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Influence of Dentifrices pH on Enamel Microhardness *In Vitro*

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ABSTRACT

Purpose was to evaluate the effect of toothpastes pH on enamel remineralization. Six fluoride toothpaste and one without fluoride were applied to the enamel slabs. Twenty eight enamel slabs were divided into seven groups and subjected to a daily cycling regimen with brushing treatments, demineralization and remineralization in artificial saliva. The surface microhardness (SMH) was calculated from the mean values obtained from six indentations (Vickers hardness number (VHN)) on the enamel surface at baseline and after 12 days. pH of the dentifrices was determined in a slurry with deionized water (1:3). Changes of the enamel surface microhardness at baseline and after remineralization stage were measured and analyzed using the Student t-test and one-way ANOVA. All groups treated with fluorides showed higher SMH values compared to control group. Toothpastes with lower pH (Pronamel, Sensodyne F, Sensodyne Rapid) were statistically superior to other fluoride dentifrices and control group after 12 days pH-cycling regimen (p<0.001). Obtained results showed that slightly acidified fluoridated toothpastes may have a positive influence on enamel remineralization process.

Key words: *enamel, fluoride, remineralization, microhardness*

Introduction

Fluorides are used in dentistry to reduce enamel demineralization and to stimulate enamel remineralization[1]. Dental caries is characterized by the dynamic process based on the demineralization and remineralization. Dental biofilm bacteria produce a large quantity of acids such as lactic acid from fermentable carbohydrates. In such conditions pH of the enamel easily falls below 5.6, and decalcification of enamel is induced. pH of the tooth environment is important factor which affects demineralization and remineralization equilibrium[2]. Fluoride from different supplements can create favorable environment to protect tooth substances from acidogenic attacks[3,4].

Toothpastes available on the market contained several fluoride compounds, including stannous fluoride, sodium fluoride, sodium monofluorophosphate (MFP) and amine fluoride as organic fluorides. Their anticaries performance is still described as very similar. Some studies showed those toothpaste containing sodium fluorides are slightly more effective than those containing MFP[5]. This is mainly attributed to the fact that MFP ions need to hydrolyze in the oral cavity to release free-fluoride ions for efficacy[6–8].

Most of the papers about the effects of toothpastes on enamel remineralization have been analyzed the amount of fluoride[9,10] or different fluoride compounds[11,12]. There are a few studies about the effects of acidic fluoridated toothpastes on enamel remineralization[12–14]. A general range of 5.5–5.0 is considered critical at which demineralization begins. It seems important to investigate the influence of fluoride toothpastes on enamel hardness under different values of pH. Preventive effect of toothpastes is related to the reduction of tooth demineralization which is due to fluoride incorporation into the apatite lattice. There is some evidence that even toothpaste with low F level can have good remineralizing action in decreased pH conditions[12].

The aim of this study is to investigate the influence of dentifrices pH with different fluoride formulation on human enamel surface microhardness in a pH-cycling model.

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Materials and Methods

Sample preparation

In the present study 28 extracted sound human teeth were analyzed. Caries free human teeth extracted for orthodontic reasons were stored in 2% formaldehyde solution at 25 °C. Sound enamel sections were cut using a water-cooled diamond saw. Enamel slabs were mounted in acrylic resin (Acryl Fix Kit, Struers) and cured for 24 hours. All blocks were progressively polished with water-cooled discs various fineness (320, 600 and 1200 grit of SiC papers; Buehler, Lake Bluff, IL, USA). These procedures are implemented to achieve parallel surfaces for Vickers tests of hardness.

De/remineralizing solutions

The composition of demineralizing solution was sodium acetate (0.1 mM CH_3COONa), potassium chloride (150 mM KCl_2), calcium chloride (1.5 mM $CaCl_2$) and potassium dehydrogen phosphate (0.9 mM KH_2PO_4). The pH was adjusted to 4.5 using hydrochloric acid (0.1mol/l). Slight elevations were adjusted with hydrochloric acid 0.1mol/l to obtain a constant pH value between 4.3 and 4.6 during the demineralization period.

The remineralizing (RM) solution (artificial saliva) contained: sodium chloride (0.50gr/l), sodium bicarbonate (4.2 g/l), sodium nitrate (0.03 g/l) and potassium chloride (0.20 g/l). The pH of artificial saliva was 7.5. Every day the pH values of demineralization and remineralisation solutions were measured with pH meter (HI 8014, HANNA instruments, Biobloch Scientific). Remineralizing solution was changed daily.

Study design

The toothpastes were applied to the enamel slabs that were exposed to 12-days demineralization/remineralization cycles. Different toothpastes were used: control paste without fluoride (Detartrine Paste-Septodont; pH 8.6), Elmex-GABA International (1250 ppm Amine fluoride; pH 4.8), Sensodyne fluoride (1400 ppm NaF; pH 5.4), Sensodyne Rapid (1040 ppm NaF; pH 6.7), Pronamel Sensodyne (1450 ppm NaF; pH 7.1), Colgate Total (1100 ppm NaF; pH 7.3) and Parodontax fluoride (1400 ppm NaF; pH 7.9).

Measurements of dentifrices pH-values

Fifteen millilitres of distilled water was added to 5 ml of toothpaste. pH was measured in each slurry. The sample included six commercially available toothpastes and Detartrine paste (Septodont) without fluoride was used as a control. Standard buffer solutions (Solutions tampons techniques 2 X pH= 4.00; 2 X pH= 7.00; 2 X pH= 10.00 (25 °C), 10 X 6 ampoules: L 4998; Cat. No. 93150, Biobloch Scientific) with nominal pH values of 4.0 and 7.0 were used to calibrate the pH meter with an accuracy of 0.01 units.

Experimental procedure

In order to standardize enamel blocks, the initial hardness was measured ((50 g, 490.3 mN or 0.05 Hv), 10 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). The surface microhardness (SMH) was calculated from the mean values obtained from six indentations on each sample. Enamel samples with a mean surface microhardness between 255.4 to 318.4 VHN were divided into six experimental groups and one control group. Baseline microhardness of enamel was obtained before the process of demineralization was initiated.

Daily pH cycling regimen was chosen to obtain partially demineralized enamel. To mimic the cycles of demineralization and remineralization of enamel that occurs under dental plaque in the mouth, a laboratory pH cycling model was previously developed. Cycling model tries to mimic the process of demineralization and remineralization by saliva in the mouth[15]. Enamel specimens were exposed to the cycles of demineralization and remineralization comprising: demineralization period of 6 hours using demineralization solution, brushing with toothpaste (two times per day) – one minute before and one minute after the demineralization period and 18 hours of remineralization in artificial saliva. Daily pH cycling regimen was repeated during 12 days. During the pH cycling regimen each of examined seven groups were manually brushed with toothpaste and standard toothbrush (Oral-B Medium). The pH model was designed to mimic daily process of a 6-hour demineralization and 18-hour remineralization repair by saliva. Two fluoride treatments per day received six experimental groups (before and after demineralization period) to evaluate the remineralization potential of the toothpaste. Samples were rinsed with distilled water for 15 s after any DM/RM period or after brushing with toothpaste. All samples were wiped dry with soft tissue paper after rinsing. Tested enamel slabs were remineralized in remineralizing solution at pH 7.5 for 18 hours at 37 °C (Cultura Vivacare Diagnostic Line-Vivadent).

The Vickers hardness number (VHN) was calculated from the mean values obtained from 24 indentations on the enamel surface in each group. Enamel microhardness was measured before and after twelve days pH-cycling regimen in each tested group. The obtained data were analyzed using commercially available software (Sigma Stats, SPSS) using Student t-test and correlation test.

Results

The percentage of surface microhardness changes (%SMH) after de/remineralization challenges are presented in Table 1. After 12 days cycling, an increased microhardness was shown at pH levels between 4.5 and 5.1. All specimens brushed with fluoridated toothpastes showed increased microhardness values compared to the control Detartrine paste ($p<0.05$, Figure 1). Sensodyne rapid even with the lower level of F showed significant improvement of the SMH and also those toothpastes with very low or high pH showed lower improvement in SMH (Table 1). Enamel samples treated with Pronamel, Sensodyne F and Sensodyne rapid showed higher SMH values than samples treated with other tested toothpastes or a control paste after 12 days pH-cycling regimen. SMH values of samples treated with Parodontax also obtained increase in SMH, but it was not statistically significant (p>0.05). Statistically significant microhardness increase was achieved in group brushed with toothpaste containing aminfluoride. The best improvement in SMH was obtained with toothpastes with pH between 5.3 and 7.3. Correlation analysis did not show significant difference (Figure 2). The negative trend was observed for percentage of surface microhardness changes (%SMH) and pH.

TABLE 1
LEVEL OF FLUORIDE AND PH OF DENTIFRICE AND PERCENTAGE OF SURFACE MICROHARDNESS CHANGES (%SMH) AFTER REMINERALIZATION

N	Toothpaste	F (ppm)	pH	% SMH	p
24	Sensodyne F	1400	5.4	18.86	p<0.05
24	Sensodyne Rapid	1040	6.7	18.15	p<0.05
24	Colgate Total	1100	7.3	13.47	p<0.05
24	Elmex	1250	4.8	6.41	p<0.05
24	Parodontax	1400	7.9	2.23	NS
24	Pronamel	1450	7.1	22.59	p<0.05
24	Detartrine	0	8.6	5.60	NS

Student t-test; p<0.05, NS – Non-significant

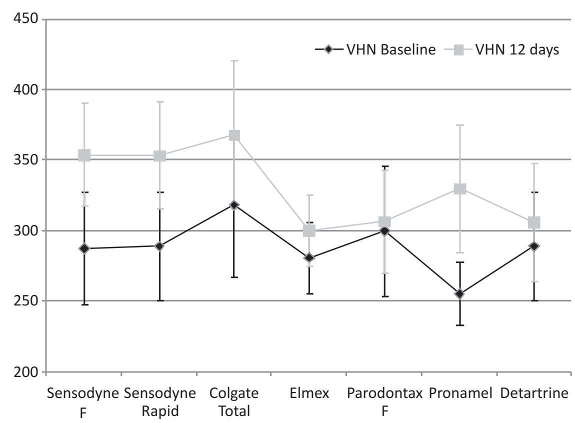

Fig. 1. Vickers hardness number (VHN) of enamel surface before and after remineralization.

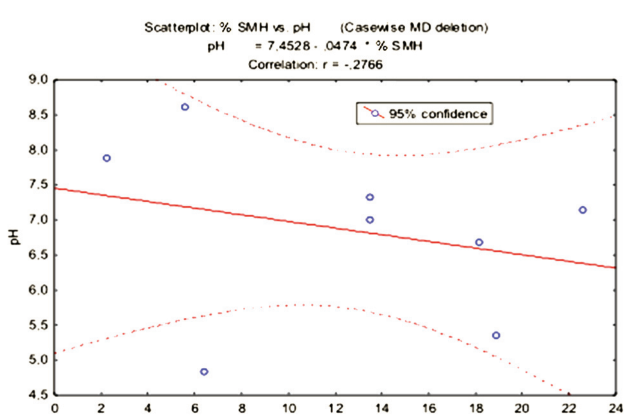

Fig. 2. Correlation between pH of dentifrice and percentage of surface microhardness changes (%SMH).

Discussion

Remineralization is defined as the redeposition of minerals lost by enamel, and this term has been used as a synonymous of enamel repair or rehardening. Mineral loss (demineralization) or gain (remineralization) by enamel is a dynamic physicochemical process occurring when oral bacteria form a biofilm[16].

This *in vitro* demineralization/remineralization cycling study evaluated the ability of various dentifrices to remineralize softened enamel surface. Enamel microhardness testing is considered to be reliable method for the provision of indirect information on mineral content changes in enamel[11]. Hardness change is an indicator of minerals gain or loss in enamel as a result of demineralization and remineralization process.

It could be assumed that the application of some of acidified toothpastes can lead to erosion of the enamel surface. This is happening at much lower pH levels where the solutions are undersaturated with respect to hydroxyapatite and also fluorapatite and therefore remineralization would not be possible due to thermodynamics[14]. Baseline microhardness of enamel was obtained before the process of demineralization was initiated.

Results obtained in this study are according to the findings in previous studies showing that toothpaste with lower F level can have strong preventive effect in conditions of decreased pH. This was obtained for the sodium F (NaF) as well as amine F contained toothpastes. Incorporation of F ions in the enamel structure depends of chemical composition of toothpaste, but it seems that low pH values can inhibit solubility of CaF_2 on enamel surface. Cuy et al.[17] found that enamel hardness varies depending on the degree of mineralization of the enamel, local variations in enamel rods and tufts, and increased porosity near the dentoenamel junction[18]. Microhardness indentation measurements can provide indirect evidence of mineral loss or gain[19]. The disadvantage of this technique (SMH) also used in this study is that it cannot quantify the amount of mineral loss or gain. However, it can provide qualitative data on mineral changes within the enamel surface. Results from this study showed that the use of the dentifrice with fluoride can be effective in increasing enamel SMH after pH cycling. Low-F (500 ppm) acidic (pH 4.5 or 5.5) NaF dentifrices have been shown in pH-cycling studies to be as effective as 1,100 ppm F neutral NaF dentifrices[20, 21]. Arnold et al.[12] showed increased remineralization at pH levels between 4.5 and 5.1 with amine fluoride toothpaste (1400 ppm). It is due to the properties of the formulation and pH will drive different ways of fluoride action. Divalent cations (Ca^{2+}) can reduce the amount of available fluoride and high phosphate levels can reduce the absorption of fluoride. Formation of calcium fluoride is pH dependent, and is less soluble at low pH[22,23]. The dentifrice formulation with a low pH and law fluoride concentration were tested *in vitro* by Brighenti et al.[21]. Research has shown that low pH and low concentration of fluoride dentifrice has the same efficacy as conventional.

The dynamic nature of the process has been modelled in a number of laboratories by various pH cycling models[10,14]. This study examined the pH cycling (alternating demineralization/remineralization) over a period of two weeks in six hours a day demineralization in pH 4.5. The demineralizing solution was partially saturated with calcium and phosphate. After demineralization, the process of remineralization was started for 18 hours during every 24 hours, in accordance with the process described previously[10].

Analysis of surface microhardness (SMH) showed that Pronamel (1450 ppm F), Sensodyne F (1400 ppm F), Sensodyne rapid (1040 ppm F) and Colgate Total (1100 ppm F) showed higher values of enamel microhardness than other fluoride toothpaste and fluoride-free placebo after 12 days pH-cycling regimen. It seems that the efficient delivery of fluoride from these toothpastes resulted in increased resistance to demineralization and remineralization efficiency.

Under the conditions created in this study, some toothpaste can significantly enhance the process of enamel remineralization. The formation of products when F reacts with the enamel depends on the F concentration, duration, pH, frequency and treatment method[24, 25]. In regard to the acidity, it is shown that the pH significantly affect the formation of fluoride products on enamel[18] as well as the anticariogenic effect of toothpaste[19].

One of the main causes of enamel demineralization is pH decrease below the critical point of hydroxyapatite dissolution[22,23]. The equilibrium between enamel demineralization and remineralization maintains an intact enamel surface. The results of Arnold et al. showed an increased remineralization at pH levels between 4.5 and 5.1 under influence of amine fluoride. It has been shown that fluorides enhance mineral uptake during enamel remineralization and inhibit mineral loss during demineralization. Calcium fluoride formation depends on pH and is less soluble at low pH values[12,22,23].

Conclusion

It could be concluded that high fluoride toothpastes (1450 ppm F) have positive effect on enamel remineralization. The results of the present *in vitro* study show that toothpastes with pH 5.5–7 are the most effective even with less F content. Under the limitations of the present *in vitro* study, it can be concluded that slight decrease of pH probably can have positive effect on F action. Further clinical trials are needed to verify whether it has clinical relevance and whether similar results can be obtained in the complex oral environment. It points that the use of fluoride dentifrice with pH 5.5–7 and even with less F content are the most effective in enamel remineralization.

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UTJECAJ PH PASTA ZA ZUBE NA MIKROTVRDOĆU CAKLINE *IN VITRO*

S A Ž E T A K

Svrha je procijeniti utjecaj pH pasta za zube na remineralizaciju cakline. Caklinski izbrusci su tretirani sa šest fluoridiranih pasta za zube i jednom bez fluorida. Uzorci su podijeljeni u sedam skupina i podvrgnuti cikličkom dnevnom režimu četkanja, tretmanu demineralizacije, a zatim remineralizacije u umjetnoj slini. Površinska mikrotvrdoća (SMH) izračunata je iz srednjih vrijednosti dobivenih od šest utisnuća (tvrdoća po Vickersu (VHN)) na površini cakline na početku i nakon 12 dana. pH paste za zube je određen u otopini s deioniziranom vodom (1:3). Promjene mikrotvrdoće na površini cakline na početku istraživanja i nakon remineralizacije pozorno su obrađene i analizirane pomoću Student t-testa i jednosmjerne ANOVA analize. Sve tretirane skupine pokazale su veće vrijednosti SMH u odnosu na kontrolnu skupinu. Zubne paste s nižim pH (Pronamel, Sensodyne F, Sensodyne Rapid) bile su statistički bolje od drugih fluoridnih pasta za zube i kontrolne skupine nakon 12 dana pH – cikličkog režima ($p<0,001$). Dobiveni rezultati su pokazali da malo zakiseljene fluoridirane paste za zube mogu imati pozitivan utjecaj na remineralizacijski proces u caklini.