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Comparison of the remineralizing potential of child formula dentifrices

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Background. Although child formula fluoridated dentifrices can be used safely by young children their remineralizing capability remains questionable.

Aims. To evaluate the remineralizing potential of child formula dentifrices on primary teeth.

Design. *In vitro* single-section technique utilizing a 7 days pH-cycling model.

Methods. Primary teeth were placed in demineralizing solution for 96 h to produce artificial carious lesions 100 μm deep, and then cut longitudinally into 50 sections 100–150 μm thick and randomly assigned to five groups. Sections in Groups A to D were treated with dentifrices containing 500 ppm AmF, 500 ppm MFP, 500 ppm MFP and xylitol, or 500 ppm NaF, respectively. Group E sections were treated with a nonfluoridated dentifrice.

Outcome measurements. Lesions were evaluated using polarized light microscopy and microradiography.

Results. Group D (500 ppm NaF) sections exhibited a significant decrease in lesion depth, whereas those in Group E (nonF) showed a significant increase in depth ($P < 0.05$, paired t-test). Decrease in lesion progression was observed in Groups A, B and C.

Conclusions. The 500 ppm NaF dentifrice demonstrated remineralization of carious lesions by virtue of a significant decrease in lesion depth; whereas dentifrices that contained AmF, MFP and MFP with xylitol decelerated the progression of demineralization.

Introduction

Many studies have shown that early carious lesions can be remineralized *in vitro*[1,2] and *in vivo*[3,4], with fluoride being one of the most significant agents for promoting remineralization[5–7]. Fluorides are added to community water supplies and are available in the form of dietary supplements, topical applications, mouthwashes, and dentifrices[8,9]. The most common source of topical fluoride for the majority of children is dentifrices[10]. Furthermore, it is also generally accepted that the use of fluoride containing dentifrices has been one of the most important factors in the decline of dental caries[11].

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In 1954, the first report of the ability of a fluoride-containing dentifrice to reduce the incidence of caries in children was published[12]. Subsequently, Holt and Murray[13] identified 100 trials which showed that brushing with a fluoridated dentifrice significantly reduced the incidence of dental caries.

A number of studies have identified early on excessive use of a fluoride dentifrice as being risk factors for dental fluorosis[14–17]. The permanent dentition is at risk of fluorosis during the first 7 years of life; excessive fluoride ingestion by children over 7 years of age is less likely to cause dental fluorosis[18]. Moreover, it has been found that the most critical period of fluorosis risk for the maxillary central incisors is from 20 to 28 months[19–24].

The objective of any fluoride preventive therapy is to attain maximum anti-caries action with the minimum risk of fluorosis. This risk is a function of both the amount of

dentifrice ingested and the fluoride concentration. Even when similar amounts of dentifrices are placed on toothbrushes, younger children ingest greater amounts of fluoride than older children, probably due to less control over their swallowing reflexes[25]. Young children are considered to ingest enough fluoride from dentifrice alone to be at risk from dental fluorosis[26].

Although small quantities of fluoridated dentifrices may carry a lower risk of fluorosis, this must be balanced against the inevitable reduction in cariostatic effects[13]. One technique for reducing the amount of fluoride ingested is to minimise the amount of dentifrice placed on the toothbrush. Another approach has been to reduce the concentration of fluoride in dentifrices to either 500 ppm or even as low as 250 ppm.

Many investigators have studied the de/remineralisation of enamel lesions in permanent teeth by conventional fluoridated and nonfluoridated dentifrices[27–30], only a few studies have tested low fluoride concentration dentifrices on primary teeth[31,32]. Consequently, the effectiveness of low fluoride dentifrices on primary teeth remains unclear. Thus, the objective of this *in vitro* study was to evaluate and compare the de/remineralizing potential of different child formula dentifrices with a low fluoride concentration when applied to artificial caries lesions in primary tooth enamel using a 7 days pH-cycling model.

Materials and methods

Dentifrices used in the study

The dentifrices and their fluoride type and concentration used in this study were as follows:

Group A = Elmex Peuter® with 500 ppm of amine fluoride (AmF),
Group B = Colgate toothpaste for Kids® with 500 ppm monofluorophosphate (MFP),
Group C = Kidodent Gel® with 500 ppm monofluorophosphate (MFP) and xylitol,
Group D = Colgate Pokeman® with 500 ppm sodium fluoride (NaF) and
Group E = Vicco® – nonfluoridated as control group.

Dentifrice slurry preparation

Dentifrice slurries were prepared in a 3 : 1 ratio of deionised water to dentifrice. To achieve this 17 g of dentifrice were dispensed from the respective tube and then transferred into five tubes to which 51 mL of deionised water were added and then stirred with a stirring rod until well mixed.

Demineralizing and remineralizing solution preparation

Demineralizing and remineralizing buffered solutions were prepared from pro-analysis reagents and deionised water. The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4 and 0.05 M acetic acid. 1 M KOH was used to adjust the pH to 4.5. The remineralizing solution, which contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4 and 0.15 M KCl had a pH of 7.0[33].

Lesion formation

Forty sound extracted or naturally exfoliated primary teeth were cleaned to remove soft tissue debris and inspected for cracks, hypoplasia, and white spot lesions. The teeth were coated with acid resistant nail varnish (Revlon®, New York, USA), leaving a narrow window, approximately 1 mm wide on the sound, intact surface of the buccal and/or lingual enamel. They were then immersed in the demineralizing solution for 96 h to produce artificial carious lesions approximately 100 μm deep. A hard tissue microtome (Leica® 1600 saw microtome, Wetzlar, Germany) was used to section the teeth longitudinally through the lesions to produce enamel specimens, approximately 100–150 μm thick. After discarding the damaged sections, ten sections were randomly assigned to each of the five experimental groups. Polarizing light microscopy (Orthoplan®, Leitz, Wetzlar, Germany) and microradiography (Softex® ISR-20, JIRA, Tokyo, Japan) were utilized to record the depth and mineral content of a lesion before and after treatment with the different dentifrice. Prior to pH-cycling, each section was coated,

under a stereomicroscope, with acid resistant nail varnish leaving only the lesion surface exposed. The enamel sections were then stored in 100% humidity until used.

pH-cycling model

The specimens were placed in the pH-cycling system on an orbital shaker (GFL®, Burgwedel, Germany) for a period of 7 days. Each cycle involved 3 h of demineralization twice daily, with 2 h of remineralization between the periods of demineralization. One minute dentifrice treatments using 3 : 1 deionized water : dentifrice, (5 mL/section) were given before the first demineralizing cycle and both before and after the second demineralizing cycle. Sections were then placed in the remineralizing solution overnight. All solutions, demineralizing, remineralizing, and the dentifrice slurries, were freshly prepared for each cycle; separate containers were used for each group throughout the experimental period. The pH level of the demineralizing and remineralizing solutions was measured before every cycle. After 7 days of pH cycling the nail varnish on each section was carefully removed with acetone before the post-treatment evaluations[34,35].

Evaluation techniques

Polarizing light microscopy measurements (PLM)

Polarizing light microscopy (Leitz®, Wetzlar, Germany), was used to record the characteristics of the lesions, both before and after the various treatments. This was accomplished by imbibing the sections in water, which promotes a clear demarcation between sound enamel and an initial carious lesion. Any changes in the lesions caused by the treatment could be evaluated from the photo-micrographs taken before and after the experiment at the same magnification, (6.3× objective lens × 8× camera lens) under the PLM. A photograph of a standardized 100 μm scale was taken to facilitate calibration of the lesion depth. After being developed, each film was mounted and its image was captured using Adobe Photoshop 6.0, which enabled automatic measurement of the depth of each lesion. Thus, an actual change, or a percent change could be calculated.

Micro-radiography measurements (MRG)

Each of the specimens was mounted onto high resolution film (Kodak USA, Rochester, New York) and exposed to Cu (Kα**)** X-rays (Softex IRS-20®, Jira, Japan) at 15 kV and 3 mA for 60 s. Standard Kodak chemistry was used to develop the exposed films. All of the films were subjected to the same developing process (60 s in the developer, 60 s rinse with water, followed by 60 s in the fixer). After developing, each film was mounted and the image captured in an image analysis system (Leica Qwin Live®, Wetzlar, Germany), which was accomplished by capturing the image on the film when observed under an optical microscope (Zeiss®, Gottingen, Germany). The microscope was connected to a colour video camera (JVC®; TK-C1 380, JVC, Tokyo, Japan) and computer system. The captured image was utilized to measure the mineral profile before and after treatment for the same lesion and also to compare the changes among the lesions in the five test groups using a computer program (Microsoft Excel®; Microsoft Corporation, Redmond, Washington DC) which calculated relative mineral density based on the data from sound enamel.

Results

Lesion depth

The lesion depth of each section was measured in three different areas, at the same sites for both before and after the treatment. With the aid of a specially designed computer program, the depth of the lesion on each section was then calculated. The mean and standard deviation of the pre-treatment lesion depth from each group ranged from 82.4 ± 13.3 μm to 93 ± 15.36 μm. Among these pre-treatment lesion depths, no statistically significant differences were obtained ($P = 0.6511$, ANOVA). This implies that even

though all of the specimens were sectioned from different teeth, the variations among the teeth did not show a major effect on the progression of demineralization. It was not unreasonable, therefore, to disregard these variations when the de/remineralization efficacy of different dentifrices were evaluated and compared after pH cycling.

The results from the lesion depth measurements after the 7 days pH cycle showed that the lesions in Groups A (Elmex Peuter®; GABA BV, Ax Almere, Netherlands), B (Colgate toothpaste for Kids®; Colgate Palmolive (India) limited, Mumbai, Maharashtra, India), C (Kidodent Gel®; Indoco Remedies limited, Mumbai, Maharashtra, India) and E (Vicco®; Vicco laboratories, Mumbai, Maharashtra, India) increased by 9%, 10%, 21% and 28%, respectively, whereas there was a reduction in lesion depth by 16% in Group D (Colgate Pokeman®) specimens. A paired *t*-test confirmed a statistically significant difference ($P < 0.05$) between the before and after lesion depth measurements in Groups D (Colgate Pokeman®; Colgate Palmolive, Petaling jaya, Selangor, Malaysia) and E (Vicco®) (Table 1).

Representative PLM photographs of the sections from each Group, before and after treatment, are exhibited in Figs. 1–5. The photo-micrographs confirmed an increase in lesion depth in the sections from Groups A (Elmex Peuter®), B (Colgate toothpaste for Kids®), C (Kidodent Gel®) and E (Vicco®), and a decrease in lesion depth in those from Group D (Colgate Pokeman®).

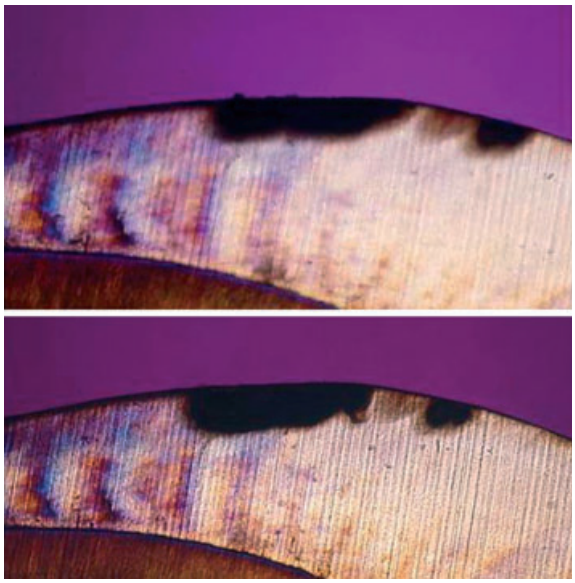

Fig. 1. Polarized light photo-micrographs of a representative enamel lesion in Group A (Elmex Peuter®), before (top) and after (bottom) 7 days of pH cycling. Note an increase in the depth of the lesion.

Mineral content and distribution

Graphs showing the relationship between lesion depth (on the *x*-axis) and relative percent (%) mineral content (on the *y*-axis) are displayed in Fig. 6. Similar to the results obtained from PLM, the mineral profiles of the lesions in Groups A (Elmex Peuter®), B (Colgate toothpaste for Kids®), C (Kidodent Gel®) and E (Vicco®) showed a decrease in mineral content. Conversely, an increase in mineral content was evident in the specimens from Group D (Colgate Pokeman®) (Fig. 6).

Table 1. The changes in mean lesion depth and the percentage change in enamel lesions after 7 days pH cycling.

Test product	F^- source	Mean lesion depth (μm ± SD)		Percentage change in lesion depth
		Pre-treatment	Post-treatment	
Elmex Peuter®	AmF 500 ppm	92 ± 25	96 ± 16	9 ± 25
Colgate toothpaste for Kids®	MFP 500 ppm	93 ± 15	100 ± 15	10 ± 25
Kidodent®	MFP 500 ppm and xylitol	82 ± 13	98 ± 20	21 ± 28
Colgate Pokeman®	NaF 500 ppm	91 ± 17†*	71 ± 13†*	−16 ± 21‡*
Vicco®	None, no xylitol	89 ± 22†*	116 ± 53†*	28 ± 29‡*

*indicates a significant difference at $P < 0.05$.
†Paired *t*-test.
‡ANOVA and Student–Newman–Keuls tests.

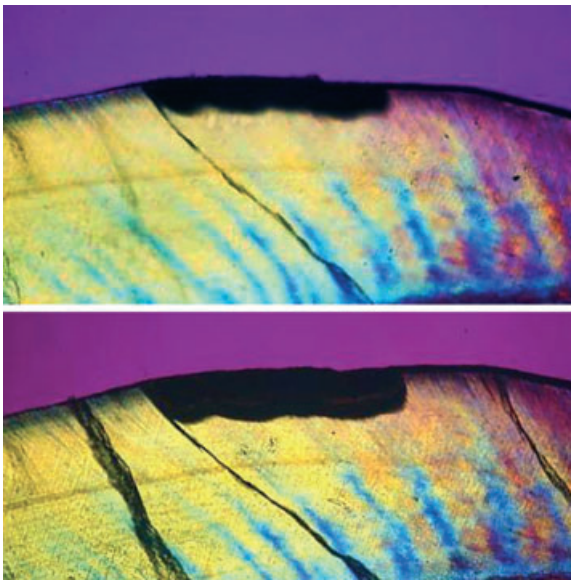

Fig. 2. Polarized light photo-micrographs of a representative enamel lesion in Group B (Colgate toothpaste for Kids®), before (top) and after (bottom) 7 days of pH cycling. Note an increase in the depth of the lesion.

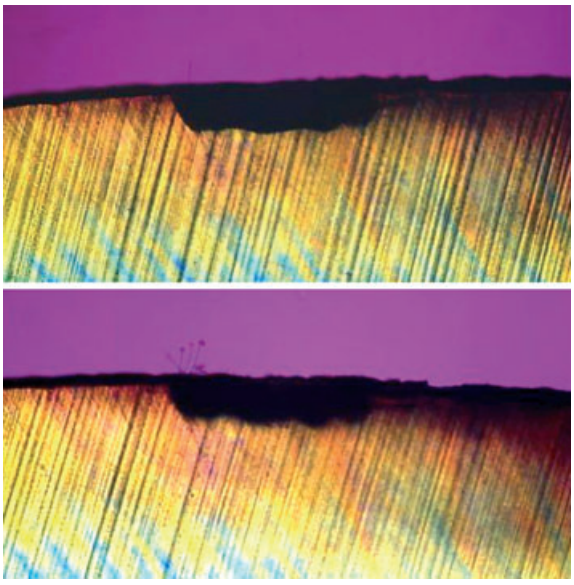

Fig. 4. Polarized light photo-micrographs of a representative enamel lesion in Group D (Colgate Pokeman®), before (top) and after (bottom) 7 days of pH cycling. Note a decrease in the depth of the lesion.

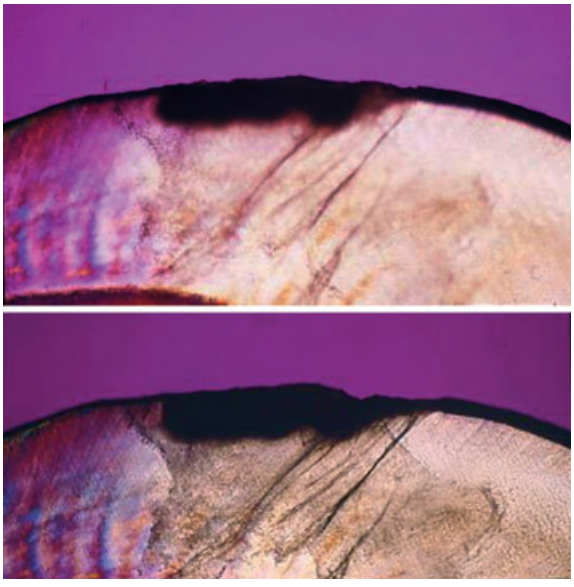

Fig. 3. Polarized light photo-micrographs of a representative enamel lesion in Group C (Kidodent Gel®), before (top) and after (bottom) 7 days of pH cycling. Note an increase in the depth of the lesion.

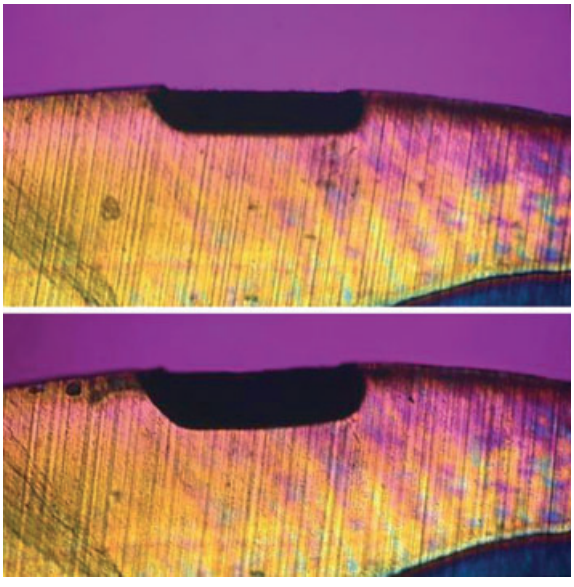

Fig. 5. Polarized light photo-micrographs of a representative enamel lesion in Group E (Vicco®), before (top) and after (bottom) 7 days of pH cycling. Note a significant increase in the depth of the lesion.

Discussion

The term 'pH cycling' refers to an *in vitro* experiment involving the exposure of specimens (enamel and/or dentine) to a combination of remineralization and demineralization. The solution concentration and pH should be kept within the range that exists in the oral fluid [33]. In this study, to avoid the risk of the solutions becoming saturated, fresh demineralizing and remineralizing

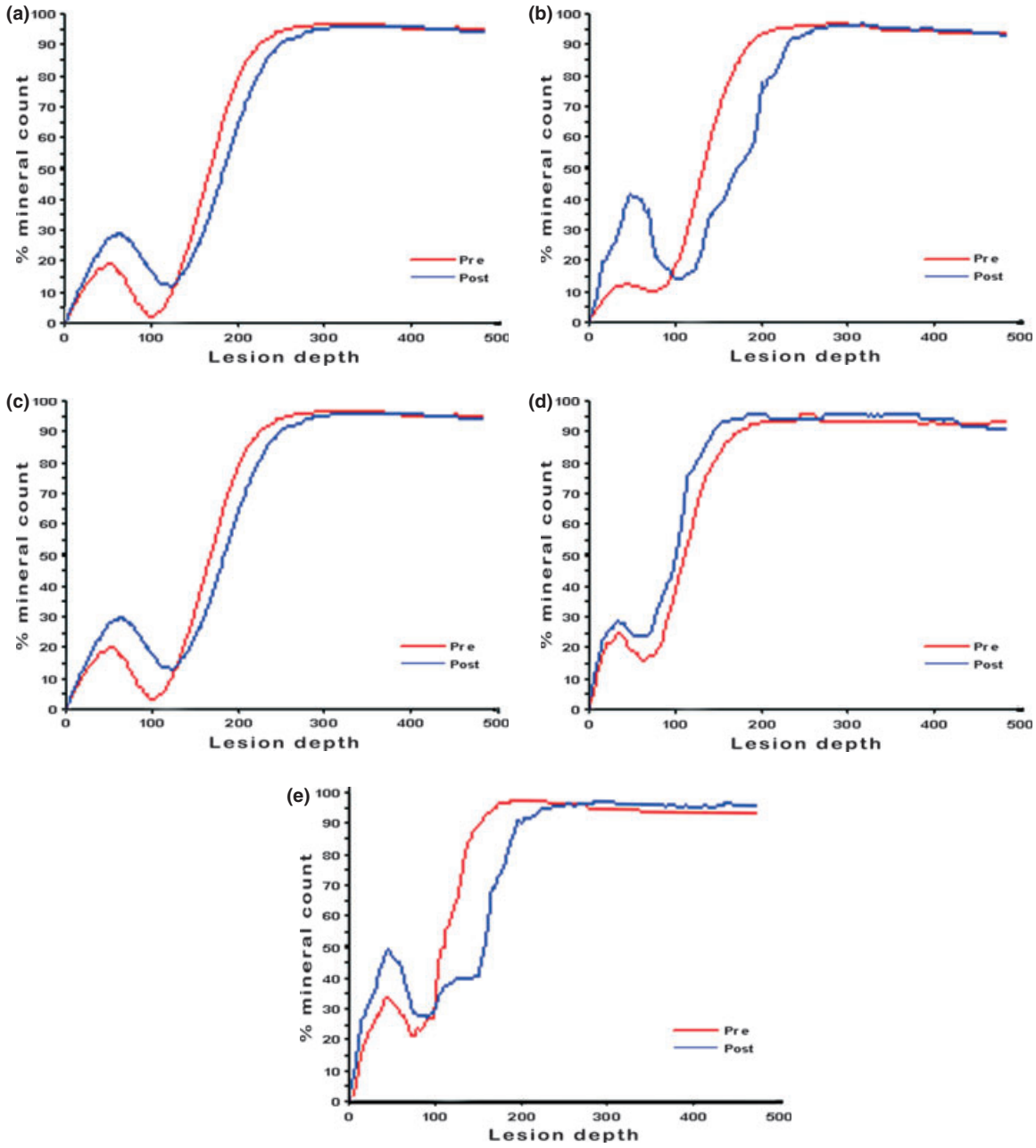

Fig. 6. (a) Graph showing the relationship between the lesion depth on the *x*-axis (μm), and the relative percentage mineral content on the *y*-axis, before and after treatment of Group A (Elmex Peuter®). Note the increase in depth of the lesion. (b) Graph showing the relationship between the lesion depth on the *x*-axis (μm), and the relative percentage mineral content on the *y*-axis, before and after treatment of Group B (Colgate toothpaste for Kids®). Note the increase in depth of the lesion. (c). Graph showing the relationship between the lesion depth on the *x*-axis (μm), and the relative percentage mineral content on the *y*-axis, before and after treatment of Group C (Kidodent Gel®). Note the increase in depth of the lesion. (d) Graph showing the relationship between the lesion depth on the *x*-axis (μm), and the relative percentage mineral content on the *y*-axis, before and after treatment of Group D (Colgate Pokeman®). Note the decrease in depth of the lesion. (e) Graph showing the relationship between the lesion depth on the *x*-axis (μm), and the relative percentage mineral content on the *y*-axis, before and after treatment of Group E (Vicco®). Note the increase in depth of the lesion.

solutions were prepared daily and the pH value was checked every time prior to use.

The single-section model system, as used in this study, had the advantage that the same tissue could be measured before and after the

experiment; thus, any changes were due to exposure to the treatment regimen. The pH-cycling model used in this study was a modified version of the one used by Thaveesangpanich and her co-workers[34,35].

Monofluorophospahte (MFP) is an effective caries inhibitor when applied topically to the tooth surfaces. This caries reducing effect has been attributed, by many investigators, to there being a chemical interaction between MFP and enamel apatite. Most of the mechanisms that have been advanced to describe this interaction postulate that fluorapatite is the end product[3,7]. The formation of pure fluorapatite, without contamination by calcium fluoride, which is less stable at physiological pH levels, supports the theoretical advantage of MFP over other fluoridated dentifrices.

The finding that the MFP dentifrice showed less remineralizing efficacy than NaF was not unexpected because our pH-cycling model consists of only an inorganic solution so the enzyme system required for MFP hydrolysis was absent.

Xylitol forms complexes with calcium ions and penetrates into demineralized enamel. It should act as a calcium ion carrier and concentrate calcium, thereby retarding demineralization by lowering the diffusion coefficient of calcium and phosphate ions from a lesion into the solution[36,37]. Furthermore, because xylitol reduces plaque acidity, it shows greater effects when used in combination[38–41]. With fluoride plus xylitol under *in vivo* conditions, the remineralization process can be accelerated as xylitol reduces plaque acidity and allows fluoride to work under less challenging conditions[39]. The less than expected response from (Kidodent Gel®) might have resulted from not only an inorganic environment, but also a chemical interaction with the other constituents of the Kidodent® dentifrice which may have rendered the fluoride unavailable. Another possibility is that the remineralization efficacy in the deep and middle layers of the enamel could not be detected with our methodology.

Researchers have speculated that amine fluoride, a surface-active fluoride typically formulated to act at a low pH, might deliver more fluoride to enamel than other fluoride compounds under *in vitro* testing conditions but this does not take account for the buffering effects of saliva [42,43].

In this study all of the test groups that contained fluoride effectively slowed down the progression of the lesion even though they did not reduce the depth of the lesions. Although, Colgate Pokeman® which contains 500 ppm NaF produced a statistically significant reduction in the depth of the lesions, the nonfluoridated dentifrice, Vicco®, showed a statistically significant increase in the depth of the lesions. These findings suggest that fluoride, even in low concentrations as in child formula dentifrices, can effectively slow down the progression of a carious lesion and/or remineralize demineralized enamel, at least *in vitro*. In general, when prescribing toothpaste for patients, it is strongly recommended that patient's caries risk and their possible risks for fluoride toxicity need to be taken into consideration[44]. Findings of this *in vitro* study however, should be interpreted in caution and in relation to the recent fluoride European guidelines[44], that suggest the use of low dosage F toothpastes, only for the very young children 2 years old and younger.

When pH cycling methods are applied to evaluate caries-preventive agents and treatment, the investigator should realize the limitations of the methods. Although it provides an advantage over traditional de/remineralization models, there are numerous dissimilarities between cycling models and *in vivo* conditions. The pH-cycling model did not entirely simulate the oral conditions where the pH fluctuates frequently and the levels attained depend upon the individual's eating habits, oral hygiene practices, fluoride usage, and the composition and quality of saliva and plaque.

Conclusions

Based on the data obtained from this study, the child formula dentifrices containing amine fluoride, monofluorophosphate and monofluorophosphate with xylitol, decreased carious lesion progression rates *in vitro*. Moreover, 500 ppm sodium fluoride showed

remineralization efficacy on artificial enamel lesions after 7 days of pH cycling.

What this paper adds

- Child formula dentifrices with low fluoride concentrations have the potential to slow down the rate of lesion progression and/or remineralize early enamel carious lesions in primary teeth.

Why this paper is important to paediatric dentists

- Paediatric dentists should know about the various fluoride formulations used in child formula dentifrices and their remineralizing potential.
- Knowledge about the remineralizing potential of child formula dentifrices will help a paediatric dentist to prescribe the appropriate formulation for children who are at high and low risk of dental caries.

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