Original Article

Effect of Fluoridated Dentifrices on Surface Microhardness of the Enamel of Deciduous Teeth

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ABSTRACT

Background: Surface microhardness is a physical property which access the effect of chemical and physical agents on hard tissues of teeth, and a useful way to examine the resistance of fluoride treated enamel against caries. The purpose of this study was to evaluate microhardness of enamel following pH-cycling through demineralization and remineralization using suspensions of dentifrices with different fluoride contents.

Methods: In this in vitro study 56 enamel blocks of primary incisors were soaked in demineralizing solution and four dentifrices suspensions including: Crest 1100 ppm F (NaF), Crest 500 ppm F (NaF), Pooneh 500 ppm F (NaF,) and Pooneh without fluoride. The means and percentage changes of surface microhardness in pre-demineralization, after demineralization and remineralization stages in four groups were measured. The findings of four groups in three stages were compared by, ANOVA, Tukey and paired t-tests. (α=0.05)

Results: Average surface microhardness changes of Crest 1100 ppm F, was higher than Crest 500 ppm F, Pooneh 500 ppm F, and Pooneh without fluoride. The percentages of surface microhardness recovery for Crest 1100 ppm F, Crest 500 ppm F, Pooneh 500 ppm F, and Pooneh without fluoride were 45.4, 35.4, 28.6, and 23.7 respectively. Demineralization treatment decreased the surface microhardness of enamel ($P<0.05$) and the surface microhardness recovery in all groups were significant ($P<0.0001$).

Conclusion: Surface microhardness of enamel after remineralization by Crest 1100 ppm F was higher than Crest 500 ppm F, Pooneh 500 ppm F, and Pooneh without fluoride.

***Keywords*:** Dentifrice, Fluoride, pH-cycling, Surface microhardness, Re/Demineralization.

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Introduction

Dental caries is still remained the most common preventable chronic oral disease. The protective and tooth resisting modalities against dental decay are incorporating fluoride ions in developing hydroxyappatite lattice and incipient caries. The excerpt of this protection is done through daily tooth brushing with fluoride dentifrices. Application of fluoride dentifrices has been effective in numerous clinical trials. It has been known as a major reason of remarkable decline in dental caries incidence and prevalence in developed countries.[1] In addition, the inherent properties of fluoride toothpaste, biological and behavioral factors, are the reasons of anticaries efficacy of fluoride products. In a systematic review

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of seventy trials, the average of reduction in dental caries attributed to fluoride dentifrices was twenty percent[1]. The daily tooth brushing frequencies, length of brushing time and availability of fluoride ions content are effective dependent factors. Degree of enamel fluorosis during permanent teeth development and application of dentifrices with high concentration of fluoride are important factors.[2-4] Systematic reduction of fluoride content in children's dentifrices and supervised application of pea sized toothpastes are the practical and reasonable strategies in decreasing dental fluorosis.[4]

Recently fluoridated dentifrices and mouth rinse products are sold over-the-counter without formal prescription. It is ordinarily recommended not to use more than 10 mg/kg fluoride to avoid undesirable degrees of dental fluorosis.[5,6] However, it has been proven in vitro that at least 500 ppm fluoride content is essentially needed to protect the enamel of permanent teeth against demineralization (artificial lesion).[7,8] The exact mechanisms of preventive effects of locally used fluoride products are debated but, arresting the progress of dental caries, diminishing the organic acid attacks, decreasing free charge enamel surfaces, changing the bacterial flora of biofilm, modifying surface hardness, and accentuation of remineralization dynamic are hypothesized and challenged.[9] It is confirmed that fluoride ions are essentially contributed to de/remineralization processes in white spot lesions and artificial caries. Conclusive evidence in literature has shown the efficacy of fluoride dentifrices in primary dentition. It seems that fluoride ions do not pass through the placenta, however, the efficacy of fluoride protection during pregnancy is dismissible.

Primary dental enamel has different microchemical structure compared to permanent teeth. Deciduous teeth exhibit a different behavior in deremineralization processes in comparison with permanent teeth. Surface microhardness of enamel is a physical property that permits the researcher to assess the effects of chemical and physical agents on enamel, dentin, and cementum.[9-11] pH-cycling systems evaluate caries lesion and mineral changes in dental hard tissues. According to Ten Cate and Duijsters[8], the best way to simulate the environment in vitro is to apply pH-cycling models that is equivalent to dynamics dental decay. The purpose of surface microhardness evaluation was supported by the finding of a strong correlation between remineralization measured by this technique and radiographic techniques[8]. Featherstone[5] reported a good correlation between enamel microhardness and mineral loss in caries lesions.

Over the counter children's toothpastes are not so common. Generally, all toothpastes have monofluoride phosphate (MFP), sodium fluoride (NaF) as anticaries and silica as abrasive agents.[12] The aim of this study was the evaluation of changes of surface microhardness following pH-cycling of Crest (Adult type) (NaF, 1100 ppm F) (standard), Crest (Children type) (NaF, 500 ppm F) (positive control), Pooneh (NaF, 500 ppm F) and Pooneh without fluoride (Negative control).

Materials and Methods

This study was executed in four phases.

Phase one:

Fifty six blocks of coronal portion of extracted primary incisors without dental caries, hypoplasia, crack, and malformation were collected. The crowns were cleaned with distilled water and soaked in formaldehyde for one month. The crowns were mounted in cylindrical plastic tubes with epoxy resin stick exposing 2 × 4 mm of enamel surface. Preparation of enamel surface was done by Boehler Abrasive Instrument (Buehler, Chicago, USA), with 600-1200 grits AL_2O_3 paper and water. The smooth surfaces were examined under light microscope (Siemens, Germany) using X 100 objective lens in order to evaluate glassy and transparent appearance of enamel surfaces.

Phase two:

The surface microhardness was measured by Buehler microhardness machine (Buehler, Chicago, USA). The force action of silver diamond was projected to enamel surface, 100 $g/20^n$ in three points distances of 500, 1000, 1500 micrometers. The average measures of three points were provided. The amount of surface microhardness was measured with Vickers Index Hardness Unit.[13-15] Through this equation, Vickers Hardness Number (VHN) = $F \times 1.85/d^2$ where F= $kg/m^2$and d=mean diameters. In this step, 56 blocks with surface microhardness of 311-330 VHN were selected.

Phase three:

The remieralizing and demieralizing solutions and four dentifrices suspensions were freshly provided in biochemical laboratory of School of Pharmacy and Pharmaceutical sciences, Isfahan University of Medical Sciences, Isfahan, Iran. The demineralizing solution contained 2.2 mM $NaHPO_4$, 1.5 M $CaCl_2$, 0.5 M KCl with pH = 7[16- 21] and reminerlizing solution contained 0.9mM NaHPO4, 1.5 CaCl2, 0.M KCL. The suspensions of Crest (NaF, 1100 ppm F), Crest (NaF, 500 ppm F), Pooneh (NaF, 500 ppm F) and Pooneh without fluoride were prepared by diluting them with distilled water to 1/3.

Phase four:

pH-cycling and remineralization process were done in 8 days with cyclic pattern. In each pH-cycle, blocks were immersed and shook in 20 ml of each solution as follow:[16]

1) At the beginning of each cycle, in each group 20ml of one of dentifrices, after homogenizing, affected on samples for one minute.
2) Each sample remained in 20 ml of deminerlising solution for 2 hours.
3) Finally, after applying 20ml of each dentifrice for minutes, samples remained in 20 ml of reminerlising solution for 22hours.

After changing each solution, samples were washed for 10 seconds by distilled water.

The mean surface microhardness was measured after demineralizing step. The range of surface microhardness was 193-220Vickers Hardness Number (VHN). The 56 blocks were randomly and blindly divided equally into four groups as Crest (NaF, 1100 ppm F), Crest (NaF, 500 ppm F), Pooneh (NaF, 500 ppm F) and Pooneh without fluoride groups. Each group was immersed in remineralizing solution and suspension of related dentifrice in pH-cycling process. We obtained the dentifrices Crest (NaF, 1100 ppm F), Crest (NaF, 500 ppm F), Pooneh (NaF, 500 ppm F) from drug stores, but Pooneh without fluoride was provided by Goltash Company in the city of Isfahan. Finally, surface microhardness of enamel blocks were measured, at the end of process, again.

The following equation was used for calculating the percentage of surface microhardness recovery:

$$SMH_R = \frac{VHN_F - VHN_D}{VHN_I - VHN_D} \times 100$$

(VHN1 is Initial VHN, VHND is Deminerization VHN, and VHNF is Final VHN).[22]

SPSS soft ware, version 11.5 was used to analyse the data. The mean, standard deviation, maximum, and minimum measures in three steps for four groups were described. We examined the findings of this study by, ANOVA, Tukey's tests and paired t-test where $\alpha < 0.05$ was considered significant.

Results

Four groups were exposed to demineralization, remineralization, and toothpaste suspensions in pH-cycling model. Mean, standard deviation, maximum and minimum surface microhardness before and after demineralization, and after remineralization are shown in table 1. Kolmogorov-Smirnoph and levene's test were examined the normality of data distribution with homoscedasticity. We applied ANOVA to evaluate the proportional changes of surface microhardness in four groups, and also we used the Tukey's test for paired group comparison (Table 2). Demineralization treatment decreased the surface microhardness of enamel (P<0.05). Then, the surface microhardness recovery was significant (P<0.0001) in all groups.

Table 1. Distribution of surface microhardness of enamel blocks according to toothpaste

Toothpaste	Stage	Mean ± SD	Max-Min
Crest (NaF, 1100 ppm F)	SMH_I	320 ± 3.9	329-311
	SMH_D	206 ± 3.3	213-204
	SMH_F	258 ± 5.6	268-251
	$\%SMH_R$	45.4 ± 3.8	52.2-41.2
Pooneh (NaF, 500 ppm F)	SMH_I	321 ± 6.4	331-313
	SMH_D	208 ± 7.9	219-198
	SMH_F	248 ± 7.6	258-235
	$\%SMH_R$	35.4 ± 6.3	42.2-19.5
Crest(NaF, 500 ppm F)	SMH_I	321 ± 4.8	328-314
	SMH_D	209 ± 7.9	220-198
	SMH_F	241 ± 9.3	258-229
	$\%SMH_R$	28.6 ± 8.9	41.6-10.3
Pooneh without Fluoride	SMH_I	325 ± 5.3	329-315
	SMH_D	210 ± 10.7	221-192
	SMH_F	238 ± 7.4	249-226
	$\%SMH_R$	23.7 ± 9.9	46.4-11.6

SMH_I: Initial Surface Microhardness, SMH_D: Demineralization Surface Microhardness, SMH_F: Final Surface Microhardness, SMH_R: Recovery Surface Microhardness.

Table2. Comparison of changes of surface microhardness in four groups (p value)

Toothpaste	Crest (NaF, 1100 ppm F)	Pooneh without fluoride	Crest (NaF, 500 ppm F)
Pooneh (NaF, 500 ppm F)	0.006	0.001	0.09
Crest(NaF, 500 ppm F)	P < 0.0001	0.341	
Pooneh without fluoride	P < 0.0001		

P < 0.05: Significant

Discussion

The purpose of the present study was the comparative evaluation of changes in surface microhardness following application of different fluoride concentration dentifrices. Data were provided in initial stage, after demineralization and after remineralization through pH-cycling process. The cariostatic efficacy of fluoridated dentifrices is indisputable. However, it has been reported that their unsupervised use in young children may result in an undesirable high fluoride uptake. Consequently, dentifrices with low fluoride content for young children have been developed. The clinical efficacy of these toothpastes in deciduous teeth is matter of dispute and particularly whether a lower caries preventive effect can be expected for the fluoride concentration below 500 ppm. . Ammari et al.[17] compared low fluoride toothpaste containing 600 ppm or less with toothpastes containing 1000 ppm or more in children and adults and reported that 250 ppm fluoride dentifrices are not as effective as dentifrices containing 1000 ppm in caries prevention. Bjarnason et al.[18] in a randomized clinical study found that the progression of already existing carious lesions was similar after using 250 ppm or 1000 ppm containing fluoride dentifrice. Our findings showed that the Crest (NaF, 1100 ppm F), Crest(NaF, 500 ppm F), Pooneh (NaF, 500 ppm F) and Pooneh without fluoride increased surface microhardness after remineralization through suspension in toothpaste solutions. In contrast to our study, Dunipace and Wefel et al.[19,20] found that mineral acquisition by initial white spot lesions in permanent teeth were increased with increasing fluoride levels in situ. Recent studies showed that 500 ppm fluoride containing dentifrices led to a similar caries reduction compared to standard and adult toothpastes in permanent teeth,[21] contradictive to our study, Crest (NaF, 1100 ppm F), (as the standard toothpaste) increased surface microhardness more than other tooth pastes in primary tooth enamel samples. The abrasive contents of our applied toothpastes were silica. In the study of Maia et al.[21], Duraphat (1.26%) was promoted the surface microhardness by 33%. Cury et al.[22] reported that the amount of increased surface microhardness was attributed to the abrasive systems in dentifrices. We could not approve or reject the role of abrasive systems on increasing remineralization in all our dentifrices groups. In the study of Queroz et al.[23], there were no significant differences between surface microhardness produced by APF and MFP contained dentifrices, that is not agree with our results. Bothenberg et al.[24] reported that the depth of indentation in the surface of enamel after application of fluoride dentifrices was more than that of dentifrices without fluoride. Tukey's test showed that the Crest (NaF, 1100 ppm F) increased the surface microhardness significantly more than Crest (NaF, 500 ppm F) and Pooneh (NaF, 500 ppm F); however, the increased surface microhardness of using Pooneh 500 ppm was significant. In the present study, Surface micro hardness recovery after application of crest (NaF, 1100 ppm F), Crest (NaF, 500 ppm F), Pooneh (NaF, 500 ppm F) and Pooneh without fluoride was 45.4% , 28.6%, 35.4% and 23.7% respectively.

In the present study, the effect of Crest (NaF, 500 ppm F) was similar to the effect of Pooneh without fluoride. The possible reasons could be the interaction of ingredients, low fluoride concentrations in the packs, inconsistent distribution and solubility of fluoride, existence of an incompatible abrasive agent with fluoride.

Conclusion

From the findings of this study, it can therefore be concluded that Crest (NaF, 1100 ppm F), Crest (NaF, 500 ppm F), Pooneh (NaF, 500 ppm F), and Pooneh without fluoride can increase the surface microhardness in primary teeth enamel. The average of changes of surface microhardness of Crest (NaF, 1100 ppm F), was higher than that of Crest (NaF, 500 ppm F), Pooneh (NaF, 500 ppm F) and Pooneh without fluoride.

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Conflict of interest

The authors declare that they have no conflict of interest. We all participated in conception, design, planning, interpretation, manuscript writing and submission of this article.

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