Original Article

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Effects of Different Fluoride-containing Toothpastes on *In Vitro* Enamel Remineralization

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ABSTRACT

Objective: Fluoride toothpaste is one of the most effective cariostatic product when used as a daily fluoride application. The purpose of this *in vitro* study was to evaluate the effect of a new fluoride-containing toothpaste on enamel surface microhardness (SMH) under a pH-cycling regimen.

Methods: Thirty-five sound human enamel samples were randomly divided into five groups (A-E) each containing seven samples as A (fluoride-free control group), B (1000 ppm NaF), C [KNO_3 (5%), 1450 ppm NaF], D (1450 ppm sodium monofluorophosphate), and E (1450 ppm NaF). After inducing caries-like lesions, each group was maintained daily for de- and remineralization cycle for seven days. During this cycle, samples were treated by the selected toothpaste for each group. Enamel mineral loss was assessed by SMH and lesion depth was analyzed by polarized light microscopy (PLM). Surface enamel microhardness was determined on the enamel blocks. SMH recovery (%SMHR) among treatments was analyzed by a two-way ANOVA.

Results: The highest values of %SMHR were observed for the 1450 ppm NaF (group C). NaF toothpastes significantly increased the microhardness of the lesions ($p<0.001$) when compared to control groups. PLM data revealed a mineral precipitation band on the surface layer of all samples but no difference was found between groups in terms of enamel remineralization layers ($p>0.05$). The results suggest that all toothpastes with similar sources/concentrations of fluoride, provide different levels of remineralization.

Conclusion: It can be concluded that new NaF compounds in toothpaste result in a clearly marked remineralization of caries-like enamel lesions.

Keywords: pH cycle, toothpaste, remineralization, demineralization

Introduction

Preventive dentistry is the most preferred research area. Though the progress of *in situ* and *in vivo* research in cariology, laboratory tests are used to examine dental caries, especially the impact of fluoride on prevention of enamel-dentin demineralization and enhancement of remineralization (1-4).

Demineralization, the first step of the decay process with the remineralization process, controls the decay process and reverses the decay. When the acidogenic bacteria reduce pH of the calculus, demineralization occurs. When Ca^{+2} and PO_4 ions in saliva increase pH in calculus, the remineralization process begins. Therefore, demineralized lesions are remineralized. However, when the demineralization is equal to or higher than remineralization, decay occurs (5).

The buffer capacity of the saliva has a great deal with Ca^{+2} and PO_4 amount inside the saliva. The amount of remineralization increases when the fluoride ions are in the saliva. Therefore, studies about the prevention of caries and reversing the decay or the demineralization process concentrate on the effect of fluoride

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ions. In recent *in vivo* and *in vitro* studies, the effect of fluoride on remineralization and demineralization has been researched (5,6).

The pH-cycling test comprises of artificial enamel lesions being treated daily with the products and of cycling in de- and remineralizing solutions to mimic oral pH-fluctuation patterns (7,8).

In studies, pH cycling models provide measurement of the amount of remineralization due to toothpastes containing different concentration of fluoride. The pH cycling model mimics the loss of mineral and the remineralization process, and needs smaller sample dimension and response variables which are performed in pH-cycling models (9,10).

The efficient concentration of Ca^{+2} and PO_4 ions and fluoride in saliva stimulate the formation of hidroxiapetite (with F^- as fluorapetite) and accelerate the remineralization. To the cope with these, fluoride is added to toothpastes, mouth rinses and drinking water.

The fluoride toothpastes are the most essential products used as a fluoride application daily (11,12). Fluoride toothpastes contain fluoride salts, such as NaF and sodium monofluorophosphate (NaMFP) (13).

According to the most researchers, toothpastes involving similar dose fluoride (500-1000 ppm) provide approximately same effect on demineralization; but 500 ppm and below fluoride concentrations are accepted as minimum dose and have minimal effect on demineralization (14,15). Higher dose of fluoride can cause fluorosis, on the other hand the lower dose has the insufficient effect on demineralization (14).

The new toothpastes including different formulas which are biocompatible to tooth structure chemically, decrease demineralization, prevent adhesion of bacteria on teeth, provide remineralization and prevent the sensitivity of dentin (6,16).

The aim of the study is to evaluate the ability of a new NaF and KNO_3-containing toothpaste on *in vitro* enamel surface microhardness (SMH) by a pH-cycling model.

Methods

Enamel Block Preparation

A total of 35 human molar teeth were extracted due to periodontal problems. The soft-tissue debris on the teeth were cleaned and re-inspected for intact surfaces free from caries, hypoplasia and white spot lesions. This study were conducted in 2012 and samples were collected from a biobank and written informed consent was not received due to the nature of this study.

Thirty-five enamel blocks (2x3 mm) which were formed from the extracted human teeth were prepared by using a diamond bur and were kept in 2% formaldehyde solution at pH 7.0 (17). The specimens were embedded in the epoxy resin and the surface of the enamel blocks was grounded flat and was polished to remove 50 µm of the surface layer with 1.2 grit waterproof silicon carbide paper and water-cooled carborundum discs. The prepared samples were submitted to the microhardness test.

Table 1. Toothpastes and fluoride concentration

Toothpastes	Ingredient	Amount
Sensodyne Mint	Fluoride free	-
Colgate® Kids	NaF	1000 ppm
Sensodyne® Pronamel™ for Children	NaF	1450 ppm
Signal White Now	Sodium monofluorophosphate	1450 ppm
Ipana 7	NaF	1450 ppm

F-Toothpaste Evaluation

Since the treatment with the different experimental dentifrices, enamel blocks were selected randomized into five groups each containing seven blocks; for group A; teeth were treated with Sensodyne Mint as the control group (SENSODYNE® MINT; GSK, USA), for group B; teeth were treated with Colgate® Kids (1000 ppm NaF), (Colgate® Kids; Palmolive Co., New York, USA), for group C; teeth were treated with Sensodyne Pronamel for Children (KNO_3 5%, 1450 ppm NaF) (SENSODYNE® PRONAMEL™; GSK, USA), for group D; teeth were treated with Signal WHITE NOW (1450 ppm NaMFP), (Signal WHITE NOW; Lever Faberge, UK) and for group E; teeth were treated with Ipana 7 (1450 ppm NaF), (Ipana 7; Procter&Gamble Co., Cincinnati, Ohio, USA). The amount of F in the experimental toothpaste was displayed in Table 1. After inducing caries-like lesions, each group was applied daily de- and remineralization cycle period for 7 days. After pH cycling, the surface was assessed and the integrated loss of the hardness of subsurface calculated. Artificial caries-like lesions were formed on specimens of intact human enamel with demineralizing solution for 32 hours.

Toothpaste Treatments and the Remineralizing pH-cycling Model

Samples were carried out five pH cycles along 7 days at 37 °C for each group (18). During pH cycling, blocks were put in a demineralization solution [demineralization solution in 75 mmol/L acetate buffer, pH 4.7; 2.2 mL/mm^2; 2.0 mmol/L $Ca(NO_3)_2.H_2O$, 2.0 mmol/L $NaH_2PO_4.H_2O$ and 0.04 µg F/mL (NaF)] for 6 hours and in a remineralization solution [remineralization solution, in 0.1 mol/L cacodylate buffer, 7.0 1.1 mL/mm^2; 1.5 mmol/L $Ca(NO_3)_2.H_2O$, 0.9 mmol/L $NaH_2PO_4.H_2O$, 150 mmol/L KCl and 0.05 µg F/mL (NaF)] for 18 hours. The treatment consisted of 1 minute soak under the agitation in 2 mL/block of toothpaste/deionized water slurries (1:3 w/w) on a daily basis before the solution was changed from demineralization to remineralization or vice versa twice a day. Deionized water was applied before each step (Figure 1). Samples were kept in the remineralization solution for 2 days.

Hardness Analysis

The hardness of the enamel surface was determined before and after pH cycling with a Digital Micro-Vickers Hardness Tester (Wilson Wolpert; Europe BV, 401 MVD, Netherlands) being

used for Surface Microhardness Analysis (SMH). It was fitted with a Vickers diamond and 25 gram load was used to make indentations in the enamel surface. The loaded diamond was allowed to rest on the surface for 10 seconds (19).

Three indentations spaced by 100 μm were formed in different parts of the enamel. SMH was determined at the baseline, after the caries-like lesions were formed (after demineralization) and after pH-cycling and percentage of SMH recovery (%SMHR) was calculated %SMHR=[(SMH3-SMH2)/(SMH1-SMH2)]x100 (SMH1: baseline SMH, SMH2: after 32 hours demineralization application, SMH3: after pH-cycling) (20).

Polarized Light Microscopy Analysis

Sections were mounted on glass-slides and the artificial caries-like lesion depth and the treatments were analyzed in a polarized light microscope (LEICA; Qwin Image Processing and Analyzing, England) as previously detailed (4). Longitudinal sections of 100±10 μm were obtained from the remaining half of each block.

Lesions were grouped in accordance with their morphological appearance after demineralization and after cycling, and as the each category, a numerical index number was designated as follows: no lesion (1), single porosities (2), interrupted lesion band (3), inhomogeneous lesion (4) and completely homogeneous lesion (5) (21).

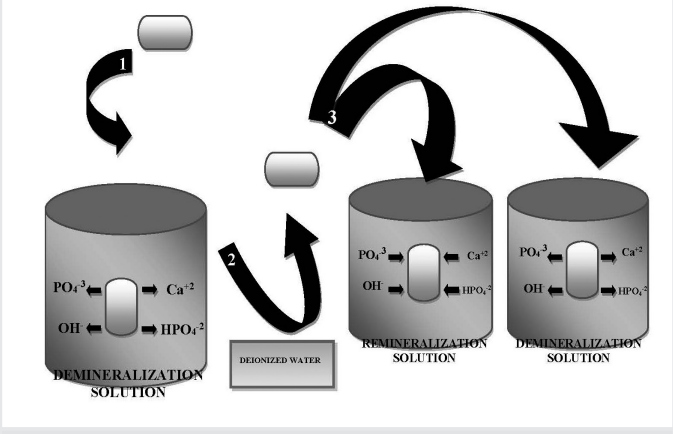

Figure 1. pH cycling model according to the flux of minerals

Statistical Analysis

Statistical analysis was evaluated by using the SPSS 16.0 software for Windows (SPSS Inc., Chicago, IL, USA). The differences between the F-toothpastes and %SMHR were performed by ANOVA. The datas were compared using the Mann-Whitney U test.

Results

The mean and standard deviation values of microhardness of the enamel at the baseline, after demineralization and after pH cycling with five different toothpastes were calculated (Table 2). The mean microhardness in group A was found to be 115.96 at baseline, 42.47 after demineralization and 56.32 after remineralization. The mean microhardness in group D was found to be 97.3 at baseline, 58.86 after demineralization and 74.47 after remineralization. There was no difference between group A and D in terms of mean microhardness at baseline, after demineralization and after remineralization (p>0.05) (Figure 2).

There was rehardening of the carious lesions in all groups (%SMHR). The percentage of %SMHR was shown in Table 3. These datas indicated that the percentages of %SMHRs were 96.48%; 67.03%; 63.39%; 60.15% and 57.77%; for groups C, D, E, B and A respectively. The highest %SMHR was found in group C, but statistically significant difference (p=0.946) was not observed for %SMHR regarding the groups.

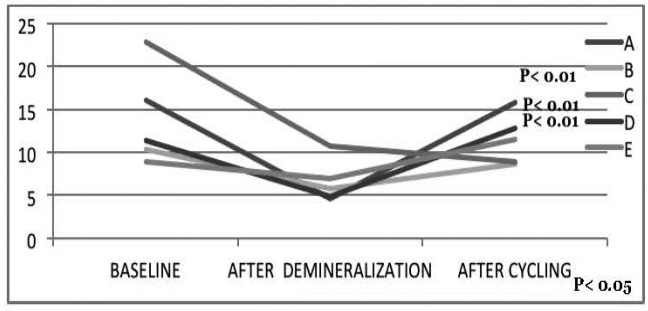

Figure 2. The surface microhardness levels of the enamel "baseline, after demineralization and after pH cycling"

Table 2. The mean and standard deviation values of surface microhardness at baseline, after demineralization and after pH cycling with five different toothpastes

Toothpastes	Baseline SMH Mean ± SD	After demineralization SMH Mean ± SD	After cycling SMH Mean ± SD	p values
Sensodyne Mint (fluoride free)	115.96±5.81	42.47±2.66	56.32±7.54	p>0.05
Colgate® Kids (NaF-1000 ppm)	71.7±5.14	50.02±4.05	69.9±6.43	p<0.001
Sensodyne® Pronamel™ for Children (KNO_3, NaF-1450 ppm)	165.45±8.60	41.24±1.35	150.4±13.37	p<0.001
Signal White Now (NaMF-1450 ppm)	97.3±9.47	58.86±6.53	74.47±6.72	p>0.05
Ipana 7 (NaF-1450 ppm)	107.44±6.31	43.96±1.52	116.32±5.54	p<0.001

SMH: surface microhardness; SD: standard deviation

Table 3. The percentage of surface microhardness recovery (%SMHR)

	%SMHR
Sensodyne Mint (fluoride free)	57.77
Colgate® Kids (NaF-1000 ppm)	60.15
Sensodyne® Pronamel™ for Children (KNO_3, NaF-1450 ppm)	96.48
Signal White Now (NaMF-1450 ppm)	67.03
Ipana 7 (NaF-1450 ppm)	63.39

SMHR: surface microhardness recovery

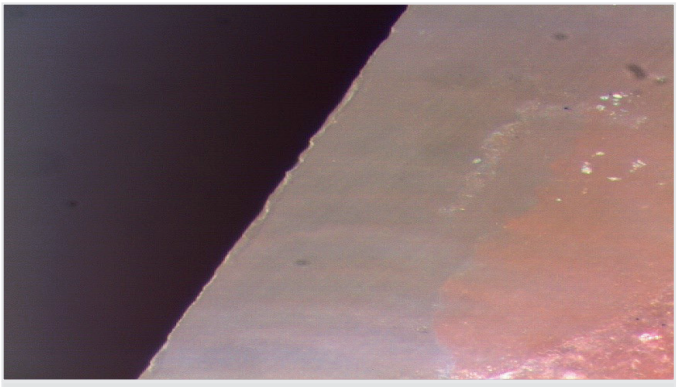

Figure 3. Remineralization effect of toothpaste in group A

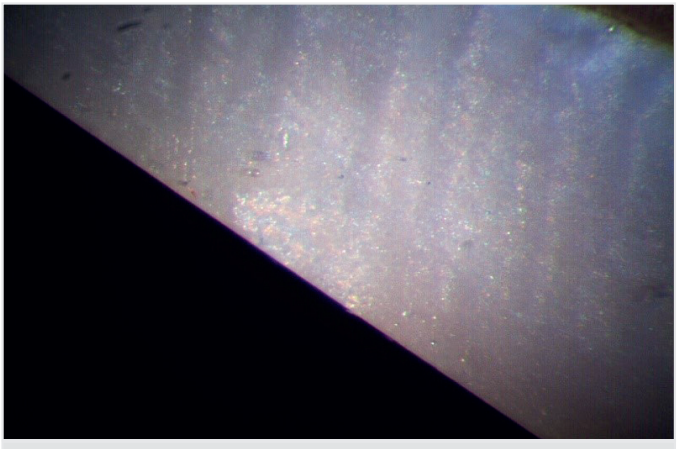

Figure 4. Remineralization effect of toothpaste in group B

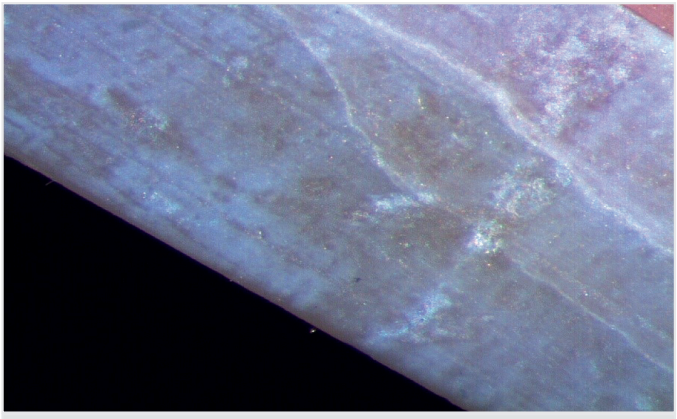

Figure 5. Remineralization effect of toothpaste in group C

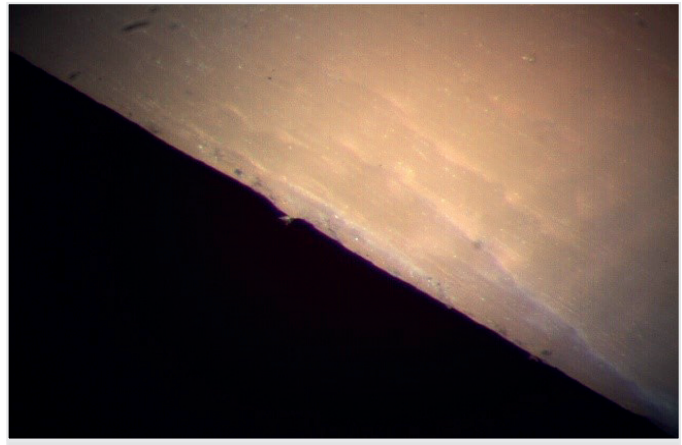

Figure 6. Remineralization effect of toothpaste in group D

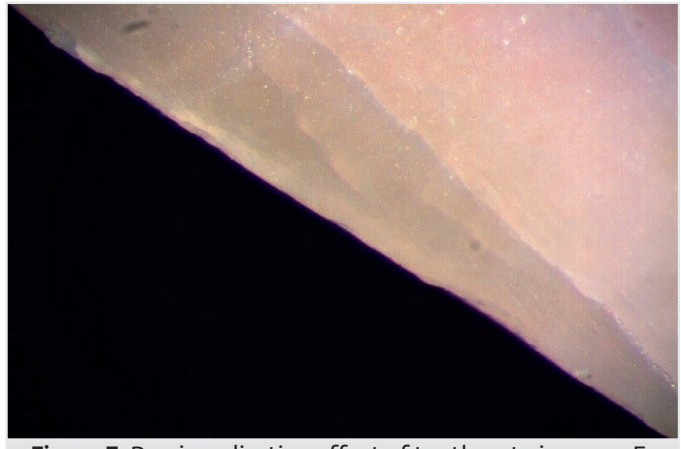

Figure 7. Remineralization effect of toothpaste in group E

Polarized light microscope analysis showed the recovery of the enamel surface hardness according to the toothpastes (Figures 3-7).

The irregular enamel surface sign after demineralization and remineralization and after pH cycling regimen were displayed in groups (Figures 8 and 9).

After demineralization; the morphological analysis by using polarized light microscopy showed interrupted bands or inhomogeneous lesions whereas the lesions expressed as single porosities or interrupted lesion bands after pH cycling with five different toothpastes (Table 4). There were no significant differences between the groups ($p>0.05$).

Discussion

The present study has demonstrated that fluoride toothpastes vary in their capability of enhancing remineralization potential as determined using an established *in vitro* 7 days pH cycling model.

Teeth brushing with F-toothpastes was first used to evaluate the dose-response effect of F on enamel. Recently, the effect of the F on enamel has been identified. However the fluoride toothpastes should be used in natural conditions to prove its usefulness. Therefore, pH cycle regimens were introduced to provide suitable media (4).

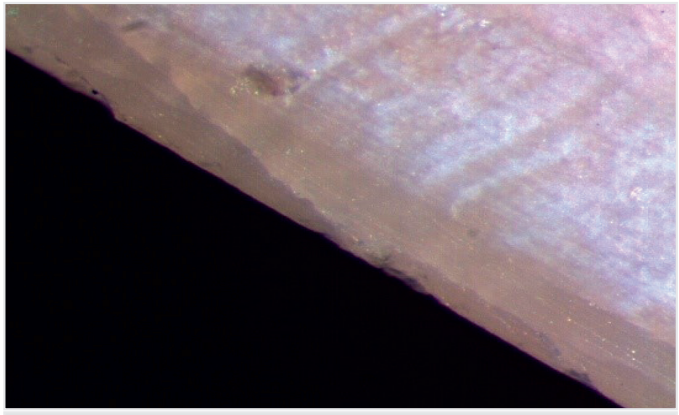

Figure 8. Toothpaste C [KNO_3 (5%), NaF-1450 ppm]: after demineralization

Figure 9. Toothpaste C [KNO_3 (5%), NaF-1450 ppm]: after pH cycling

Table 4. Number of lesion categories after demineralization and after pH cycling and morphological code numbers in the different groups

	After demineralization					After pH cycling				
	1	2	3	4	5	1	2	3	4	5
Sensodyne® Pronamel™ for Children	-	-	1	5	1	-	3	4	-	-
Sensodyne® Mint	-	-	4	3	-	-	-	1	6	-
Signal White Now	-	-	4	2	1	-	1	5	1	-
Ipana 7	-	-	2	4	1	-	-	3	3	1
Colgate® Kids	-	-	-	4	3	-	-	4	3	-

The response variables that can be employed in pH-cycling models are more sensitive than those using in the clinical situation. pH-cycling studies are intended to be extrapolated for the clinical situations. The short period of pH-cycling may produce results that inadequately display the natural process of de- and remineralization. The factors that influence the length of the pH-cycling are the fluoride concentration of the de- and remineralizing solutions (22).

Furthermore Newby et al. (23) demonstrated the importance of formulation effects on driving performance in *in vitro* models.

The findings that the NaMFP toothpaste, which has a definite protective effect, showed less remineralizing efficacy than NaF was not unexpected because pH cycling model consists of only an inorganic solution (24,25). The model choosen to mimic remineralization events is not adequate to estimate the anticaries potential of toothpastes containing NaMFP, because its hydrolysis occurs by phosphatase enzymes, this produced unfavorable results for group C.

The new NaF toothpaste (C-KNO_3, NaF 1450 ppm) showed in this model demonstrated the importance of fluoride compound and formulation excipients on driving remineralization *in vitro*.

KNO_3 helps reducing tooth sensitivity and it has a neutral pH and a low abrasivity (26). Using an *in situ* erosion remineralization model and a microhardness test, Zero et al. (27) concluded that fluoride toothpaste containing KNO_3 dramatically enhanced the remineralization of enamel.

Newby et al. (23) showed that a 1150 ppm NaF test toothpaste protected enamel specimens better (with higher SMH) than a 1100 ppm NaF and a fluoride-free samples at both 10 days and 20 days ($p<0.05$).

Allegrini et al. (28) used polarized light microscopy to determine bone formation in the presence of hydroxyapatite in their study. Similar to our study, Arnold et al. (29) used polarized light microscopy to evaluate crystalline layer of enamel after applying fluoridated milk in their study.

This study demonstrated that fluoride toothpastes can increase the protection of enamel. The present studies also demonstrate the importance of formulation effects on driving performance *in vitro* models.

The *in vitro* model described in the present study should be further used to investigate the effect of enamel SMH of toothpastes.

Study Limitations

This study has no limitations.

Conclusion

This study suggests that the pH-cycling models are enough for studying effect of fluoride on enamel *in vitro* by measuring the change in SMH or performing polarizing microscopy analysis. The average of changes in SMH with KNO_3 containing toothpaste was higher than with other toothpastes.

Ethics

Ethics Committee Approval: The study were conducted in 2012 and samples were collected from a biobank.

Informed Consent: Written informed consent was not received due to the nature of this study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.H., G.Ö.Y., Concept: Z.H., G.Ö.Y., B.K., Design: Z.H., G.Ö.Y., B.K., Data Collection or Processing: Z.H., G.Ö.Y., Analysis or Interpretation: Z.H., G.Ö.Y., Literature Search: Z.H., Writing: Z.H.

Conflict of Interest: No conflict of interest was declared by the authors.

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