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Original Article

An *in vitro* Comparative Evaluation of Fluoride Uptake by Demineralized Enamel Exposed to Four Different Low Fluoride Dentifrices

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ABSTRACT

Background: Topical fluoride application has an important role in caries prevention. The aim of the present study was to evaluate and compare fluoride uptake of tooth enamel after exposure to four commercially available low fluoridated toothpastes.

Methods: The present *in vitro* study was conducted on 60 sound extracted premolar teeth. The teeth were covered with acid-resistance nail polish except at a 5×5 mm area on the buccal and lingual surface of each tooth (for experimental and control group respectively). After demineralization of the window area for 2 days, the teeth were immersed in toothpaste slurry containing: Sodium Fluoride 1000ppm (Group A), Soduim monofluorophosphate 1000ppm (Group B), Soduim monofluorophosphate 500ppm (Group C) and Sodium fluoride 500ppm (Group D). The pH of the dentifrices was measured. The acid biopsy technique and fluoride ion-specific electrode was used for fluoride ion estimation.

Results: All of the applied toothpastes significantly increased fluoride content of the enamel compared with the control group (P <0.001). There was a significant difference among the four groups of toothpastes in the mean fluoride uptake and group A showed maximum uptake of fluoride (5.5920 ppm), followed by group B, C and D respectively. According to Pearson correlation analysis, there was not any significant relationship between the pH of the dentifrices and uptake of fluoride.

Conclusion: There was a positive correlation between the fluoride concentration of dentifrices and the fluoride uptake on demineralized enamel. The toothpastes containing NaF are more effective than toothpastes containing NaMFP. Moreover, dentifrice pH had no influence on fluoride uptake by enamel.

Keywords: Dentifrices, Enamel, Fluoride

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Introduction

As indicated by the WHO report, dental caries is a major problem in most industrialized nations affecting 60-90% of school children. The report shows that the frequency of dental caries will increase. The important explanations for this increase are developing sugar consumption and inadequate exposure to fluoride. One of the focuses of the WHO oral health preventative strategies is to prevent dental caries among all population and social groups (1, 2).

Whether dental caries progresses, stops, or reverses is dependent on a balance between demineralization and re-mineralization. Demineralization can be reversed in its early stages through uptake of calcium, phosphate, and fluoride (3). Fluoride acts as a catalyst for the diffusion of calcium and phosphate into the tooth, which re-mineralizes the crystalline structures in the lesion (4).

Regarding the self-applied fluoride, fluoridated toothpaste is the most commonly used form of fluoride delivery. Several reviews shows that fluoride toothpastes are efficient to control caries and have played an important role in caries reductiont (5, 6).

Tooth brushing with fluoride toothpaste controls caries through at least a two-fold action; firstly, fluoride can reduce demineralization after binding the enamel surface, as fluoridated enamel is less acid-soluble than intact enamel; secondly, it can promote re-mineralization of partially demineralized enamel in the presence of saliva-derived calcium and phosphate ions, both bound to the enamel surface and present in the fluid overlying the lesion (7). In addition, fluoride has been shown to interfere with bacterial metabolism *in vitro*, which may inhibit plaque acid production (8).These have been the reasons for suggesting the utilization of fluoride toothpastes for improving oral health.

The children toothpastes should contain different concentrations of fluoride and have maximized fluoride availability and low abrasivity (9). The use of fluoridated toothpastes in children is depended on caries risk assessments. The decision of what fluoride levels to use for young children should be balanced against the risk of fluorosis (10). To reduce the ingestion of fluoride from toothpaste, it is recommended that children use a pea-sized amount of fluoride toothpaste twice daily, with the difference that children less than 2 years of age should brush with 500 ppm fluoride toothpaste, and children up to 6 years of age should brush with 1000 ppm fluoride (11). Studies have shown that different fluoride toothpastes and gels have different uptake of fluoride by tooth enamel (9, 12).

To assess the fluoride uptake, several methods have been employed, including acid etch enamel biopsy, enamel micro-hardness, confocal laser scanning, quantitative transversal micro-radiography, and iodine permeability. According to the literature, an *in vivo* acid etch biopsy method was developed by Hotz *et al*. (1970) to determine fluoride uptake and retention in the outer surface of enamel (13). Santos *et al.* analyzed the total fluoride and total soluble fluoride concentrations in toothpastes intended for children and showed that only 2 of 12 toothpastes available in the market presented soluble fluoride concentrations capable of preventing dental caries (14). The results of the Shanbhog *et al.* study showed 1400-ppm fluoridated dentifrice was more effective compared to1000-ppm fluoridated dentifrice and non-fluoridated dentifrice in increasing surface micro hardness of fluorosed enamel (15). From studies, it seems that fluoride in tooth-paste causes considerable different uptake of fluoride by tooth enamel; therefore, the purpose of this study was to evaluate and compare fluoride uptake by demineralized enamel following exposure to four commercially available fluoridated toothpastes used by children at the different stages of their development.

Materials and Methods

This study was approved by the Research and Ethics Committee of Ardabil University of Medical Sciences (IR.ARUMS.REC.1396.175). The present *in vitro* study was conducted on 60 sound extracted premolar teeth. All teeth had been extracted for the orthodontic purposes in the last month. The teeth that had caries lesion, crack line and white spot were excluded from the study. The enamel surfaces of teeth were cleaned with a rotating rubber cap and pumic slurry. The teeth were disinfected by soaking in 10% buffered formalin for 2 weeks and were stored in deionized distilled water which was replaced every week. Then, each of the teeth was cut vertically into two halves with a water-cooled diamond wafering blade (Buehler, Warwick, U.K). The buccal side in each tooth served as the experimental and the lingual side as the control.

Except the two 5×5 mm areas in the middle of buccal and lingual surface of the enamel of each tooth (for experimental and control group), other areas were covered with acid-resistance nail polish. The teeth were immersed in 500ml demineralization solution containing 0.1M lactic acid, 3mM calcium chloride, 1.8 mM potassium dihydrogen phosphate and 0.2%*w/v* Carbopol C907 (BF Goodrich Co., Akron, OH, USA). The pH was carefully adjusted to 4.5 with concentrated NaOH (16).

The teeth were kept inside the solution for 2 days at 37˚C in an incubator (16). The solution was changed every 12 h to avoid variation of the pH of the demineralizing solution. The specimens were withdrawn after the demineralization, washed with deionized distilled water and air-dried. Then the lingual half of all teeth (control group) were stored in de-ionized distilled water for 4 days at 37˚C in an incubator and the buccal half of all teeth were randomly divided into four group containing 15 teeth in each group.

Group A: toothpaste with sodium fluoride, 1000ppm (Folktandkräm Baby, proxident, Sweden)

Group B: toothpaste with soduim monofluorophosphate, 1000ppm (frice, Dr. akhavi lab Co., Iran)

Group C: toothpaste with soduim monofluorophosphate, 500ppm (2080 kid, aekyung, South Korea)

Group D: toothpaste with Sodium fluoridell, 500ppm (signal kid, unilever, France)

Toothpaste solutions were prepared with the same concentrations (20g toothpaste per 100cc of distilled water) and the experimental group of tooth were stored in this solutions for 4 days at 37˚C in an incubator which simulates 4 years of tooth brushing twice daily for 2 min per day (11, 17). The pH of the slurries was measured before immersion of samples. After this period the teeth were washed with de-ionized distilled water and dried. The acid biopsy technique was used for fluoride ion estimation. The samples were etched for 60s in 10 ml of 0.5 M perchloric acid separately in the experimental and control window (18). Then 10 ml of total ionic strength adjustment buffer (TISAB-II) was added. The fluoride content in the solution was measured with a fluoride ion selective electrode (Mettler Toledo, United State). Before proceeding with the test, the fluoride electrode had to be calibrated. This was done by using a sodium fluoride stock solution with a concentration of 1000 ppm fluoride. This solution was then diluted in stages with distilled water to produce standard solutions of 15 ppm, 10 ppm, 5 ppm, and 2.5 ppm and1ppm fluoride.

The Pearson correlation analysis was used to determine the relation between the pH of the dentifrices and uptake of fluoride. The data from fluoride uptake analysis were examined statistically by two-way ANOVA and the Tukey test, with a level of significance at 0.05.

Results

According to two Way-ANOVA test, all toothpastes significantly increased the fluoride content of the demineralized enamel compared with the control group (P <0.001). A summary of the results are given in table 1 and figure 1. According to Tukey posthoc analysis test, there was a significant difference in the mean fluoride uptake among four groups of applied toothpastes and group A (contained 1000ppm NaF) showed maximum uptake of fluoride (5.5920 ppm), followed by group B (3.8920 ppm), group C (3.8454 ppm) and group D (3.4990 ppm) respectively (P <0.001). The results showed no significant difference in the mean content of fluoride among the four types of control group (P >0.05). The pH of toothpaste was 8.46, 5.98, 6.65 and 7.14 for groups A, B, C and D, respectively. According to Pearson correlation analysis, there was no significant relationship between the pH of the dentifrice and uptake of fluoride (P >0.05).

Table 1. Comparison of experimental and control windows in terms of fluoride uptake in different groups

window		Group A	Group B	Group C	Group D	P-value
mean± SD	case	5.5920±.78	3.8920±.40	3.8454±.61	3.499±.63273	<.001
	control	1.728±.42	1.7850± .46	1.68±.47	1.828±.30626	

Fluoride values were expressed in mg/L (equivalent to ppm F)

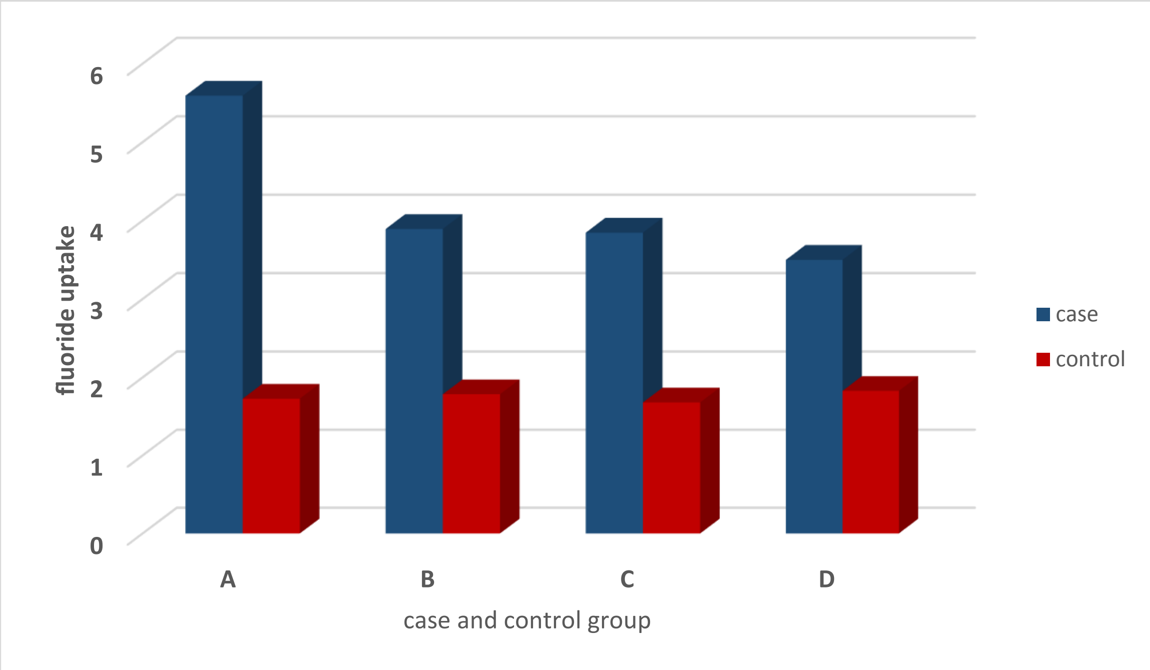

Figure 1. Comparison of fluoride uptake between experimental and control windows among the four group

Discussion

In the present study, there was uptake of fluoride by demineralized tooth enamel surfaces in the all experimental groups compared to their control groups. Although all dentifrices showed the higher values of fluoride we found when 1000 ppm fluoride dentifrice was used higher concentration of fluoride was uptake, since a positive correlation between the fluoride concentration in dentifrices and the fluoride concentration in teeth. Similar results have been reported in *in vitro* studies (19, 20). Walash *et al.* suggest that children's toothpaste should have lower concentration of fluoride, although the evidence for a caries preventive effect of formulas with less than 500 ppm fluoride are insufficient (10). As suggested in Ammari *et al.* meta-analysis, 250 ppm F toothpastes are not as effective as 1,000 ppm F pastes in preventing dental caries in the permanent dentition(21). Fluoride toothpastes have played a major role in the pronounced caries decline observed worldwide due to their widespread availability (5, 6, 9). Fluoride is delivered from the dentifrice into the oral cavity during the brief periods of tooth brushing. When fluoride toothpaste is used, a high concentration of fluoride is maintained in the mouth for some minutes. In saliva, fluoride concentration takes 1 or 2 hours to reach the baseline, pre-brushing values (22). In the biofilm, increased fluoride values are maintained even 10 to 12 hours after brushing when fluoride toothpaste is used regularly (23). Dentist who recommended the use of fluoridated toothpaste ensure that a balance is maintained between maximizing the protective effect against dental caries and minimizing the risk of dental fluorosis (10). Some strategies have been proposed to increase the anti-caries efficacy of low-F dentifrices such as the addition of sodium trimetaphosphate, xylitol and the pH reduction (24, 25).

The bioavailability of fluoride is very important for the caries-preventive effect which in turn is dependent on the solubility of fluoride-containing compounds leading to the adhesion of that fluoride compound to the tooth surface. Other factors influencing the efficacy of dentifrice include the formulation and brushing behavior such as brushing frequency, brushing time and post-brushing rinsing practices (6).

Different studies have shown that the symmetrical areas in one tooth have the same basic fluoride content. So, in this study, the symmetrical areas in one tooth were evaluated. The acid biopsy technique was used for fluoride ion estimation in the present study. This technique is based on *in vitro* studies that have been carried out to evaluate the enamel uptake of fluoride (19).

The main fluoride compounds currently found in toothpastes are sodium fluoride and sodium monofluorophosphate, although stannous fluoride and amine fluoride are also used (26). In terms of anti-caries action, all forms of fluoride described above have similar efficacies, with minor differences (26).In this study, the highest uptake of fluoride by enamel was in samples treated with 1000ppm NaF toothpaste rather than the 1000ppm NaMFP toothpaste. These results are in good agreement with the findings reported by Altenberger *et al.* (2010) and Hattab (2013) who reported higher fluoride uptake by NaF toothpaste than NaMFP (27, 28). This could be due to the fact that NaF, rather than the covalently bonded NaMFP toothpaste, is ionically bonded, which appears to be critical for fluoride uptake. However, in contrary to this, in a study by Patil *et al.* (2014), a higher F uptake has been observed for NaMFP-based toothpaste compared to NaF toothpaste (18).

In this study, the pH of the toothpaste was measured prior to the immersion of the sample in slurry water. The highest uptake of fluoride was seen in the NaF 1000ppm group with a pH value of 8.46 followed by NaMFP 1000ppm with a pH value of 5.98, NaMFP with a pH value of 6.65 and NaF 500ppm with a pH value of 7.14. There was no significant correlation between pH value and enamel uptake of fluoride. Buzalaf *et al.* in their study showed that reduction of dentifrice pH did not affect fluoride concentration (29), but Altenberger *et al.* in 2010 (27) and Patil *et al.* in 2014 (18) reported that fluoride uptake by enamel is higher at lower pH. The mechanisms by which the reduction of pH could increase the anti-caries efficacy of the dentifrice still are not completely understood; eventhough, the increased formation of CaF2-like deposits has been suggested (30). However, a recent study revealed that caries in children using the acidic toothpaste in the concentration of 550 μg F/g was not significantly different from that represented in children using a neutral toothpaste in the concentration of 1100 μg F/g (31).

Further detailed *in vivo* studies with larger sample sizes may be required for evaluating the uptake of fluoride by tooth enamel with different fluoride formulations in the dentifrice.

Conclusion

Regardless of the limitations of the present study, it seems there is a positive correlation between the fluoride concentration of dentifrices and the fluoride uptake by enamel. The toothpastes containing NaF are more effective than toothpastes containing NaMFP and dentifrice pH has no influence on fluoride uptake by enamel.

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