

# Remineralization effects of two pediatric dentifrices and one regular dentifrice on artificial carious lesion in primary teeth: An *in vitro* study

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## Abstract

**Aim:** The aim of the following study is to know the efficacy of remineralization of two pediatric dentifrices and one regular dentifrice on artificial carious lesions in primary teeth. **Materials and Methods:** A total of 21 teeth coated with nail varnish leaving a window of 1 mm were subjected to demineralization for 72 h. These 21 teeth were then sectioned into two equal parts with a diamond disc. The 42 sections obtained were then evaluated under the stereomicroscope and the demineralization values were noted. The 42 sections were divided into three groups: Group 1: Kidodent, Group 2: Cheeriogel, Group 3: Colgate Total and subjected to remineralization respectively for 7 days. The specimens were again evaluated under the stereomicroscope for the remineralisation values. **Results:** All the three dentifrices showed remineralization with artificial carious lesions. Colgate Total showed higher remineralization rates compared with the other two pediatric dentifrices. Kidodent showed a slightly higher remineralisation rate compared with Cheeriogel which was not significant. **Conclusion/Hypothesis:** The pediatric dentifrices also showed remineralization with artificial carious lesions. Hence, we conclude that, this amount of remineralization was more or less, when compared to the regular dentifrice which showed higher remineralization rates, which could lead to adverse effects, like fluorosis if not used judiciously. On basis of which we hypothesize: Pediatric dentifrices have an appropriate fluoride content, as required by the children, and also does not minimize the cariostatic effects.

**Key words:** Demineralization, pediatric dentifrice, remineralization

## INTRODUCTION

“An ounce of prevention is worth a pound of cure” says an old proverb. It is reasonable to prevent a disease from both the ethical point of view as well as its cost-effectiveness. Prevention is one of the main concepts and forms the cornerstone of health care. It has been recognized as one of the most important characteristics of public health.<sup>[1]</sup> The applications of

preventive measures are relevant to management of many orofacial diseases including dental caries.<sup>[2]</sup>

Dental caries is said to be a disease of civilization. And a civilized society cannot allow a preventable disease to attack almost every man, woman and child. According to the World Health Organization expert committee,<sup>[3]</sup> the decline of dental caries prevalence observed in many countries can be attributed to the widespread use of toothpastes that contain fluoride.

Remineralization is, in the most literal sense mineral deposition after mineral loss during or after a carious attack. Throughout the dental caries process beneath the enamel, it is subjected to repeated demineralization and remineralization, cycles of unknown intensity and duration. The clinical significance of remineralization is where the chemical destruction of enamel can be stopped or reversed

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without the use of restorative material. In children, the potential for demineralization at low oral pH is greater while remineralization at normal oral pH, is lower than in adults.<sup>[4]</sup>

The concept of *in vitro* pH cycling was first proposed by Ten Cate and Duijsters in 1982,<sup>[5]</sup> in experiments where they exposed artificial carious lesions in enamel to a combination of remineralizing and demineralizing solutions. These experiments were designed to stimulate the dynamic variations in mineral saturation and pH associated with the natural carious process.

As fluoride dentifrices have a dose – response relationship; this is important because dentifrices for children usually contain between 250 and 500 ppm fluoride, in order to reduce the risk of fluorosis. But we also need to make sure that these low fluoride dentifrices do not comprise the cariostatic effect.

Hence an *in vitro* study was conducted with a purpose, to compare the remineralization effects of two pediatric and one regular dentifrice on artificial carious lesions in primary teeth.

## MATERIALS AND METHODS

The required data for the study was collected from 42 sections derived from non-carious extracted or exfoliated primary teeth obtained from the Department of Pedodontics and Preventive Dentistry, KLES VK Institute of Dental Sciences, Belgaum. Criteria for inclusion were caries free primary teeth (either extracted or exfoliated). Carious, hypoplastic discolored teeth and teeth with cracked areas and white spots were excluded.

### Storage

Extracted or naturally exfoliated primary teeth devoid of soft-tissue debris were stored in 10% formalin until further use.

### Demineralisation

Teeth were then dried and coated with an acid resistant nail varnish, leaving a rectangular window 4 cm × 3 mm wide for demineralization, on the buccal or lingual surface. They were then completely immersed in demineralizing solution (10 ml/tooth) for 96 h to produce artificial carious lesions. This was in accordance to Ten Cate and Duijsters pH cycle.<sup>[5]</sup>

Demineralizing solution was prepared using the following chemicals: 2.2 mM calcium chloride, 2.2 mM

potassium hydrogen orthophosphate, unstirred solution of 0.05 M acetic acid and 1 M potassium hydroxide pH at 4-5. 1050 ml of distilled water was taken in a beaker and 2.2 g of calcium chloride was added to it. To this, 2.2 g potassium hydrogen orthophosphate, 3 g of acetic acid and 56 g of potassium hydroxide was added. This was in accordance the demineralizing solution used in the pH cycle used by Ten Cate and Duijsters.<sup>[5]</sup>

### Sectioning and division into groups

The teeth were then longitudinally sectioned using either a diamond disc or a hard tissuemicrotome in bucco-lingual direction, such that a part of the demineralized area and normal enamel is present in each section. After discarding the damaged sections, a total of 42 sound sections were obtained, which were then divided into three groups, with 14 sections in each group. The three groups will be as follows:

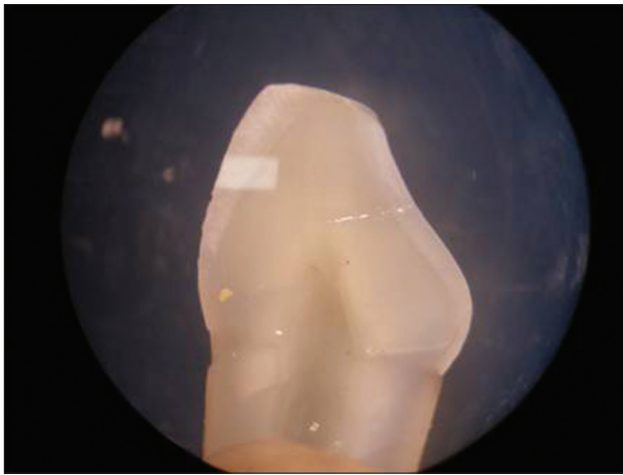
- Kidodent (Warren, Bombay) - Pediatric Dentifrice  
Content: Sodium monofluorophosphate 0.38%, fluoride up to 458 ppm.
- Cheeriogel (Dr. Reddy's, Hyderabad) - Pediatric Dentifrice  
Content: Sodium monofluorophosphate 0.35%, Fluoride up to 500 ppm.
- Colgate Total (Colgate, Himachal Pradesh) - Regular Dentifrice  
Content: Triclosan, Sorbitol, Fluoride up to 1000 ppm.

### Recording demineralisation rates

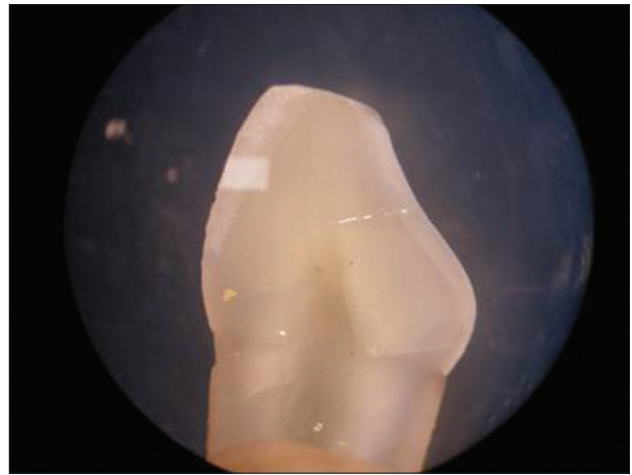
These 42 demineralized sections were then studied under the stereomicroscope to evaluate the lesion depth [Figure 1]. The evaluated values were noted and tabulated. The values were noted by 3 examiners were taken and the average value of all 3 were noted. The cut inner part of the section was then painted with nail varnish except the artificial carious lesion surface for further exposure to remineralization.

### Remineralisation

Remineralizing Solution was prepared using the following chemicals: 0.5 mM Calcium Chloride, 0.9 mM sodium dihydrogen phosphate, 0.15 M potassium chloride pH adjusted at 7. 152 ml of distilled water was taken and 0.1665 g of calcium chloride, 0.108 g of sodium hydrogen phosphate and 11.25 g of potassium chloride was added to it. This was in accordance to the remineralizing solution used in the pH cycle used by Ten Cate and Duijsters.<sup>[5]</sup>



**Figure 1:** Demineralized sections: Viewed under the stereomicroscope



**Figure 2:** Remineralized sections: Viewed under the stereomicroscope

### pH cycle

Dentifrices solution was freshly prepared, by thoroughly mixing a 3:1 ratio of water and dentifrice, centrifuged at 4000 rpm for 20 min.

Each specimen was then subjected to different solutions for a specific period of time in a pH cycle. The pH cycle used, as in accordance to Ten Cate and Duijsters, was as follows:

- Step 1: Dentifrice supernatant solution (5 ml/section) for 60 s.
- Step 2: Demineralizing solution (10 ml/section) for 3 h.
- Step 3: Remineralizing solution (10 ml/section) for 2 h.
- Step 4: Dentifrice supernatant solution (5 ml/section) for 60 s.
- Step 5: Demineralizing solution (10 ml/section) for 3 h.
- Step 6: Dentifrice supernatant solution (5 ml/section) for 60 s.
- Step 7: Remineralising solution (10 ml/tooth) overnight.
- Step 8: This pH cycle continues for 7 days.

### Recording the remineralization rates

After 7 days the remineralized sections were studied under the stereomicroscope evaluated for lesion depth, [Figure 2]. The evaluated values will be noted and tabulated. The values were noted by 3 examiners were taken and the average value of all 3 were noted.

The evaluated values for the demineralized (control group) sections and remineralized sections were noted in units by using “micromotor eyepieces.” The units value were then converted into micrometer and millimeter using the following formulas:

$$\frac{\text{No. of units} \times \text{Zoom}}{\text{Eye piece magnification}} = \text{Millimeter}$$

$$\frac{\text{No. of units} \times \text{Zoom}}{\text{Eye piece magnification } 1000} = \text{Micrometer}$$

### Evaluation and statistical analysis

The remineralized values were compared with the evaluated demineralized (control group) values of the same specimen. This was done for all the three groups. The results of comparison of the three test groups were done using Anova test, Student Newmann Keyes Test and Bonterroni multiple comparison test and comparison between the two pediatric dentifrices and between the two pediatric dentifrices and between each pediatric dentifrice and regular dentifrice was done.

### RESULTS

The present study was designed to test the effects of three pre-selected pastes on enamel caries initiation and lesion depth progression *in vitro* condition. Tooth sections were obtained from extracted human molar teeth were subjected to experimental application and subsequent *in vitro* acid challenge. The depths of the artificial carious produced in enamel were measured in millimeter using stereomicroscope and the different test groups were compared with the control group as well as with each other at the end of each phase.

The mean lesion depths were not significantly different when compared between three groups after demineralisation [Table 1].

The mean lesion depth was significantly higher for the pediatric dentifrices groups compared to the regular

dentifrice group when compared after remineralisation [Table 2].

The mean difference (between demineralisation and remineralisation) was highest for the regular dentifrice group when compared with each of the pediatric dentifrice group [Table 3].

The results of comparison of the three test groups were done using ANOVA test, Student Newmann Keyes Test and Bonterroni multiple comparison test [Table 4 and Figure 3].

## DISCUSSION

Survival of all higher forms of life is dependent on ingestion of food to fuel life's processes. Teeth are tools that have evolved to ensure survival of species. Nature has fashioned the hard enamel covering to serve this function. It is a paradox that though enamel is the hardest biological substance, its acellular and avascular nature makes it incapable of any natural defense mechanism based on cellularity.<sup>[6]</sup>

When tooth erupts in the oral cavity, the hypomineralized enamel encounters a high complex ecology. Imbalance in this complex environment holds the clue to the origin of this unique disease called dental caries. The onset of dental caries requires the establishment of necessary physicochemical conditions for mineral dissolution.<sup>[7]</sup>

In a primary tooth, enamel caries progresses rapidly into the underlying dentine. This is probably due to the relatively thin enamel, the lower mineral content and the higher organic content of the enamel of primary tooth enamel compared to that of permanent tooth enamel.<sup>[8]</sup> As a consequence of the trend towards caries prevention, rather than treatment, it is necessary to gain a better understanding of mechanisms that can enhance remineralization or reduce demineralization.

Demineralization models utilize a wide variety of substrates like sections of sound teeth, sections with lesions and lesions pre-treated with chemical agents. Topical treatments of sound or carious enamel can be made followed by secondary acid exposures, to measure the effect of this topical application on subsequent demineralization.<sup>[9]</sup> For the carious process to proceed, the pH and the ionic activities of calcium and phosphate in plaque fluid are critical because they determine the stability of the tooth structure under cariogenic attack.<sup>[10]</sup> The plaque fluid of a caries free population

has been found to be supersaturated with respect to enamel.<sup>[11]</sup> Thus, it can be postulated that the mineral lost when the oral environment is undersaturated could be replenished by the precipitation of new minerals.

As fluoride dentifrices have a dose – response relationship;<sup>[12]</sup> this is important because dentifrices for children usually contain between 250 and 500 ppm fluoride, in order to reduce the risk of fluorosis. The anticariogenic efficacy of a 550 ppm fluoride designed for children was found to be similar to that of 1100 ppm fluoride dentifrice.<sup>[12]</sup> However, studies have shown that remineralization of enamel by a 1000 ppm is achieved more rapidly than with a 250 ppm fluoride.<sup>[11,12]</sup>

**Table 1: Mean lesion depths of the three groups after demineralization**

| Dentifrice    | Values after demineralization |
|---------------|-------------------------------|
| Kidodent      | 0.83±0.29                     |
| Cheeriogel    | 0.98±0.56                     |
| Colgate total | 1.11±0.34                     |

**Table 2: Mean lesion depth of the three groups after remineralization**

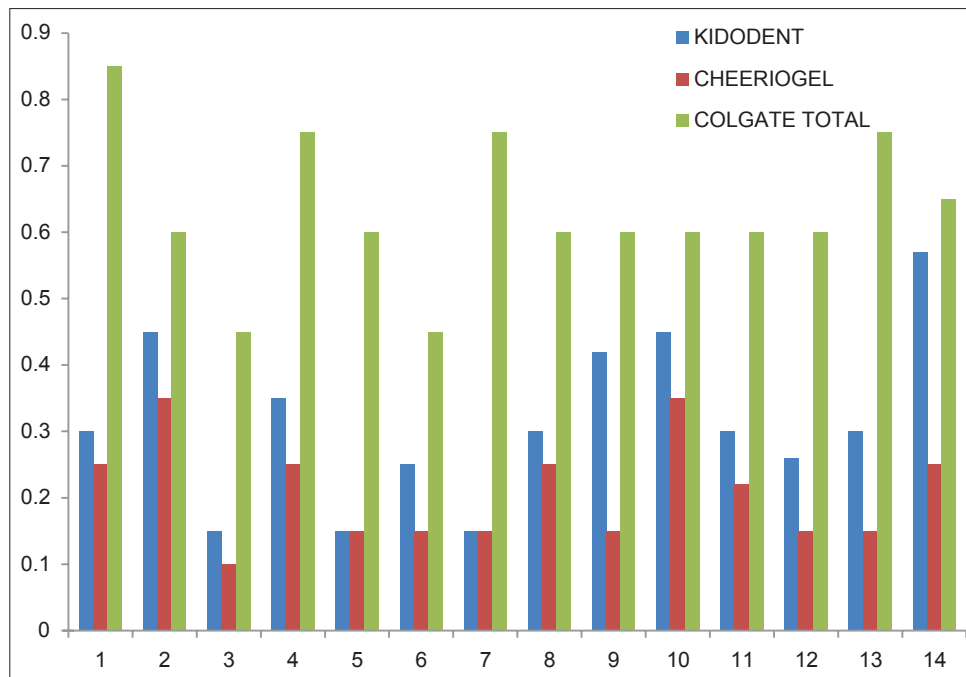
| Dentifrice    | Values after remineralization |
|---------------|-------------------------------|
| Kidodent      | 0.55±0.17                     |
| Cheeriogel    | 0.77±0.50                     |
| Colgate total | 0.57±0.29                     |

**Table 3: Mean difference between the demineralization and remineralization values for each of the three groups**

| Dentifrice    | Difference |
|---------------|------------|
| Kidodent      | 0.28±0.23  |
| Cheeriogel    | 0.21±0.17  |
| Colgate total | 0.54±0.16  |

**Table 4: Comparison between the three dentifrices, regular dentifrice (Colgate Total), pediatric dentifrice (Kidodent) and pediatric dentifrice (Cheeriogel)**

| Student-Newmann Keyes test          |         |
|-------------------------------------|---------|
| Phases                              | P value |
| After demineralization              | 0.223   |
| After remineralization              | 0.222   |
| Difference                          | 0.000   |
| Bonterroni multiple comparison test |         |
| Comparison                          | P value |
| Colgate with Kidodent               | 0.005   |
| Colgate with Cheeriogel             | 0.000   |
| Kidodent with Cheeriogel            | 0.961   |



**Figure 3:** Comparison of remineralization effect between three dentifrices. X axis: Specimen number, Y axis: The remineralization effect (Deminerilization value – Remineralization value)

The fluoride contents of the three dentifrices were Kidodent with 500 ppm fluoride, Cheeriogel with 458 ppm fluoride, Colgate Total with 1000 ppm fluoride. From the presented results it can be hypothesized that fluoride content of Cheeriogel was less sufficient to remineralized the carious lesions in the specimens compared to the other two.

### Comparative evaluation between two pediatric dentifrices (Kidodent and Cheeriogel)

An *in vitro* study was done in Brisbane, Australia where effects of three different dentifrices (Perio Children’s toothpaste, Colgate Pokemon and Vocco) were observed on 21 specimens of primary teeth. The effects were observed using polarized light microscopy and microradiography to evaluate lesion depth, before and after 7 days of pH cycle. It was concluded that Colgate Pokemon remineralized initial carious lesions while other two failed to do so.<sup>[13]</sup>

In contrast, the present study, when an individual comparison between the two pediatric dentifrices was done, all the three dentifrices showed remineralization of carious lesions. Kidodent showed a higher healing effect on remineralizing artificial carious lesions compared to Cheeriogel.

An *in vitro* study was done to evaluate the effectiveness of two pediatric dentifrices on enamel of primary teeth. The effects were observed using a potentiometer and a spectrometer. Fluoride uptake was lower in Poonch

pediatric toothpaste as compared to the other test ADA approved pediatric dentifrice, but considerably higher than the control group.<sup>[14]</sup>

In our study where Kidodent showed more or less similar efficacy than Cheeriogel. This can be attributed to fact due to similar levels of fluoride and sodium monofluorophosphate content.

### Comparative evaluation between each pediatric dentifrice (Kidodent, Cheeriogel) and regular dentifrice (Colgate total)

An *in vivo* study was done, where the anticaries effect was seen on primary dentition using two dentifrices containing 250 ppm and 1450 ppm respectively. The effects were observed by calculating the Decayed, Missing Filled Surfaces (DMFS) and Decayed, Missing Filled Teeth increments. It was observed that the mean DMFS increment was lower in high fluoride group whereas the mean DMFS increment excluding occlusal surfaces were lower in high fluoride group.<sup>[15]</sup>

It was in accordance to the present study, where the high fluoride group showed more effect then the other dentifrices.

An *in vitro* and *in situ* study was conducted in Amsterdam, The Netherlands where the effects of three dentifrices (non-fluoridated dentifrice, fluoridated control dentifrice and a dentifrice containing 1000 ppm

F, 0.3% Triclosan and 0.75% zinc citrate trihydrate. It was observed that there were no differences between the two fluoride toothpastes in the *in vitro* studies. In, *in situ* studies the paste containing Triclosan significantly inhibited caries progression as compared to the fluoridated dentifrice. There was no significant difference in the remineralization effects between the three dentifrices.<sup>[5]</sup>

In contrast, in the present study, the regular dentifrice (Colgate Total) showed a significantly higher effect than each the pediatric dentifrices (Kidodent, Cheerigel). This could be attributed to the higher amount of fluoride present in Colgate Total (1000 ppm) compared to Kidodent (500 ppm) and Cheerigel (458 ppm).

The natural regimen carried out in brushing primary teeth is to use pea sized regular dentifrice with a fluoride content of 1000 ppm. The intention behind this is to limit the excess of exposure of fluoride. Instructing parents to use a “pea size” or “smear” of fluoride toothpaste is not universally effective to reduce the amount applied to the toothbrush.<sup>[16]</sup>

The other regimen is to use a regular length of a pediatric dentifrice with a fluoride content of almost half of the regular dentifrice. This should again been done without minimizing the cariostatic effects of the dentifrice.<sup>[17]</sup>

This study using two pediatric dentifrices and a regular dentifrice hypothesizes that regular dentifrice does remineralize the artificial carious lesions but at the cost of a high fluoride level (1000 ppm). The pediatric dentifrices too have an effect on artificial carious lesions, with an additional benefit of a low fluoride level.

*In vitro* studies are good in analyzing selected properties of agents in single variable experiments. However *in vitro* designs are mechanistically limited in three key ways as listed by White.<sup>[18]</sup>

1. Inadequate simulation of complex and diverse intra-oral conditions
2. Difficulty in simulating volume and composition of saliva and tooth surface area encountered
3. Lesion depths of 100-250 um created here are created in short periods of few days whereas in clinical situation such lesion progression would take 12 months.

The other limitations were:

1. Small sample size
2. No control group.

These findings also have dental health education implications. Dental health education should be directed more towards ensuring tooth brushing with a fluoride containing toothpaste twice a day rather than focusing only on dietary restrictions which is more difficult to achieve.

The lesions produced in the study especially in initiation and first progression phases are very small which clinical examination cannot detect. Some of the agents tested in this study did show ability to resist early lesion development. Prevention of such early lesions can be considered as primary level of prevention because such lesions can be successfully dealt in such a way that they never progress to a stage where they are diagnosed. Such surfaces will remain caries free at clinical and radiographic levels.<sup>[19]</sup>

## CONCLUSION

The following conclusions can be drawn from the present study that:

1. All the three dentifrices showed a remineralizing potential
2. The regular dentifrice (Colgate Total) showed a higher healing potential compared to the pediatric dentifrices (Kidodent, Cheerigel), which could be attributed to the high fluoride content.

The following hypothesis can be made:

1. High fluoride levels may lead to deleterious effects if not used judiciously in children
2. Pediatric dentifrices have an appropriate fluoride content, as required by the children, and also does not minimize the cariostatic effects.

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