DOI: 10.14235/bs.2018.1810

Manuscript Type: Original Article

Turkish Title: Farklı Fluoridli Diş Macunlarının Mine Remineralizasyonu Üzerine Etkileri: In Vitro

Turkish Running Head: Farklı Fluoridli Diş Macunlarının Mine Remineralizasyonu Üzerine Etkileri

Title: Effects of Different Fluoride- Containing Toothpastes on Enamel Remineralization in Vitro

Running Head: Effects of Different Fluoride- Containing Toothpastes on Enamel Remineralization

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Cite this article as: Hatipoğlu Z, Yavlal Özbay G, Kargül B. Effects of Different Fluoride- Containing Toothpastes on Enamel Remineralization in Vitro. Bezmialem Science 2018. DOI: 10.14235/bs.2018.1810

Öz

Amaç: Fluoridli diş macunu, günlük fluorid uygulaması olarak kullanıldığında, en etkili diş çürüğü oluşumunu azaltan ürünlerden biridir. Bu çalışmada yeni bir fluoridli diş macununun diş minesinin yüzeysel mikrosertliği (YMS) üzerindeki etkisinin in vitro pH döngüsü şartları altında değerlendirilmesi amaçlanmıştır.

Yöntemler: Otuzbeş sağlam insan diş minesi, rastgele A-E olarak adlandırılan 5 grupta 7 ayrı örnek içermektedir. Grup A fluordan yoksun, Grup B 1000ppm NaF, Grup C Potasyum nitrat(%5)ve 1450ppm NaF, Grup D 1450ppm SodyumMonofluorofosfat ve Grup E 1450ppm NaF içermek üzere hazırlanmıştır. Örneklerde çürük benzeri lezyonlar oluşturduktan sonra her grupta günlük demineralizasyon ve remineralizasyon döngüsü 7 gün boyunca devam ettirilmiştir. Bu döngü esnasında her gruptaki örneklere seçilmiş değişik diş macunu uygulanmıştır. Diş minesinden mineral kaybı, örnek mine bloklarının yüzeyinin mikrosertliği ölçülmüş ve çürük benzeri yapay lezyon derinliği ve yüzeyi polarize ışık mikroskopu(PIM) ile analiz edilmiştir. Yüzey mikrosertlik geri kazanımı (%YMGK)iki-yollu ANOVA ile değerlendirilmiştir.

Bulgular: En yüksek %YMGK 1450 ppm NaF içeren Grup C örneklerinde görülmüştür. NaF içeren diş macunları uygulanması sonucunda mikrosertlik artışı kontrol grubu ile karşılaştırıldığında istatistiksel açıdan anlamlı bulunmuştur ($P<0,001$). PIM ile yapılan ölçüm verileri ile örneklerin diş minesi üzerinde bir mineral çöküntü tabakası görülmüş fakat minedeki remineralizasyon tabakası arasında bir fark görülmemiştir ($P>0.05$). Sonuçlar benzer kaynaklı ve konsantrasyonlu fluorid içeren diş macunlarının değişik derecede remineralizasyona neden olduğunu göstermiştir.

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Sonuç: Sonuç olarak yeni tip NaF içerikli diş macunlarının diş minesinde oluşturulan yapay çürük lezyonlarında remineralizasyonu sağladığı net olarak görülebilmektedir.

Anahtar Kelimeler: pH siklus, Diş Macunu, Remineralizasyon, Demineralizasyon

Abstract

Objective: Fluoride toothpaste is one of the most effective cariostatic products when used as a daily fluoride application. The aim of this in-vitro study is to evaluate the influence 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 group having seven samples as follows: A (fluoride-free control group), B (1000ppmNaF), C (Potassium nitrate (5%),1450ppm NaF), D (1450ppm SodiumMonofluorophosphate), and E (1450ppm NaF). After inducing caries-like lesions, each group was maintained daily for demineralization-remineralization cycle for seven days. During this cycle, samples were treated by the selected toothpaste for each group. Enamel mineral loss was assessed by surface microhardness and lesion depth was analyzed by Polarized Light Microscopy (PLM). Surface enamel microhardness was determined on the enamel blocks. Surface microhardness 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 toothpastes; however, when compared to treated lesions, no statistical difference among the groups (*P*>0.05) were found. The results suggest that toothpastes with similar sources/concentrations of fluoride, obtain different levels of remineralization.

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

Keywords: pH cycle, Toothpaste, Remineralization, Demineralization

INTRODUCTION

Preventive dentistry is the one of the most preferred research subjects. In many of *in situ* and *in vivo* research projects in cariology, laboratory tests are used to examine dental caries, with a focus on the impact of fluoride (F) on prevention of enamel-dentin demineralization and remineralization.[1, 2, 3, 4]

Demineralization is the first step in dental decay process, while remineralization controls and reverses the decay process. Demineralization occurs when the acidogenic bacteria reduce pH of the calculus. On the other hand, when Ca^{+2} and PO_4 ions in saliva increase the pH in calculus,

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remineralization process begins. This allows demineralized lesions to become remineralized. However, when demineralization is equal to or higher than remineralization, decay occurs.[5]

The buffer capacity of the saliva depends on the concentration levels of its Ca^{+2} and PO_4 ions. The amount of remineralization increases when the fluoride ions are in the saliva. Therefore, studies that deal with the prevention of caries and reversing the decay or the demineralization process are focused on the role of fluoride ions. In recent *in vivo* and *in vitro* studies, the effect of fluoride on remineralization and demineralization has been researched.[5, 6]

In pH-cycling test, artificial enamel lesions are treated with oral hygiene products in demineralization and reminieralization cycles to mimic oral pH-fluctuation patterns.[7, 8]

In studies, pH cycling models provide a means to measure the amount of remineralization of toothpastes that contain different concentrations of fluoride. The pH cycling model mimics the demineralization and remineralization procedures, that need smaller sample dimensions and response variables that are performed in pH-cycling models.[9, 10]

When combined with fluoride, Ca^{+2} and PO_4 ions can stimulate the formation of hydroxyapetite (with F^- as fluorapetite) and accelerate remineralization. That is why fluoride is added to toothpastes, mouth rinses and drinking water.

Fluoride toothpastes are one of the most essential products for daily fluoride application.[11, 12] They contain fluoride salts, such as sodium fluoride (NaF) and sodium monofluorophosphate (NaMFP).[13] According to most researchers, toothpastes that contain a similar dose of fluoride (500-1000 ppm) provide approximately the same effect on demineralization; however, fluoride concentrations of 500 ppm and under are accepted as minimum dose and have minimal effect on demineralization.[14, 15]

Higher doses of fluoride can causefluorosis, while lower doses can have insufficient effect on demineralization.[14]

The new toothpastes including different formulas which are biocompatible to tooth structure chemically, decrease demineralization, prevent adhesion of bacteria's 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 potassium nitrate (KNO3)-containing toothpaste on in-vitro enamel surface microhardness (SMH) by a pH-cycling model.

MATERIAL AND 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 inspected for intact surfaces that are free from caries, hypoplasia, and white spot lesions. The study protocal was reviewed and approved by an Independent Ethics Committee and the consents of patients were taken.

35 enamel blocks (2x3mm) were formed from extracted human teeth using a diamond bur and 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 were grounded flat. Later the surface of enamel blocks were polished using a 1,2 grit waterproof silicon carbide paper and water-cooled carborundum discs so that 50µm of the surface layer was removed. The prepared samples were submitted to the microhardness test.

F- Toothpaste evaluation

After treatment with different experimental dentifrices, enamel blocks were randomly selected into five groups of seven; in group A: teeth were treated with Sensodyne Mint as the control group (SENSODYNE® MINT; GSK, USA); in group B: teeth were treated with Colgate® Kids (1000 ppm NaF), (Colgate® Kids; Palmolive Co., New York, USA); in group C: teeth were treated with Sensodyne Pronamel for Children (KNO3 5%, 1450 ppm NaF) (SENSODYNE® PRONAMEL™®; GSK, USA); for group D: teeth were treated with Signal WHITE NOW (1450 ppm Sodiummonofluorophosphate), (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 is displayed in Table 1. After inducing caries-like lesions, daily demineralization and remineralization cycles were applied for 7 days. After pH cycling, the surface was assessed and the integrated loss of subsurface hardness calculated. Artificial caries-like lesions were formed on specimens of intact human enamel after demineralizing solution was applied for 32 hours.

Toothpaste treatments and the remineralizing pH-cycling model

Samples were applied to five pH cycles along 7 days at 37° C.[18] As part of the 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 included a bi-daily 1-minute soak in 2 ml/block of toothpaste/deionized water slurries (1:3 w/w) under agitation daily before the solution was changed

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from demineralization to remineralization or vice versa. 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 via the Surface Microhardness Analysis (SMH) before and after pH cycling with a Digital Micro-Vickers Hardness Tester (Wilson Wolpert; Europe BV, 401 MVD, Nedherland).The Digital Micro-Vickers Hardness Tester was fitted with a Vickers diamond and 25 gram load was used to make indentations on the enamel surface. The loaded diamond was allowed to rest on the surface for 10 seconds.[19]

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

Polarized light microscopy analysis

Sections were mounted on glass-slides and the depth of artificial caries-like lesions were analyzed before and after treatment 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 cycling. a numerical index number was designated to each category as follows: no lesion (1), single porosities (2), interrupted lesion band (3), inhomogeneous lesion (4) and completely homogeneous lesion (5).[21]

Statistical analysis

Statistical analysis was evaluated by using the SPSS 16.0 software for Windows (SPSS Inc., Chicago, IL, USA). ANOVA was performed to analyze thedifference between the F-toothpastes and %SMHRThe data were compared using the Mann-Whitney test.

RESULTS

The mean and SD values were calculated for microhardness of the enamel at the baseline, after demineralization and after pH cycling with five different toothpastes (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. While the mean of microhardness in Group D was found to be 97.3 at baseline, 58.86 after demineralization and 74.47 after remineralization. The mean of microhardness was not found to be significantly different for groups A and D at baseline and after demineralization. Similarly, no significantly difference was found for groups A and D f between demineralization and remineralization stages (P>0.05). (Figure 2)

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

Polarized Light Microscope analysis showed the recovery of the enamel surface hardness according to the toothpastes (Figure 3-7).

Irregular enamel surface after demineralization and remineralization after pH cycling regimen were displayed in groups in Figure 8 and 9. The morphological analysis that was carried out by PLM showed interrupted bands or inhomogeneous lesions after demineralization; however, lesions that underwent Ph cycling with five different toothpastes were expressed as single porosities or interrupted lesion bands(Table 4). There were no significant differences among the groups ($P>0.05$).

DISCUSSION

The present study has demonstrated that fluoride toothpastes vary in their capability of enhancing remineralization potential via and established in vitro 7 days pH cycling model.

Brushing with F-toothpaste was first used to evaluate the dose-response effect of F on enamel. Recently the effect of the F ion on enamel has been identified. However, the fluoride toothpaste should be used in natural conditions to prove its usefulness. Therefore, regiments of pH cycle were introduced to provide suitable media.[4]

The response variables that can be employed in pH-cycling models are more sensitive than those used in clinical situations. pH-cycling studies are intended to be extrapolated for 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 C.S. et al. demonstrated the importance of formulation on performance of *in vitro* models.[23]

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The finding 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] Choosing the model that mimics remineralization events is not adequate to estimate the anticaries potential of toothpastes containing sodium monofluorophosphate, because hydrolysis of samples occurs by phosphatase enzymes, as shown by the unfavorable results for group C.

The new NaF toothpaste (C- KNO3, NaF 1450 ppm) showed in this model demonstrates the importance of fluoride compound and formulation excipients on driving remineralization in vitro. Potassium nitrate helps reducing tooth sensitivity and it has a neutral pH as well as a low abrasivity.[26] Using an in situ erosion remineralization model and a microhardness test, Zero D. et al. said fluoride toothpaste containing potassium nitrate dramatically enhanced the remineralization of enamel.[27] Newby CS. showed that a 1150 ppm NaF toothpaste protected enamel specimens more than a 1100 ppm NaF toothpaste and fluoride-free samples at both 10 days and 20 days as indicated by the greater SMH ($p < 0.05$).[23]

Allegrini S. et al. used polarized light microscopy to determine bone formation in the presence of hydroxyapatite in their study.[28] Similar to our study, Arnold WH. et al. used polarized light microscopy to evaluate crystalline layer of enamel after applying fluoridated milk in their study.[29] This study demonstrated that fluoride toothpastes can increase the protection of enamel. The present study also demonstrates the importance of formulation effects on driving performance in *in vitro* models.

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

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CONCLUSION

Our findings suggest that Fluoride varnish is effective in remineralizing the early enamel caries at the surface level. There is significantly difference between Fluoride-free toothpaste and fluoride-containing toothpaste on remineralisation potential of the surface of enamel. The average of changes of surface microhardness of potassium nitrate containing toothpaste was higher than other toothpastes.

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Table 1. Toothpastes and fluoride concentration

TOOTHPASTES	INGREDIENT	AMOUNT
Sensodyne Mint(A)	Fluoride free	-
Colgate® Kids (B)	NaF	1000 ppm
SENSODYNE® PRONAMEL™ ® for Chidren (C)	NaF	1450 ppm
Signal WHITE NOW (D)	Sodium Monofluorophosphate	1450 ppm
Ipana 7 (E)	NaF	1450 ppm

Table 2. The Mean and SD Surface Microhardness Values at Baseline, After Demineralization and After pH Cycling with Five Different Toothpastes

TOOTHPASTES	Baseline SMH mean ± SD	After Dem SMH mean ± SD	After Cycling SMH mean ± SD	P
A (Fluoride free)	115.96 ± 5.81	42.47 ± 2.66	56.32 ± 7.54	p>0.05
B (NaF- 1000 ppm)	71.7 ± 5.14	50.02 ± 4.05	69.9 ± 6.43	p < 0.001
C (KNO3, NaF-1450 ppm)	165.45 ± 8.60	41.24 ± 1.35	150.4 ± 13.37	p < 0.001
D (NaMF-1450 ppm)	97.3 ± 9.47	58.86 ± 6.53	74.47 ± 6.72	p>0.05
E(NaF- 1450 ppm)	107.44 ± 6.31	43.96 ± 1.52	116.32 ± 5.54	p < 0.001

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

	%SMHR
A (F -)	57.77
B (NaF- 1000 ppm)	60.15
C (KNO3, NaF-1450 ppm)	96.48
D (NaMF-1450 ppm)	67.03
E (NaF-1450 ppm)	63.39

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 Chidren			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	

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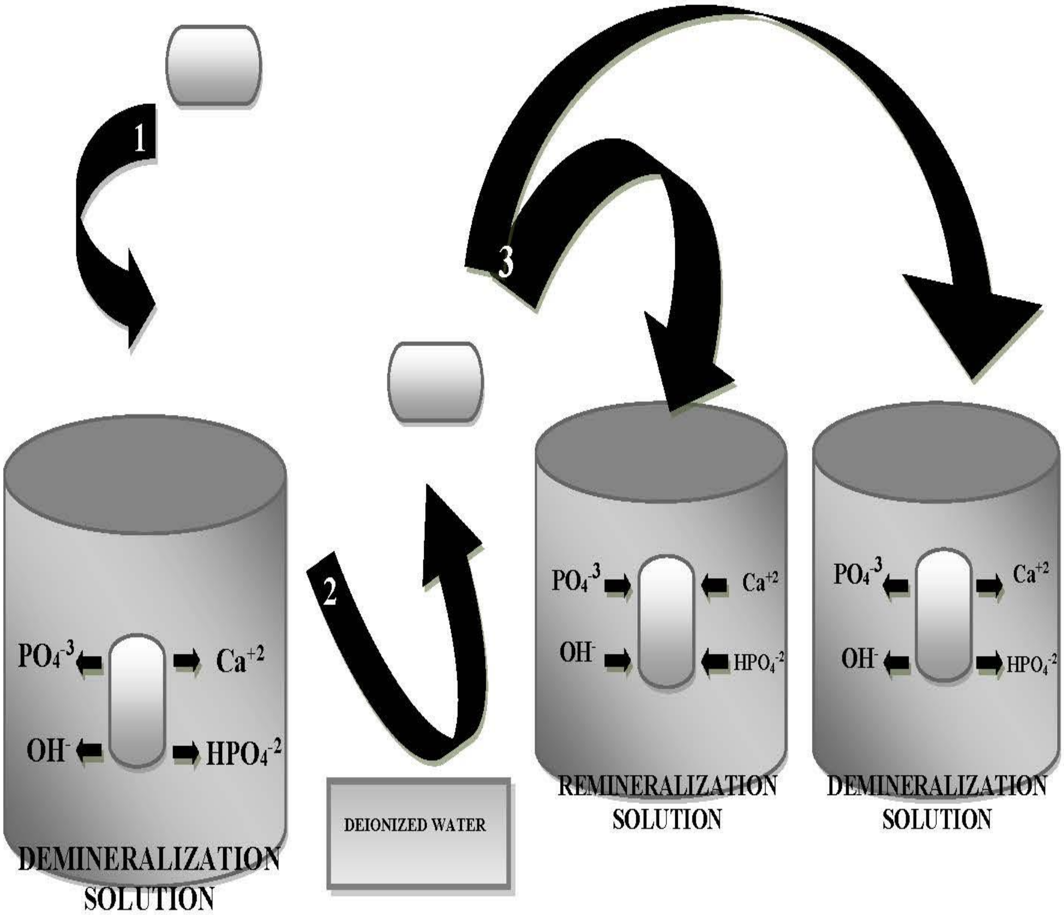

Figure 1: pH cycling model according to the flux of minerals

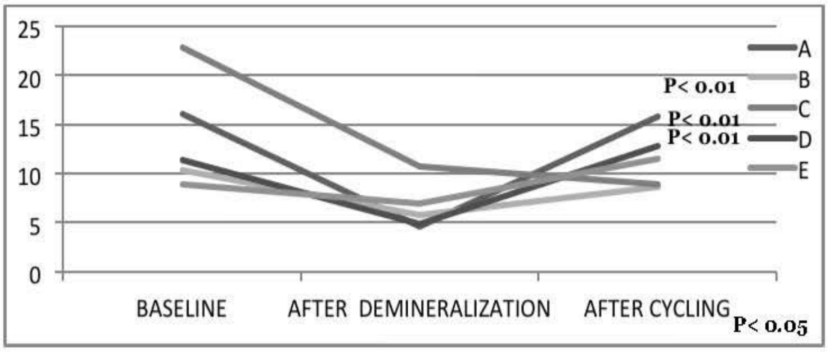

Figure 2: The SMH Levels of the Enamel 'Baseline, After Demineralization and After pH Cycling '

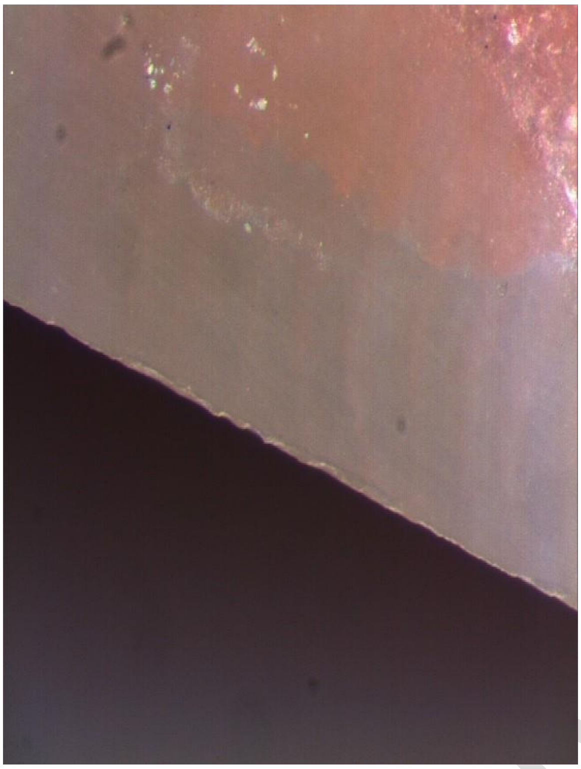

Figure 3

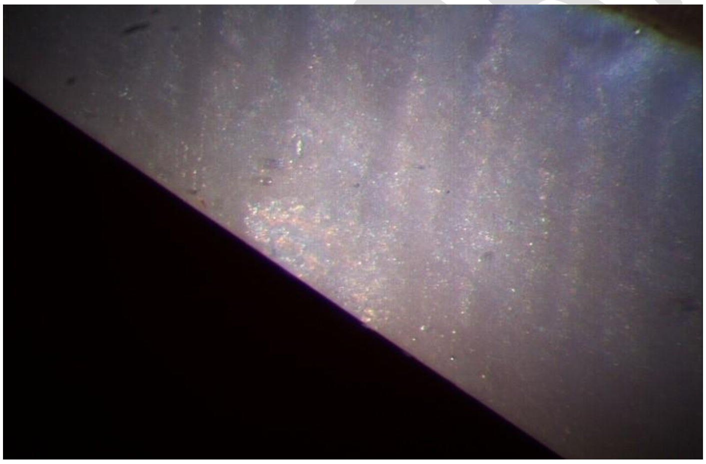

Figure 4

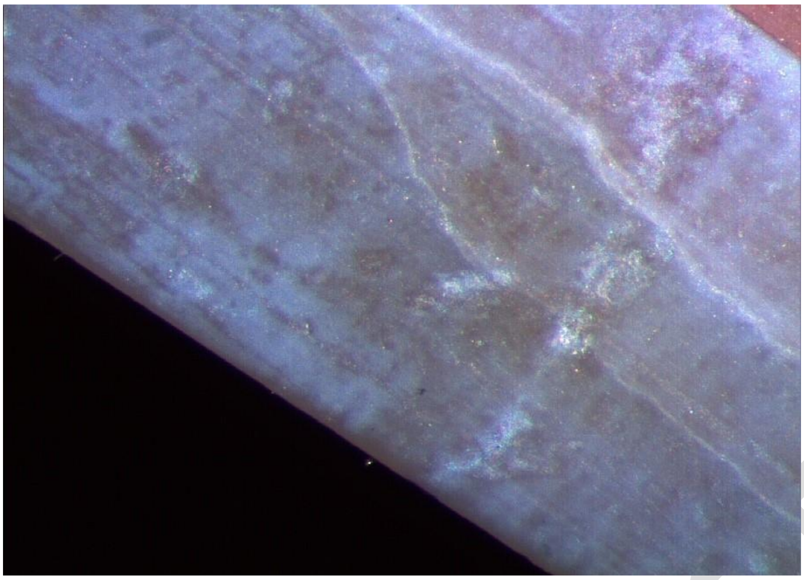

Figure 5

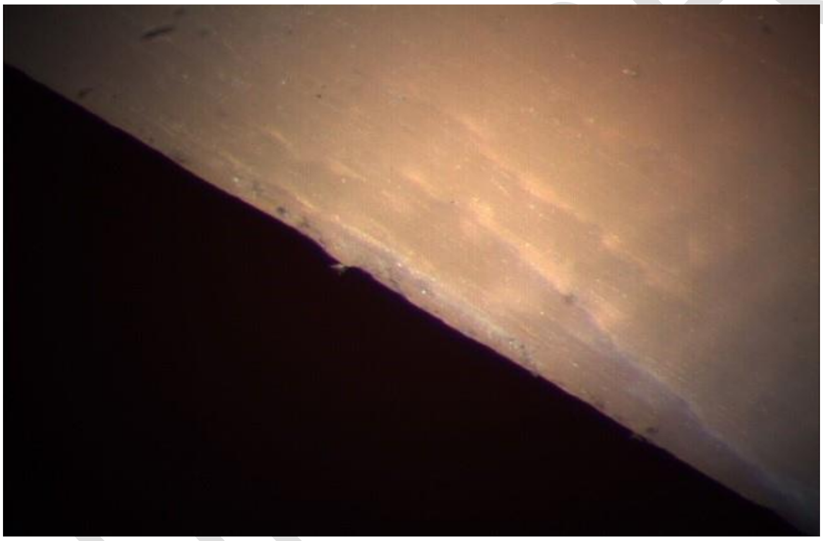

Figure 6

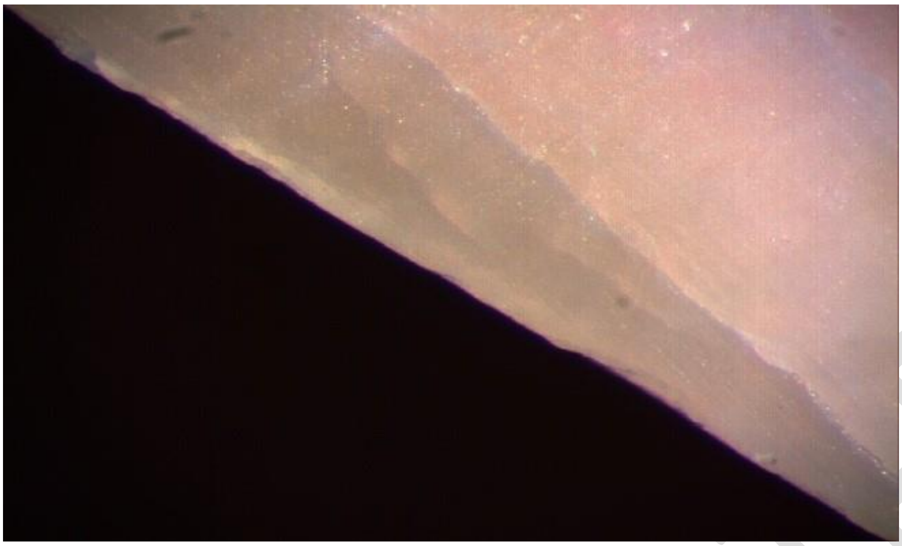

Figure 7

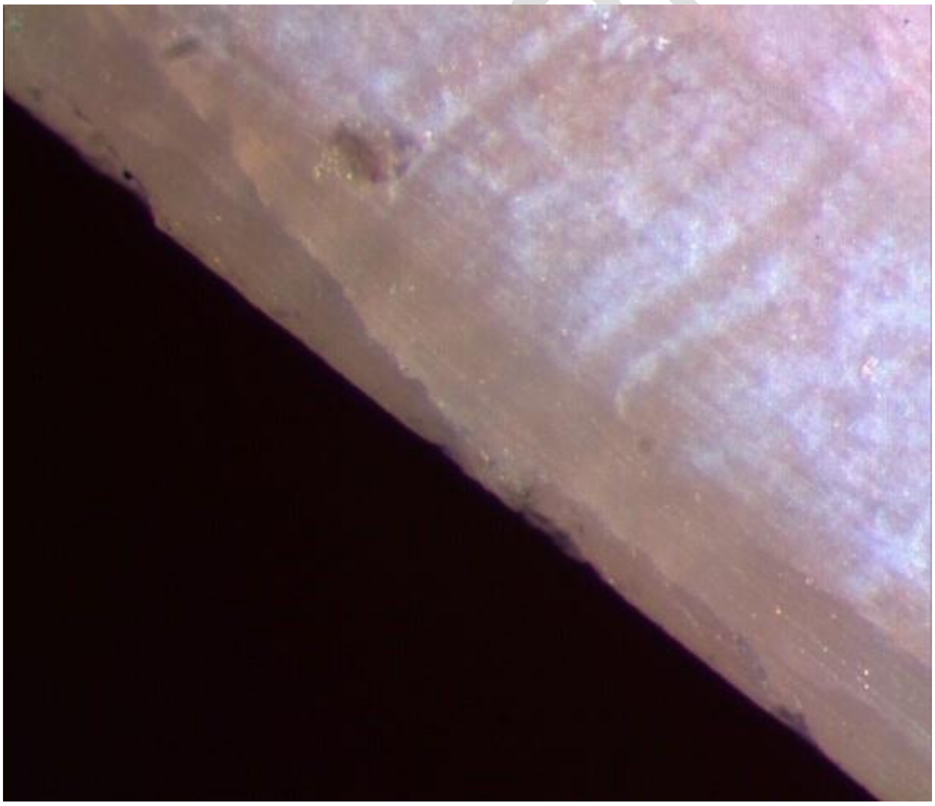

Figure 8

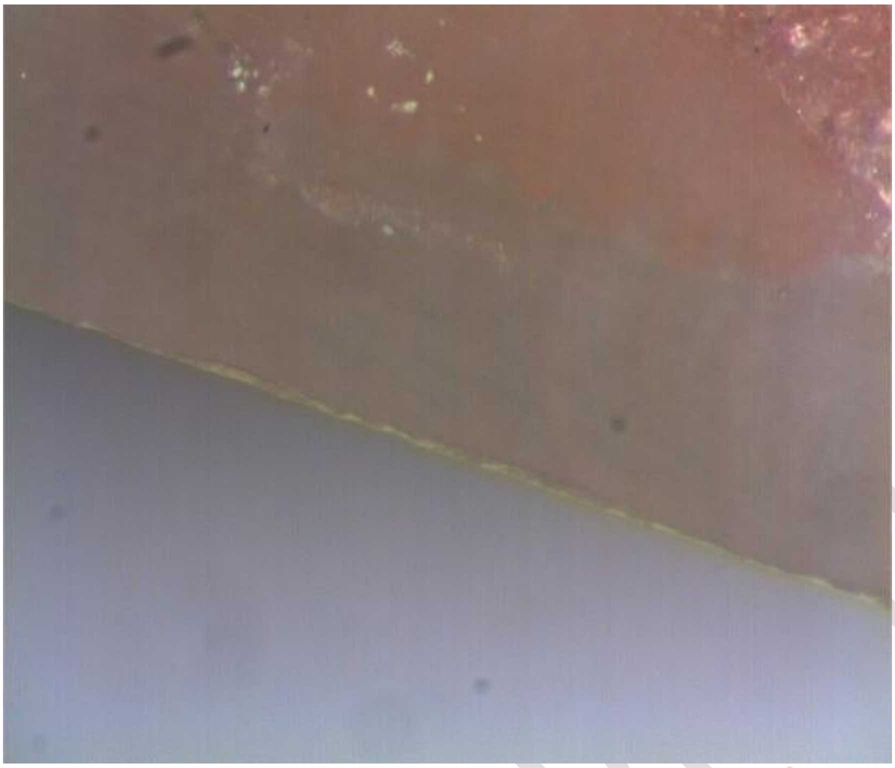

Figure 9

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Hatipoğlu Z, Yavlal Özbay G, Kargül B. Effects of Different Fluoride- Containing Toothpastes on Enamel Remineralization in Vitro. Bezmialem Science 2018. DOI: 10.14235/bs.2018.1810