Appraisal of the remineralizing potential of child formula dentifrices on primary teeth: An *in vitro* pH cycling model

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Abstract

Aim: To evaluate the remineralizing potential of child formula dentifrices on primary teeth using an *in vitro* 7 days pH cycling model. **Materials and Methods:** Twenty-one primary teeth were placed in demineralizing solution for 96 h to produce artificial carious lesions; then cut longitudinally into 100–150 μm thick sections and randomly assigned to three groups. Sections in Group A were treated with dentifrice containing 458 ppm monofluorophosphate (MFP) and sections in Group B with 500 ppm sodium fluoride (NaF). Group C sections were treated with a nonfluoridated dentifrice. **Results:** Group A (458 ppm MFP) and Group B (500 ppm NaF) showed significant decrease in lesion depth, whereas Group C (non F) showed a significant increase in depth ($P \leq 0.05$, paired *t*-test). **Conclusion:** Though dentifrices containing 458 ppm MFP and 500 ppm NaF demonstrated remineralization of carious lesions, it was not complete. Therefore, it is also important to emphasize on other preventive methods in the prevention and/or reversal of carious lesions.

Keywords: Artificial caries, fluoride dentifrices, pH cycling, primary teeth

Introduction

Caries prevention is one of the hallmarks of contemporary dental practice. According to the World Oral Health Report, dental caries remains a major public health problem in most countries, affecting 60–90% of school going children and a vast majority of adults,[1] and this may be due to the changing lifestyles, dietary habits, increased sugar consumption and inadequate exposure to fluorides. The primary teeth are more susceptible to caries development than permanent teeth because of lower mineral and higher organic content of enamel.[2] Compared to adults, the demineralization potential at low oral pH is greater while the remineralization potential at normal pH is lower in children.[3] Hence, the progression of caries will be faster, and reversal will be slower in children, as they depend upon the balance between demineralization and remineralization.

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Fluorides have the ability to remineralize early carious lesions, and can be used as active anticariogenic agents,[4] which are available in the form of dentifrices, mouth rinses, varnishes, gels, and foams. Fluoride dentifrices are the most widely used products that deliver topical fluoride to the oral environment. Most fluoride dentifrices contain fluoride predominantly in the form of sodium fluoride (NaF) or sodium monofluorophosphate (MFP) and other formulations are with amine fluoride or stannous fluoride. The content of fluoride varies between 500 and 1500 ppm and are categorized into low-fluoride (<600 ppm F), standard (1000 ppm F) or high-fluoride (1500 ppm F) dentifrices.[4] The daily use of a fluoridated dentifrice will provide sufficient fluoride to maintain appropriate levels in saliva and plaque to actively influence remineralization, but the major drawback of fluoride dentifrices is the risk of dental fluorosis in children. Fluoride toothpastes contribute approximately 57% of the total daily amount of fluoride ingested by 4- to 7-year-old children, which occurs because of less control over swallowing especially in preschool children.[5] Preventive measures to reduce the ingestion of fluorides from toothpastes are necessary, such as reducing the amount of toothpaste used, supervised brushing in preschool children and developing low-fluoride toothpastes.[6] The American Academy of Pediatric Dentistry recommends low-fluoride dentifrices for children aged 2–6 years twice daily. The amount to be used should be of a small pea or of smear size.[7]

Many studies were conducted to test the de/remineralizing efficacy of fluoridated and nonfluoridated dentifrices on the enamel of permanent teeth,[8-11] with only a few studies conducted on the de/remineralization efficacy of low-fluoridated dentifrices on carious lesions in primary teeth.[12-16] Hence, there is an increasing need to know the effect of fluoride dentifrices on the carious lesions of the enamel in

primary teeth. Therefore, the present study was undertaken to evaluate and compare the de/remineralization potential of different child formula dentifrices on artificial carious lesions in primary teeth using a 7 days pH-cycling model.

Materials and Methods

Dentifrices used

- Cheerio gel®: A fluoride dentifrice manufactured by Group Pharmaceuticals Limited, Malur and marketed by Dr. Reddy's Laboratories Ltd, Solan, Himachal Pradesh, India (contents: 0.35% sodium MFP USP in a flavored gel base)
- Colgate Pokémon toothpaste®: A fluoride dentifrice manufactured by Colgate Palmolive, USA (contents: 0.11% NaF, sorbitol, silica abrasive)
- Children natural toothpaste®: A nonfluoridated dentifrice manufactured by Pigeon Company, Korea (contents: Calcium phosphate, glycerine, maltilol, carrageenan, flavor), which served as a negative control.

De/and remineralizing solutions

The demineralizing solution was prepared to create the artificial lesions. It contained 2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4, and 0.05M acetic acid. 1M KOH was used to adjust pH to 4.4. The remineralizing solution contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15M KCl and pH of 7.[17]

Dentifrice supernatants

The dentifrice supernatants were prepared by thoroughly mixing a 3:1 ratio (by weight) of deionized water and dentifrice, which was then centrifuged at 4000 rpm for 20 min.

Lesion formation

Twenty-one sound primary teeth indicated for extraction due to preshedding mobility were collected, and soft tissue debris was cleaned and then stored in 0.2% thymol solution. The teeth were inspected for cracks, hypoplasia and white spot lesions and then coated with an acid resistant nail varnish, leaving a narrow window, approximately 1 mm wide on the sound, intact surface of the buccal or lingual enamel. Then, they were immersed in demineralizing solution for 96 h to produce artificial carious lesions of 150–200 μm deep. The teeth were embedded in self-cure acrylic resin blocks. A hard tissue microtome (Leica 1600 Saw Microtome®, Germany) was used to section the teeth longitudinally through the lesions to produce enamel specimens of approximately 100–150 μm thick. The damaged specimens were discarded, and the rest of the specimens were randomly assigned for each of the three groups (Groups A, B and C). Polarizing light microscopy was utilized to record the depth of the lesions. The sections were painted under a stereomicroscope with acid resistant nail varnish leaving the lesion surface exposed for exposure to experimental solutions. The specimens were suspended with dental floss in a beaker containing deionized water and sealed with paraffin wax to achieve 100% humidity until usage.

pH-cycling model

All of the specimens in a particular group were placed in the pH-cycling system on an orbital shaker (Kemi Company™, Kadavil Electromechanical Industries, Ernakulam, Kerala) for a period of 7 days. Each cycle involved 3 h of demineralization twice daily, with 2 h of remineralization between periods of demineralization. Dentifrice supernatant was treated for 60 s before the first demineralization and both before and after the second demineralization. Sections were then placed in the remineralizing solution overnight.

The demineralizing, remineralizing solutions and dentifrice supernatants were freshly prepared for each cycle and stored in separate containers designed for each group throughout the experimental period. Before a topical treatment with supernatant solutions, the teeth were removed from the de-/remineralizing solutions and thoroughly washed with deionized water. The de-/remineralizing solutions and supernatant solutions were changed daily to prevent depletion or saturation of the solutions and accumulation of enamel dissolution products. The sections were then studied under polarized light microscopy to evaluate the lesion depth before and after 7 days.

Evaluation techniques

Polarizing light microscopy measurements

For clear demarcation between sound and carious enamel, the specimens were imbibed in water and then recorded using polarizing light microscope both before and after pH-cycling, to evaluate qualitatively the lesion depth in each enamel section [Figures 1-3]. The depths of the lesions were measured with a computerized calculation method using a software program (ProgRes®, Germany).

Results

Seven sections of Group A were treated with cheeriogel* and the mean score, and standard deviation (SD) was 180 ± 26 before pH-cycling and 175 ± 22 after pH-cycling ($P = 0.01$). Similarly in seven sections of Group B treated with Colgate pokeman*, the mean score and SD was 191 ± 2l before pH-cycling and 173 ± 16 after pH-cycling ($P = 0.03$). In seven sections of Group C treated with children's natural, the mean score and SD was 183 ± 30 before pH-cycling and 201 ± 18 after pH-cycling ($P = 0.04$) [Table 1]. In all the groups, there exists a statistical significance ($P < 0.05$).

Inter group comparison revealed that, the mean and SD in Groups A and B after pH Cycling had decreased than the mean and SD before pH cycling. Only mean and SD for Group C had increased after pH cycling.

The means and SD's of the pretreatment lesion depths between Groups A, B and C were not significantly different from each other ($P = 0.745$) as shown in Table 2. This shows that, even though, the specimens were sectioned from different primary teeth, the variations among the

Table 1: Comparison of the depth of the lesion before and after pH cycling in Group A (cheerio gel), Group B (Colgate paokeman) and Group C (children's natural)

Groups	Number of samples	Treatment	Mean	SD	*t*-test	*P*	% change
A	7	Before	180.778	26.0341	−2.95	0.0135*	−3.0714
		After	175.391	22.3048			
B	7	Before	191.277	21.266	1.303	0.035*	−10.4281
		After	173.214	16.420			
C	7	Before	183.680	30.491	1.386	0.042*	9.0503
		After	201.958	18.620			

SD: Standard deviation, *P*<0.05 statistically significant, *Statistically significat

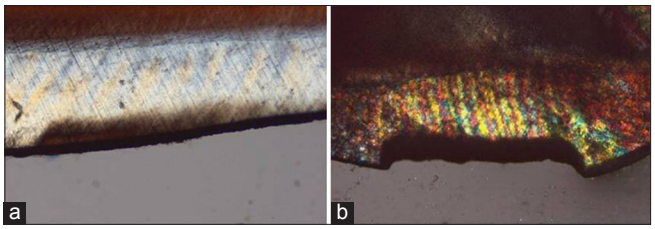

Figure 1: Polarized light micrographs of enamel lesions before (a) and after (b) pH cycling for Group A

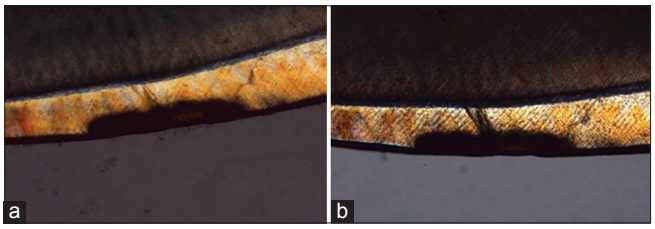

Figure 2: Polarized light micrographs of enamel lesions before (a) and after (b) pH cycling for Group B

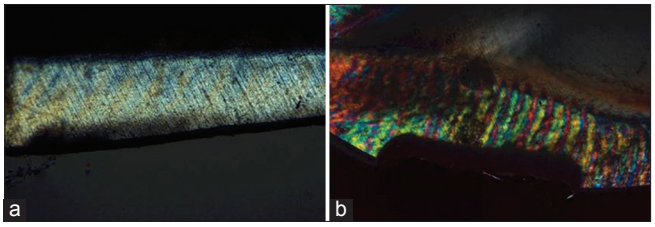

Figure 3: Polarized light micrographs of enamel lesions before (a) and after (b) pH cycling for Group C

teeth did not show a major effect on the progress of demineralization.

On comparison, the posttreatment lesion depths of Groups A, B and C showed Mean and SD as 175 ± 22, 173 ± 16 and 201 ± 18 respectively, which was found to be statistically significant ($P = 0.012$) as shown in Table 3.

On overall comparison, the lesion depths in Groups A and B decreased by 3% and 10% respectively, while Group C demonstrated an increase in lesion depth by 9% [Table 1]. Comparisons using ANOVA and paired *t*-test showed that Groups A and B were significantly different from Group C, but

Table 2: Comparison of the depth of the lesion among the pretreatment Groups A, B and C using ANOVA

Groups	Mean	SD	*F* *P*
A	180.7761	26.03408	
B	191.2765	21.26562	0.300 0.745^{NS}
C	183.6824	30.49277	

NS: Not significant; SD: Standard deviation; *P*<0.05 statistically significant

Table 3: Comparison of the depth of the lesion among the posttreatment Groups A, B and C using ANOVA

Groups	Mean	SD	*F* *P*
A	175.3914	22.30506	
B	173.214	16.41744	5.0214 0.012*
C	201.9586	18.62285	

*Represents significance at 0.05 level. SD: Standard deviation; ANOVA: Analysis of variance

there was no statistical significance between Groups A and B. On using Duncan's multiple range test, it was observed that Group A differ from Group C ($P < 0.05$) but not with Group B ($P > 0.05$), whereas Group B differs significantly from Group C ($P < 0.05$).

Discussion

The caries process is a continuum resulting from an imbalance between many cycles of demineralization and remineralization rather than a unidirectional demineralization process. Fluoride has been shown to have a greater inhibitory effect on caries progression than on caries initiation.[18] The levels needed to significantly reduce caries or, at a mechanistic level to shift the balance from caries initiation and progression to caries reversal are apparently in the sub ppm range.[19] Hence, the present study was undertaken to evaluate and compare the remineralization effects of different child formula dentifrices on artificial carious lesions in primary teeth using a 7 days pH-cycling model.

Artificial early caries-like lesions of the enamel showed all the principal histological features of natural caries and had

been successfully used to study the remineralization of enamel *in vitro*. These artificial lesions of the enamel were more homogenously reproducible than natural lesions and thus provide a reliable experimental model,[20] hence; carious lesions were artificially produced in the present study.

Extracted or naturally exfoliated primary teeth (molars, canines, and incisors) were used for lesion formation in this study. Though there are variations in the morphology of individual teeth, it was hypothesized that these variations among the teeth do not play a significant role in caries formation,[16] and in the present study too, the depths in the pretreatment test groups were not statistically different. This implies that, even though, the specimens were sectioned from different teeth, the variations among the teeth did not show a major effect on the progress of demineralization.

Single-section model, as used in this study had the advantage that a single section was fully evaluated prior to the experimental period and then again after the exposure period. Thus, any change was only due to exposure of the experimental solutions. The de/remineralizing solutions and supernatant solutions were changed daily to prevent depletion or saturation of the solutions and accumulation of enamel dissolution products.

The concept of *in vitro* pH cycling was first proposed by ten Cate and Duijsters in 1982, in experiments where they exposed artificial carious lesions in enamel to a combination of remineralizing and demineralizing solutions.[17] Two types of pH-cycling models are used, the 7-day pH-cycling and the 10-day pH-cycling. A 10-day pH-cycling model can be used on the enamel of permanent teeth whereas a 7-day pH-cycling or 10-day cycling with added 0.25 ppm fluoride can be used for primary teeth.[9,14,15,20,21] In the present study, pH-cycling was done for 7 days without the addition of fluoride, because the addition of fluoride could have interfered with the hypothesis being tested.

Fluoride dentifrices remain the most widely used method of delivering topical fluoride. NaF and sodium MFP contain fluoride in chemically distinct forms, and they will differ in their mode of action with respect to caries reduction. The reason for greater retention of oral fluoride from NaF than from MFP could be due to: (1) Fluoride ions diffuse faster from NaF than MFP, by a factor of 1000 in dental enamel; (2) there is no MFP analogue of calcium fluoride, which is important in oral fluoride retention; (3) fluoride ions from MFP bind to a lesser extent to tooth mineral and plaque bacteria than NaF.[4,22,23]

An extensive series of *in vitro* and clinical trials have tested the anticaries efficacy of dentifrices containing NaF or MFP. Many *in vitro* studies[11,16,24-33] suggest that dentifrices containing NaF perform better than dentifrices containing MFP whereas a study[34] concluded no statistically significant difference between the two. Clinical studies[35,36] concluded that NaF was as equally effective to MFP in caries reduction. The efficacy of fluoride toothpastes in clinical trials is potentially influenced by several factors, namely: Fluoride concentration, frequency of use, amount used and rinsing behavior whereas in the *in vitro* studies, the sole factor that plays key role is the fluoride concentration used.[5] This might be the reason for less effectiveness of MFP in the *in vitro* studies than *in vivo* studies. This again might be due to the absence of a key mechanistic step in the *in vitro* studies in determining the clinical efficiency of MFP, namely the hydrolysis of MFP to fluoride ions. These are in accordance with the present study where, both the MFP and NaF dentifrices showed decrease in the lesion depths and the difference between the two was not statistically significant. But the NaF had showed a greater decrease in lesion depth confirming its superior anticaries efficacy over MFP.

Conclusion

Based on the results obtained from the present study, we could conclude that the child formula dentifrices containing NaF and sodium monofluorophosphate have the ability to remineralize the initial carious lesions in the primary teeth as both reduced the depth of the artificial carious lesions. But, it is also important to emphasize other preventive methods in the prevention and/or reversal of caries as the child formula dentifrices could not completely remineralize the carious lesions.

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