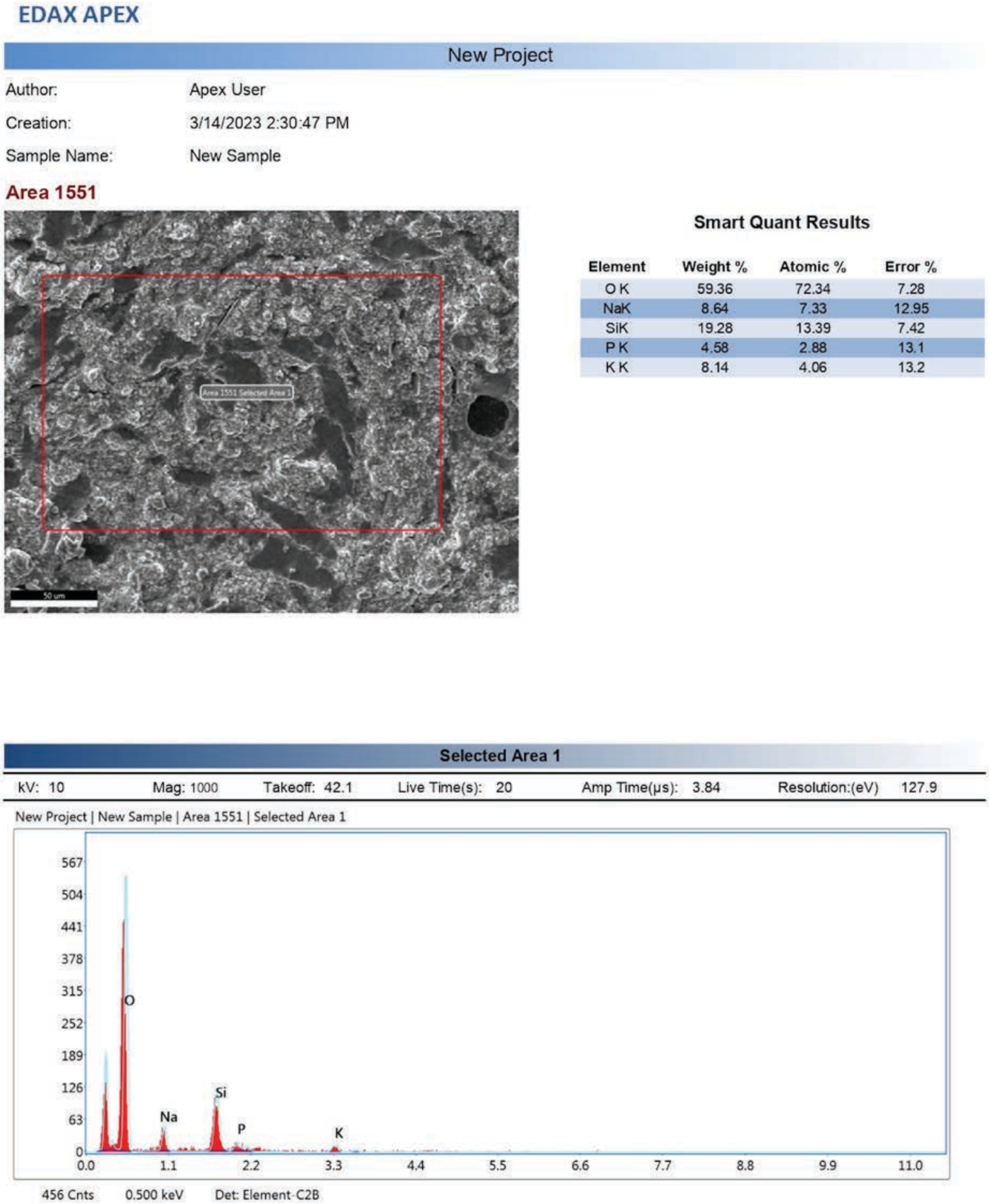

Figure 1. SEM and Elemental analysis of sound enamel sample by EDX (first measurement for group 1)

EDAX APEX

New Project

Author: Apex User

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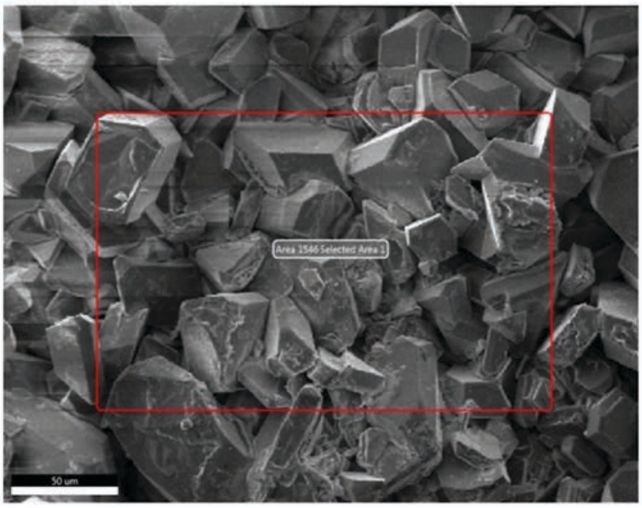
Sample Name: New Sample

Area 1546



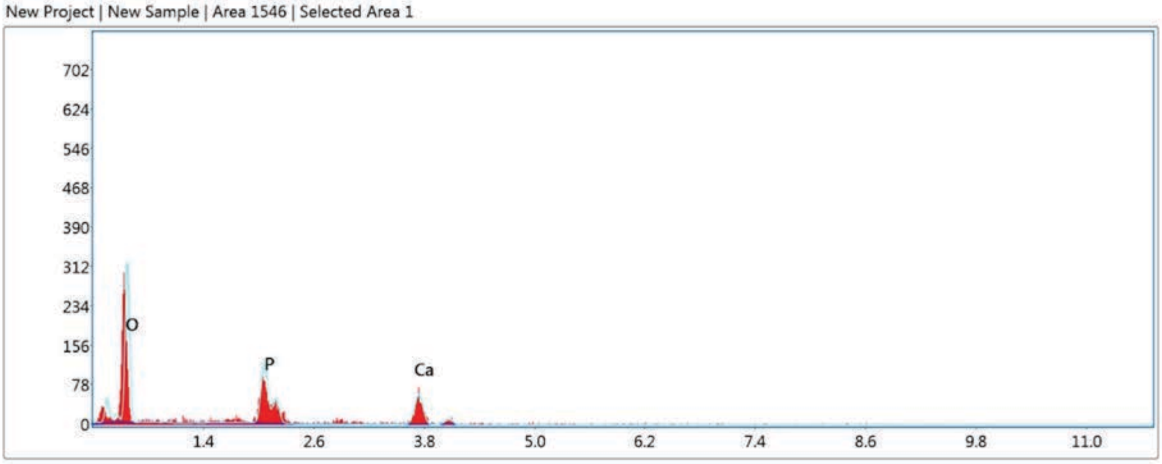
Smart Quant Results

Element	Weight %	Atomic %	Error %
O K	44.78	64.61	11.04
P K	21.18	15.78	5.81
CaK	34.04	19.61	8.36

Selected Area 1

kV: 10	Mag: 1000	Takeoff: 42.1	Live Time(s): 20	Amp Time(μs): 3.84	Resolution:(eV) 127.9

New Project | New Sample | Area 1546 | Selected Area 1



12 Cnts 1.720 keV Det: Element-C2B

Figure 2. SEM and Elemental analysis of sound enamel sample by EDX (first measurement for group 2)

EDAX APEX

New Project

Author:	Apex User
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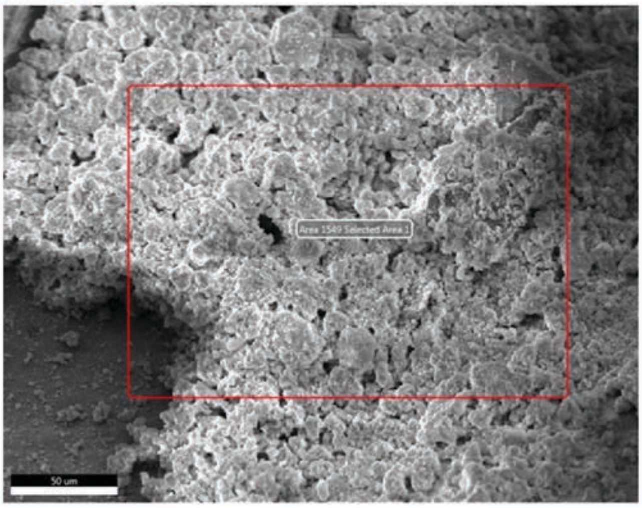
Sample Name:	New Sample

Area 1549



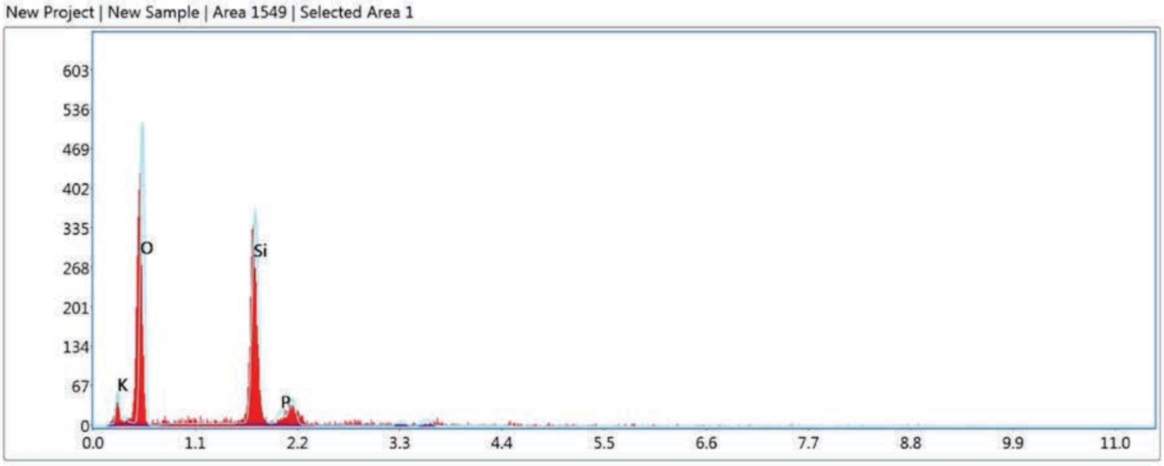
Smart Quant Results

Element	Weight %	Atomic %	Error %
K L	0	0	13.65
O K	47.17	61.27	7.6
SiK	47.62	35.23	4.83
P K	5.22	3.5	9.89

Selected Area 1

kV: 10	Mag: 1000	Takeoff: 42.1	Live Time(s): 20	Amp Time(µs): 3.84	Resolution:(eV) 127.9

New Project | New Sample | Area 1549 | Selected Area 1



42 Cnts 0.250 keV Det: Element-C2B

Figure 3. SEM and Elemental analysis of sound enamel sample by EDX (first measurement for group 3)

EDAX APEX

New Project

Author: Apex User

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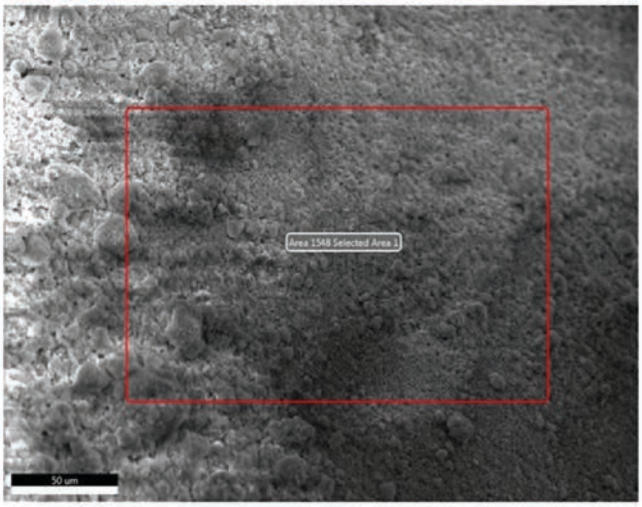
Sample Name: New Sample

Area 1548



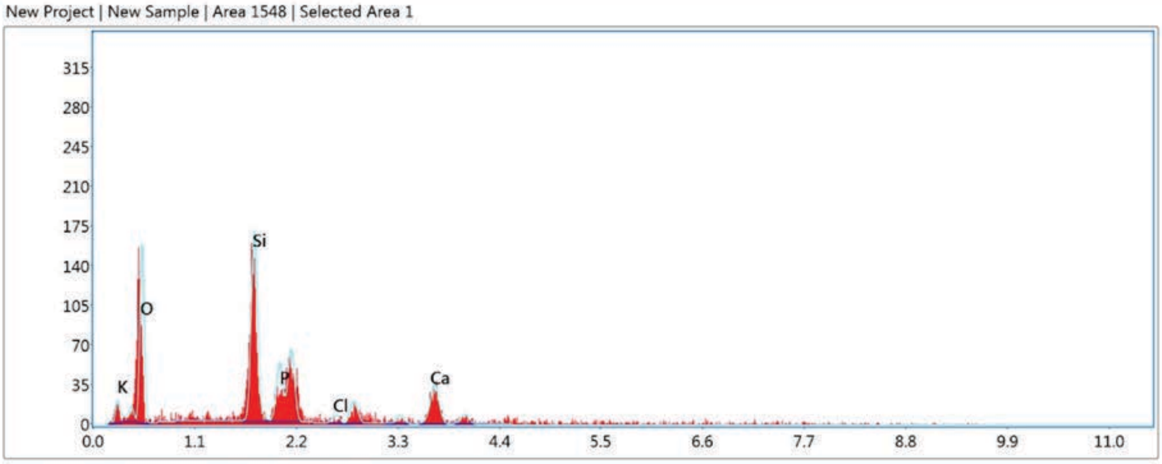
Smart Quant Results

Element	Weight %	Atomic %	Error %
K L	0	0	19.4
O K	30.09	46.81	12.19
SiK	26.97	23.91	6.37
P K	13.23	10.64	7.63
ClK	2.45	1.72	16.88
CaK	27.25	16.93	11.3

Selected Area 1

kV: 10	Mag: 1000	Takeoff: 42.1	Live Time(s): 20	Amp Time(μs): 3.84	Resolution:(eV) 127.9

New Project | New Sample | Area 1548 | Selected Area 1



20 Cnts 0.250 keV Det: Element-C2B

Figure 4. SEM and Elemental analysis of sound enamel sample by EDX (first measurement for group 3)

EDAX APEX

New Project

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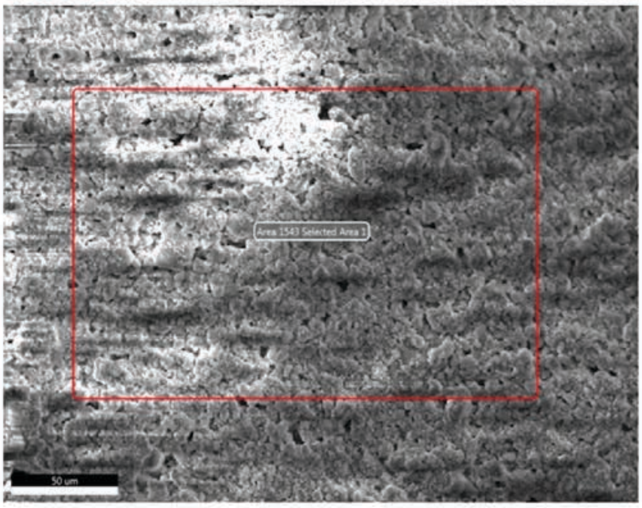
Sample Name:	New Sample

Area 1543



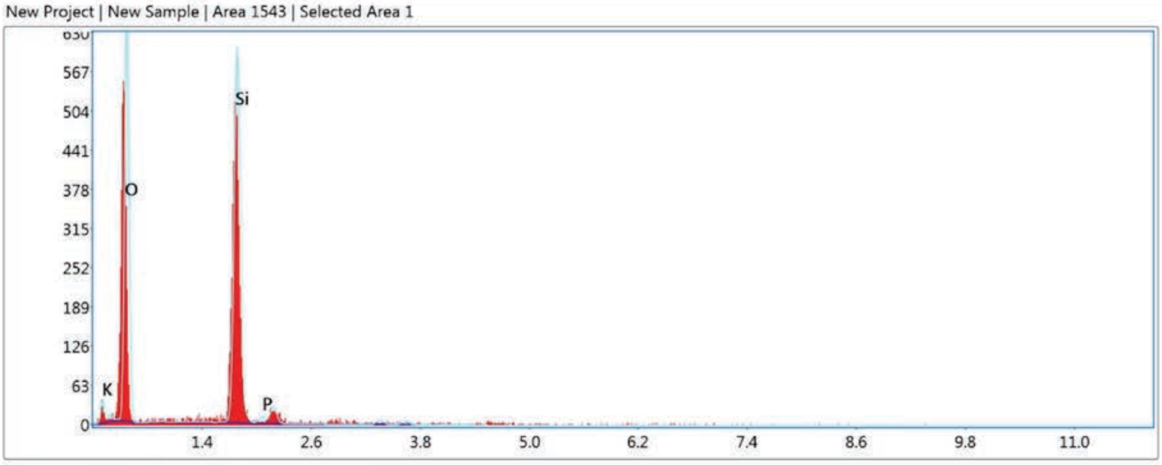
Smart Quant Results

Element	Weight %	Atomic %	Error %
K L	0	0	15.04
O K	44.21	58.24	7.57
SiK	54.23	40.7	4.18
P K	1.56	1.06	15.61

Selected Area 1

kV: 10	Mag: 1000	Takeoff: 42.1	Live Time(s): 20	Amp Time(µs): 3.84	Resolution:(eV) 127.9

New Project | New Sample | Area 1543 | Selected Area 1



6 Cnts 2.020 keV Det: Element-C2B

Figure 5. SEM and Elemental analysis of sound enamel sample by EDX (second measurement for group 1)

EDAX APEX

New Project

Author:	Apex User
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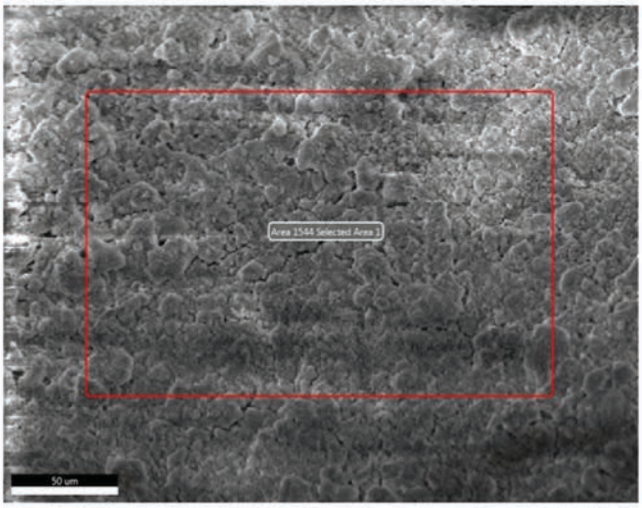
Sample Name:	New Sample

Area 1544



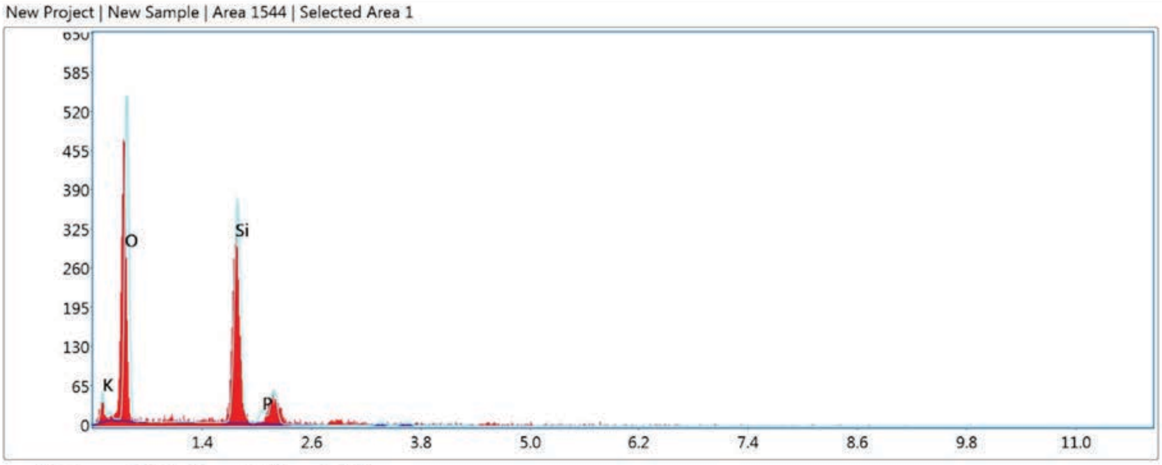
Smart Quant Results

Element	Weight %	Atomic %	Error %
K L	0	0	13.71
O K	47.94	61.97	7.72
SiK	47.44	34.94	4.79
P K	4.62	3.09	9.39

Selected Area 1

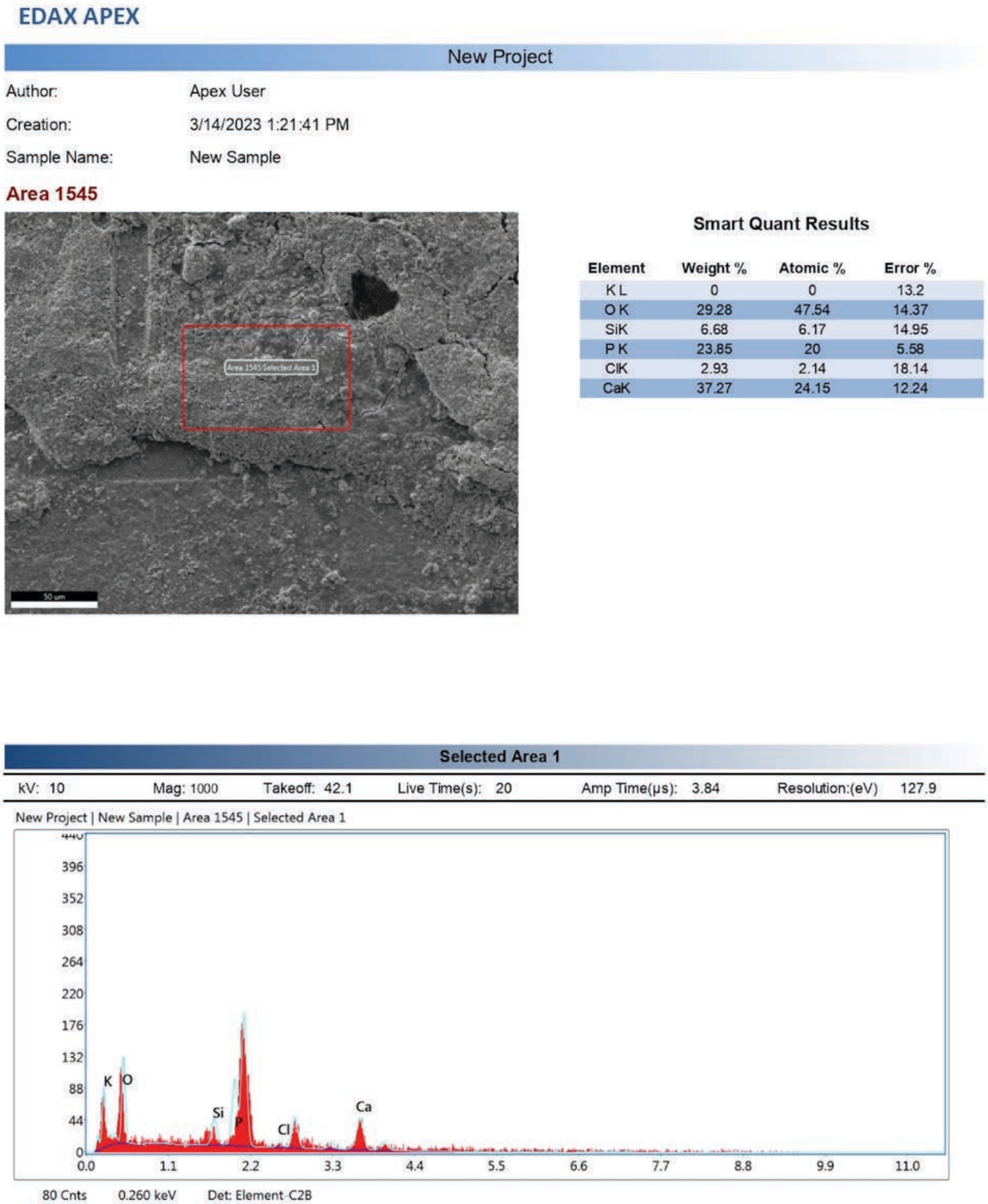
kV: 10	Mag: 1000	Takeoff: 42.1	Live Time(s): 20	Amp Time(µs): 3.84	Resolution:(eV) 127.9

New Project | New Sample | Area 1544 | Selected Area 1



7 Cnts 3.000 keV Det: Element-C2B

Figure 6. SEM and Elemental analysis of sound enamel sample by EDX (second measurent for group 2)



EDAX APEX

New Project

Author: Apex User

Creation: 3/14/2023 1:21:41 PM

Sample Name: New Sample

Area 1545

Smart Quant Results

Element	Weight %	Atomic %	Error %
K L	0	0	13.2
O K	29.28	47.54	14.37
SiK	6.68	6.17	14.95
P K	23.85	20	5.58
ClK	2.93	2.14	18.14
CaK	37.27	24.15	12.24

Selected Area 1

kV: 10 Mag: 1000 Takeoff: 42.1 Live Time(s): 20 Amp Time(μs): 3.84 Resolution:(eV) 127.9

New Project | New Sample | Area 1545 | Selected Area 1

80 Cnts 0.260 keV Det: Element-C2B

Figure 7. SEM and Elemental analysis of sound enamel sample by EDX measurement group 3)

EDAX APEX

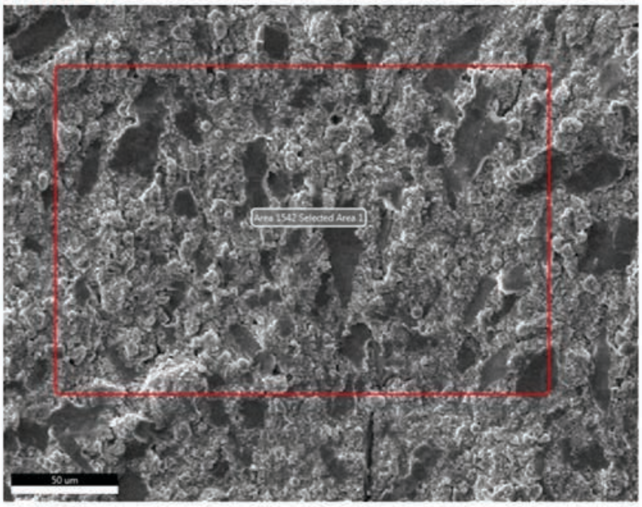
New Project

Author: Apex User

Creation: 3/14/2023 12:59:17 PM

Sample Name: New Sample

Area 1542



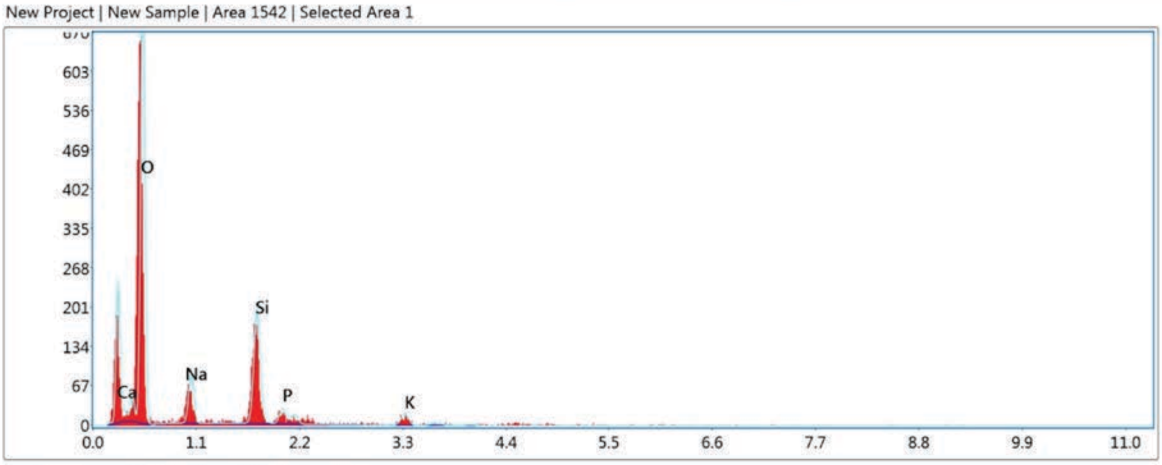
Smart Quant Results

Element	Weight %	Atomic %	Error %
CaL	10.72	5.46	18.92
O K	54.34	69.28	8.25
NaK	8.06	7.15	11.09
SiK	17.64	12.81	6.34
P K	3.51	2.31	11.98
K K	5.73	2.99	19.02

Selected Area 1

kV: 10	Mag: 1000	Takeoff: 42.1	Live Time(s): 20	Amp Time(µs): 3.84	Resolution:(eV) 127.9

New Project | New Sample | Area 1542 | Selected Area 1



18 Cnts 0.370 keV Det: Element-C2B

Figure 8. SEM and Elemental analysis of sound enamel sample by EDX (second measurement for group 4)

Discussion

In our study, the remineralization potential evaluated based on calcium mineral loss according to the measurements done before and after remineralization in artificially created carious lesions in sound enamel tissue, was determined using SEM-EDX. Arginine is a natural peptide found in human saliva. Arginine is secreted in free form with an average distribution of 50 µM. Arginine is also a regulator of structured ammonia metabolism. Many cellular bacteria, including oral streptococci, lactobacilli, and spirochetes, catabolize arginine to ornithine, ammonia, and CO[2]. Ammonia production can alkalize the oral microenvironment and maintain the oral microbial ecology[10]. Many beneficial oral bacteria can convert arginine, the peptide that can buffer acidic pH, into ammonia via the deiminase pathway. Therefore, presenting arginine to the oral microbiome in pastes and similar products is expected to stimulate arginine-sensitive microorganisms, thus increasing the buffering capacity of saliva and reducing acidogenicity in the mouth. In addition, in vivo studies evaluating the effect of arginine on periodontal pathogens are continuing and their progress will be very effective for understanding the arginine metabolism[11].

According to the studies based on the process of potassium nitrate preventing dentine sensitivity in vital teeth, potassium ions, one of its main active components, have been shown to be effective. The depolarization produced by the painful stimulus activates dentin sensory nerve activity, while potassium nitrate reduces the effects of this activity. Such effects may be better observed in clinical or in vivo studies on dentin sensitivity[12-13].

Based on the research conducted by Zawaideh *et al*., products containing Potassium nitrate have been shown to have increased resistance to the acid attacks on both primary and permanent teeth and are recommended for use by both children and adults for prophylaxis[14].

According to the study performed by Freda *et al*., the use of calcium sodium phosphosilicate in percentages up to 5 percent has been determined to be safe in preventing dentin hypersensitivity. But in this study, calciumsodium phosphosilicate is just one of many interventions to minimize dentin sensitivity, including toothpastes containing potassium, fluoride and arginine. All of these have been reported to be effective to varying degrees in the treatment of hipersensitivity treatment. In the study of Freda *et al*., aimed at treating sensitivity, similar results were obtained from the experimental groups containing calcium-sodium phosphosilicate, fluoride and arginine[15]. The toothpastes containing 5% calciumsodiumphosphosilicate has been determined to be optimal as a therapeutic home treatment to relieve dentin sensitivity. It has not been proven that toothpastes containing 15% CSPS continue to be overexpanded after periodontal treatment in the experimental group. In addition, the effectiveness of the high calcium-sodium phosphosilicate amount should be increased in lower daily intensity numbers[15-16]. CSPS can be applied in two different ways: home application or clinical application. The amount of CSPS for home treatment use ranged from 2.5% to 7.5% (optimum %5)[17]. However, the amount of professional distribution orientation is generally higher. Professionally applied prophylaxis paste should be applied by doctors in clinical use. Its use is more complex and aims to minimize sensitivity by preventing blockage in the dentinal tubes[18].

To the study comparing the effectiveness of fluorinol and calcium sodium phosphosilicate in preventing dentin sensitivity, it was shown that the use of fluorinol was more effective than calcium sodium phosphosilicate. In this study, the effectiveness of fluorinol varied over time and reached its maximum level in the 3rd and 4th weeks[16].

According to the metanalysis written by Bruwell *et al*., calciumsodiumphosphosilicate, Novamin as its trade name, has a great remineralization mechanism on the ion transfer in dentinal tubules. Besides that, this article focuses on the biocompatibility of Novamin and the areas that has been safely used like bone regeneration etc[15,19].

Conclusions

The use of micro-particles like arginine, potassium nitrate, calcium sodiumphosphosilicate in toothpastes show ability to increase the remineralization potential of the enamel and acquire a valuable prevention against the superficial subsurface lesions.

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Received on November 2, 2023.
Revised on December 19, 2023.
Accepted on December 20, 2023.

Conflict of Interests: Nothing to declare.
Financial Disclosure Statement: Nothing to declare.

Human Rights Statement: None required.
Animal Rights Statement: None required.

Correspondence

Pelin Kukey
Department of Restorative Dentistry
Faculty of Dentistry, Marmara University, Turkey
e-mail: pelinkukey@marmara.edu.tr