Original Article

Remineralizing Effect of Child Formula Dentifrices on Artificial Enamel Caries Using a pH Cycling Model

Beheshteh Malekafzali[1], Maliheh Ekrami[2], Armin Mirfasihi[3], Zahra Abdolazimi[4]

[1]Associate Professor, Department of Pediatric, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Post Graduate Student, Department of Operative, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Dental Student, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Post Graduate Student, Department of Pediatric, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding author:
Z. Abdolazimi, Department of Pediatric, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran

zahraabdolazimi@yahoo.com

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Abstract

Objectives: Although fluoridated toothpastes are among the most accessible fluoride sources for caries prevention, their remineralization potential remains questionable. This study sought to compare the effects of 5 different child formula dentifrices on remineralization of artificial primary enamel caries using a pH cycling model.

Materials and Methods: Twenty sound primary canine teeth were immersed in demineralizing solution for 96 hours to produce 100µm-deep artificial caries. The teeth were then longitudinally sectioned into 100-150 µm-thick slices and randomly divided into 5 groups and treated as follows: group A. Calcium phosphate toothpaste, group B. Pooneh children's toothpaste, group C. Biotin toothpaste, group D. Crest children's toothpaste and group E. Darougar children's toothpaste. The specimens underwent a pH cycling model for 10 days. The degree of demineralization before and after treatment and its changes were evaluated under a polarized light microscope and a stereomicroscope and data were statistically analyzed using repeated measures ANOVA and post-hoc test.

Results: Stereomicroscopic analysis showed that only group C had a significant difference with other groups ($P<0.001$) and no significant differences were found between the remaining groups ($P>0.05$). Polarized light microscopic analysis revealed that in addition to group C, a significant difference was detected between groups A and B ($P=0.02$) and calcium phosphate toothpaste showed higher efficacy.

Conclusion: All the understudy toothpastes had remineralizing effect but calcium phosphate children's toothpaste had the highest and biotin toothpaste had the lowest efficacy.

Key words: Fluoride; Toothpastes; Tooth remineralization

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INTRODUCTION

Although preventable, tooth decay still remains a major dental health hazard in developing countries [1, 2]. Tooth caries is known as the most prevalent chronic disease of the childhood with a prevalence rate 5 times that of asthma and 7 times that of Hay fever [3]. The progression of primary caries may be stopped by proper enamel surface remineralization treatments. This issue signifies the importance of modern concepts regarding the remineralization/demineralization of enamel surfaces [4]. Increased remineralization and decreased demineralization of enamel depend on the fluoride, phosphate, hydroxide and calcium contents of the saliva [5, 6]. However, fluoride, especially in its topical form, is the most effective substance to enhance enamel remineralization [4]. Fluoride ions in presence of sufficient amounts of calcium and phosphate ions in the saliva can thermodynamically enhance the growth of enamel crystals and remineralize the demineralized enamel; also, by interfering with the metabolism of plaque microorganisms, fluoride ions may prevent acid production by these bacteria [3, 7, 8]. Fluoride is usually added to water sources in different communities and is also available as nutritional supplements, topical agents and also in the form of mouth rinses and dentifrices. Fluoride toothpastes are the most common source of topical fluoride for the majority of children. Moreover, fluoride toothpastes are among the most important cariostatic agents and are considered as the most common vehicle for fluoride delivery to the oral cavity [9]. The first study on the efficacy of fluoride toothpastes for decreasing the prevalence of caries was published in 1954 [10]. Holt and Murray demonstrated a significant reduction in incidence of caries following tooth brushing with fluoride toothpastes in 100 studies [11]. Rirattanapong et al, in their study found that the depth of carious lesions in primary teeth treated with non-fluoride toothpaste was significantly higher than that in group treated with fluoride toothpaste [12].

Yimcharoen et al. indicated greater efficacy of a dentifrice containing 500ppm fluoride compared to casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) toothpaste and a dentifrice with 260ppm fluoride for prevention of caries progression in primary teeth [4]. However, aside from the beneficial effects of fluoride on prevention of caries, numerous studies have shown that excessive use of fluoride toothpastes may increase the risk of dental fluorosis [9].

The remineralizing capacity of products produced by foreign manufacturers has been extensively studied but to the best of our knowledge, no study has investigated the remineralizing potential of domestically manufactured toothpastes. Considering the high cost of foreign-made dental hygiene products and the national manufacturers' claims regarding the high fluoride content of their products, this in-vitro study sought to assess the effects of 5 different child formula dentifrices on remineralization of artificial caries in primary teeth using a pH cycling model.

MATERIALS AND METHODS

Toothpastes used in the present study:

The 5 toothpastes, their fluoride concentration and type of fluoride compound in the 5 treatment groups were as follows:

Group A. Treated with calcium phosphate children's toothpaste (GC MI Paste Plus, 900 ppm, 0.2% NaF, GC Corporation, USA)

Group B. Treated with Pooneh children's toothpaste (Paaksun, 0.38% sodium monofluorophosphate, Tehran, Iran)

Group C. Treated with biotin children's toothpaste (non-fluoride, Laclede, USA)

Group D. Treated with Crest children's toothpaste (Crest, 500 ppm, NaF, Procter & Gamble, Germany)

Group E. Treated with Darougar children's toothpaste (Kaf Co., sodium monofluorophosphate, Tehran, Iran)

Slurry of each toothpaste was prepared by 3:1 weight ratio of deionized water to toothpaste; 17g of each toothpaste was transferred to 5 tubes containing 51 mL of deionized water and then mixed using a vibrator (Vortech, Korea).

Preparation of demineralizing and remineralizing solutions:

The demineralizing solution contained 0.05M acetic acid, 2.2 Mm $ClCa_2$, 2.2 Mm NaH_2PO_4 and 1M KOH for adjusting the pH as 4.5. The remineralizing solution contained 1.5 mM

$CaCl_2$, 0.15M KCl and 0.9 mM NaH_2PO_4 and had a pH of 7.

Artificial carious lesion formation:

This in-vitro experimental study was conducted on 20 extracted sound primary canine teeth. Debris and tissue appendages were removed and the teeth were visually examined to ensure absence of white/brown lesions, cracks, hypoplasia, fluorosis and pigmentation. The selected teeth were stored in saline solution. The surfaces of specimens were entirely coated with one layer of acid-resistant varnish (Max Factor, France) except for a 1x1mm window on the buccal enamel surface; which remained intact.

The specimens were immersed in the demineralizing solution at 37˚C for 96 hours to produce artificial carious lesions with an approximate depth of 100µm. Specimens were mounted in transparent acrylic blocks (for the accuracy of sectioning) and longitudinally cut in buccolingual direction through the middle of the window into 100-150µm thick sections by a hard tissue microtome (Accutom-50; Struers, Copenhagem, Denmark). After excluding the defected sections, the remaining 40 were randomly divided into the above-mentioned groups. Before starting the pH cycling model, the surface of specimens was painted with acid-resistant varnish (Max Factor, France) under a stereomicroscope and only the surface of lesions remained intact (uncovered) for surface treatments. The specimens were stored in 100% humidity.

The pH cycling model:

All specimens were subjected to pH cycling models for 10 days. Each specimen was immersed in the demineralizing solution (10mL for each specimen) twice a day for 3 hours and for 2 hours in the remineralizing solution (10mL for each specimen) in-between the demineralization cycles. Each specimen was immersed in the toothpaste slurry (5mL for each specimen) for 60s before the onset of the first demineralization cycle and before and after the second demineralization cycle.

Next, all specimens were stored in the remineralizing solution in an incubator at 37˚C overnight. After applying each phase of the pH cycling model, specimens were rinsed with deionized water for 30 seconds in order to prevent the cross reaction of solutions. Fresh toothpaste slurry was used for each cycle of remineralization and demineralization. The pH of solutions was measured daily by a pH meter (Jenway 3310,UK). Separate plates were used for each group of specimens in all phases of the experiment. After completion of 10-day pH cycle and post-treatment assessments, acid-resistant varnish was completely removed using acetone. Images were obtained of all teeth sections before and after exposure to treatment regimens under a polarized light microscope and a stereomicroscope. The images were transferred to a software program where the depth of lesions was measured. Data were entered into SPSS version 16 software and descriptive statistics were reported for different groups in two phases of before and after treatment using a polarized light microscope and a stereomicroscope. Repeated measures ANOVA was applied for the assessment of changes in depth of lesions before and after treatment with different toothpastes using a polarized light microscope (Zeiss, Germany) and a stereomicroscope (SZX, Olympus, Japan). Considering the inequality of variances, Tamhane's post-hoc test was applied for pairwise comparison of understudy groups. $P \leq 0.05$ was considered statistically significant.

RESULTS

The depth of lesion was measured in 3 different sites in each section before and after treatment (at similar sites) and calculated by a software program. The mean and standard deviation of degree of demineralization before and after treatment with different toothpastes and the mean and standard deviation of changes in the degree of demineralization under a polarized light microscope and a stereomicroscope are presented in Tables 1 and 2. Evaluation of changes in the degree of demineralization after treatment with the toothpastes by the two mentioned techniques (using repeated measures ANOVA) revealed significant differences in this respect between different toothpastes in both techniques (P<0.05). Tamhane's post-hoc test showed that only biotin toothpaste had significant differences with the other toothpastes (P<0.05) and no difference was observed between other groups (P>0.05) based on stereomicroscopic analysis. Tamhane's post-hoc test showed that biotin group had significant differences with others (P<0.05). Moreover, a significant difference was found between the calcium phosphate and Pooneh toothpaste groups based on polarized light microscopic analysis (P=0.002)(Table 2). The remineralizing capacity of calcium phosphate toothpaste for artificial enamel lesions was significantly greater than that of Pooneh toothpaste.

DISCUSSION

This in-vitro experimental study aimed to compare the effects of 5 children's toothpastes (calcium phosphate, Pooneh, Biotin, Crest and Darougar) on the remineralization of artificial enamel lesions in primary teeth using a pH cycling model. The degree of demineralization before and after treatment and its changes were calculated using a stereomicroscope and a polarized light microscope. Based on the results of both methods, the biotin toothpaste group had statistically significant differences with other groups. Polarized light microscopic analysis indicated the higher efficacy of calcium phosphate toothpaste compared to Pooneh toothpaste for the remineralization of artificial enamel caries in primary teeth.

In-vitro models facilitate the acquisition of quantitative data for designing clinical trials. The pH cycling model is a powerful tool for evaluation of the preventive effect of different materials on the demineralization/ remineralization process of artificial caries [13].

Table 1. The degree of demineralization and its changes before and after treatment with different toothpastes based on stereomicroscopic analysis

Tooth paste	Depth of lesions		Changes in depth of lesions
	Before treatment	After treatment	
Calcium phosphate	46.06±4.00	38.95±6.81	-7.11±5.89
Pooneh	48.52± 5.68	45.01±6.31	-3.50±3.04
Biotin	49.87 ±19.38	52.57±18.62	2.69±2.54
Crest	52.20±12.35	36.77±5.75	-15.43±10.17
Darougar	51.01±5.58	47.41±5.55	-3.59±2.40

&. Level of significance P≤0.05

Table 2. The degree of demineralization and its changes before and after treatment with different toothpastes based on the polarized light microscopic analysis

Tooth paste	Depth of lesions		Changes in depth of lesions
	Before treatment	After treatment	
Calcium phosphate	101.04±11.3	48.35±13.05	-52.68±16.58
Pooneh	92.25±18.40	79.03±13.35	-13.22±14.76
Biotin	92.61±16.21	101.51±15.99	8.89±1.42
Crest	100.91±29.38	50.02±25.45	-50.89±28.15
Darougar	99.53±16.94	77.47±16.21	-22.06±21.78

&. Level of significance P≤0.05

The pH values of the demineralizing and remineralizing solutions used in our study were equal to those in similar previous studies and also equal to the pH established when brushing with the respective toothpastes in the oral cavity [9]. In order to prevent the risk of reaching the saturation threshold, fresh remineralizing and demineralizing solutions were used and the pH of solutions was measured daily. Use of single sections in our study is of great value in laboratory studies allowing the researcher to evaluate each specimen before and after exposure to the therapeutic regimen and thus, each observed change can be attributed solely to the effect of therapeutic regimen [9]. However, it should be noted that the majority of previous studies in this regard are limited to permanent teeth and foreign-made dental hygiene products. Number of studies on primary teeth and their microscopic changes is scarce [1], making it difficult to compare the results. The organic content of the primary tooth enamel is higher than that of the permanent tooth making the primary tooth enamel softer and more porous and consequently more susceptible to caries compared to permanent enamel [4]. Based on our results, the progression of demineralization was prevented in all groups except for the biotin toothpaste group. In both microscopic techniques, the biotin toothpaste group had significant differences with other groups and biotin toothpaste could not efficiently reverse the process of demineralization. This finding is somehow in accord with the results of previous studies. In a study by Ekambaram et al (2011), the progression of caries was prevented in all groups treated with various concentrations of fluoride. In their study, treatment with children's toothpaste containing 500 ppm NaF caused a significant reduction in depth of caries. Depth of artificial carious lesions significantly increased in group treated with non-fluoride children's toothpaste [9]. In a study by Itthagarun et al (2007), the depth of artificial carious lesions in the groups treated with a pea-sized portion of a non-fluoride toothpaste and half-pea-sized portion of a toothpaste containing 500 ppm fluoride increased by 60%; whereas, the depth of artificial caries in group treated with pea-sized portion of 500ppm fluoride toothpaste increased by 19% [14]. Thaveesangpanich et al (2005), demonstrated that pea-sized portion of 500ppm fluoride toothpaste was more effective than non-fluoride toothpaste for prevention of demineralization in primary tooth enamel using 7 and 10-day pH cycling models [15]. Based on the results of Lynch et al, in 2004 only toothpastes with 0.014ppm fluoride or higher can decrease the degree of demineralization [16].

A meta-analysis of 70 studies on the efficacy of fluoride toothpastes for prevention of tooth caries in children indicated that fluoride toothpastes significantly decrease the incidence of caries in the permanent dentition [4]. Although the mentioned study did not provide much information about the efficacy of fluoride toothpastes for primary teeth, the results of our study and those of previous ones indicated that incorporation of fluoride into the child formula dentifrices even in small amounts can effectively decrease the progression of caries and at least under in-vitro conditions lead to remineralization of artificial carious lesions. Biotin toothpaste does not contain fluoride; which may explain its ineffectiveness for prevention of demineralization in artificial enamel caries in our study.Based on the stereomicroscopic analysis, except for biotin toothpaste, no significant difference was found between the remaining groups. However, based on the polarized light microscopic analysis, in addition to biotin toothpaste group, a significant difference was also found between calcium phosphate and Pooneh toothpastes. Polarized light microscope is a standard tool for demineralization/remineralization studies and for the assessment of the depth of lesions and their extension. It can provide valuable information about the interaction of materials with enamel, dentin and demineralized hard tissue [17].

Thus, since this method has been used in several studies [2, 9, 14, 19], the results obtained by this method seem to have higher reliability in comparison with stereomicroscopic assessments. Based on the results of Karlinsey et al (2010), it appears that simultaneous incorporation of 500ppm fluoride and tricalcium phosphate into toothpastes has higher cariostatic efficacy compared to the incorporation of fluoride alone or use of non-fluoride toothpastes [2]. Schemehorn et al (1999) showed that addition of calcium and phosphate to toothpastes and mouth rinses improves the process of remineralization and increases fluoride uptake [18]. The results of the afore mentioned studies are in concord with our findings and the difference between these two toothpastes may be due to the specific formulation of calcium phosphate that contains CPP-ACP in addition to fluoride. On the other hand, Yimcharoen et al, in 2011 reported more favorable effects of 500ppm fluoride toothpaste compared to calcium phosphate toothpaste and 260ppm toothpaste on decreasing the progression of artificial caries. This finding is in contrast to our findings [4]. Quiroz et al, (2008) reported dose-dependent effects of fluoride toothpastes on the demineralization/remineralization process [19]. This finding may be attributed to the fluoride content of Pooneh children's toothpaste, which was too low to effectively prevent the progression of caries. Fluoride has been incorporated into calcium phosphate toothpaste in the form of sodium fluoride; while Pooneh toothpaste contains sodium monofluorophosphate. In order to have adequate efficacy in the oral cavity, fluoride ions need to be freely accessible. Sodium fluoride has this characteristic; whereas, sodium monofluoride first has to be hydrolyzed by microbial or salivary phosphatase and thus, there is a possibility that fluoride in Pooneh toothpaste may not be effectively released [8].

However, when using the pH cycling model for the assessment of cariostatic effects of agents, the researchers should be well aware of the limitations of this method and generalization of in-vitro results to the clinical setting. Despite the superiorities of the pH cycling model over the traditional demineralization/remineralization models, some differences exist between this model and the oral environment. The pH cycling model cannot completely simulate the oral conditions because some differences exist in pH variations based on the nutritional habits, hygienic measures, fluoride sources consumed and the composition and quality of saliva. On the other hand, some confounding factors also play a role in the oral cavity. For example, fluoride is diluted with saliva and thus, exposure to fluoride products and their wash out from the oral cavity can never be completely simulated under in-vitro conditions.

CONCLUSION

All the understudy toothpastes had remineralizing effect, but calcium phosphate children's toothpaste had the highest and biotin toothpaste had the lowest efficacy.

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