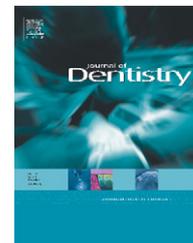


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## Preventive effects of dentifrice containing 5000 ppm fluoride against dental erosion in situ

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### ABSTRACT

**Objective:** To evaluate the effectiveness of a dentifrice with 5000 ppm fluoride in preventing dental erosion by orange juice in situ in comparison to a control dentifrice with 1450 ppm fluoride.

**Methods:** This was a double-blind and randomized clinical study with a cross-over design. Sixteen subjects wore an intra-oral appliance containing two enamel disks with an exposed surface of approximately 2 mm × 5 mm. Enamel disks in the study group were treated with a dentifrice with 5000 ppm fluoride and in the control group with 1450 ppm fluoride. The subjects rinsed with slurries of study dentifrices for one minute before immersing the enamel disks in 250 ml orange Juice four times in an 8-h period daily. The treatment procedure was repeated for three 5-day phases for each dentifrice. Enamel erosion was measured after each 5-day treatment phase using a focus-variation 3D scanning microscopy. Medians and inter-quartile ranges (IQR) of mean erosion depth were compared between the groups.

**Results:** The mean erosion depths of enamel varied greatly amongst the subjects. Enamel treated with 5000 ppm fluoride had less erosion (median 5.7 μm, IQR 4.5 μm) as compared to the control (median 12.6 μm, IQR 12.3 μm) after 15 days of fluoride treatment and erosive challenge cycles ( $p < 0.05$ ).

**Conclusions:** Enamel treated with 5000 ppm fluoride had significantly improved resistance to erosion by orange juice. Periodic application of 5000 ppm fluoride may be beneficial in individuals at risk of acidic erosion associated with soft drink consumptions.

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Dental erosion in children and adults is a growing concern in dentistry, especially as it relates to the rising consumption of acidic beverages. Studies reported an increase in the loss of hard dental tissue after the consumption of acidic soft drinks including fruit juices and carbonated beverages.<sup>1–5</sup> With the

advent of new methodologies in quantifying surface enamel changes,<sup>6–9</sup> we have gained significant insights in the relationship between soft drinks and tooth surface demineralization. Soft drink as a significant etiological factor in dental erosion is receiving more and more attention due to its rapid

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increase in daily consumption in the past two decades.<sup>10,11</sup> The commercial sale of soft drinks has increased dramatically in recent decades. It is estimated that an average American consumes more than 56 gallons of soft drinks per year, one and half 12 oz cans per day,<sup>12</sup> and that the consumption of soft drinks will keep rising at 2-3% a year in the foreseeable future.<sup>11</sup> Adequate acidity, often signifying a pH below 4.0, is essential to maintain the pleasant taste of the soft drinks and crucial to prevent rapid bacteria growth. For these reasons, the pH of almost all soft drinks is significantly below the critical threshold value that initiates enamel demineralization and leads to enamel surface softening and tissue loss. As the hard tissue loss associated with dental erosion is irreversible and often involves the entire dentition, effective prevention of enamel surface softening and tissue loss should be the major goal for the management of dental erosion. Besides abstinence from the usage of soft drinks, improving the resistance of surface enamel to acid attacks is the obvious choice for dental professionals to approach this goal. In this regard, experiences gained through decades of research in caries prevention may be translated into prevention of demineralization caused by acidic soft drinks. Topical application of fluoride should be a preferred strategy owing to its ease of use and its proven efficacy in prevention of enamel demineralization.

However, the efficacy of fluoride in prevention of dental erosion has been an issue of controversy in scientific literature in recent years. Some studies showed that modification of the soft drinks with relatively high concentrations of fluoride was not effective against acidic erosion of enamel by soft drinks,<sup>13,14</sup> whilst others demonstrated that addition of fluoride could statistically significantly reduce the erosive potential of a range of soft drinks.<sup>15,16</sup> Some authors have doubts about the possibility of using fluoride to prevent enamel erosion by soft drinks and fruit juices because of the high acidity and low pH of these beverages and high solubility of the enamel apatite structure.<sup>17</sup> In comparison to fluoridation of soft drinks, topical applications of fluoride have had more encouraging outcomes. It has been shown that fluoride could minimize the erosive effects of soft drinks when applied as a vanish,<sup>18-20</sup> a mouthwash,<sup>21</sup> a topical gel<sup>11,22</sup> or as a dentifrice.<sup>23,24</sup> A dose-response effect has been observed when using fluoride dentifrices for treatment of enamel erosion *in situ*<sup>24</sup> and *in vitro*.<sup>25</sup> Enamel treated by dentifrices with higher fluoride concentrations was significantly more resistant to erosive challenges than those with lower fluoride concentrations. As the anti-erosive effects of fluoride are mainly attributed to a protective layer of calcium fluoride precipitate on the enamel surface following topical applications,<sup>26</sup> increasing the concentrations of fluoride agents have been shown to have an improved effect against erosion,<sup>11,27,28</sup> presumably owing to the formation of a thicker and more stable layer of such protective precipitate.<sup>29</sup> It was found that treatments with an acidic gel containing fluoride in high concentration (12,500 ppm) could increase resistance of enamel to acidic erosion whilst treatment with fluoride dentifrice in lower concentration (1250 ppm) was not as effective.<sup>11</sup> Ganss et al. have also demonstrated that the addition of 12,500 ppm fluoride gel to a mouthrinse treatment regime produced significantly better preventive effects against enamel erosion *in vitro* when compared to treatment with

dentifrice containing 1500 ppm fluoride.<sup>27</sup> A recent study showed that 5000 ppm and 19,000 ppm fluoride were effective but 250 ppm and 1450 ppm fluoride were not effective against enamel erosive wear *in vitro*.<sup>30</sup> Though these studies clearly support a dose-dependent effect of fluoride against enamel surface erosion and erosive wear, other studies showed that increasing the concentrations of fluoride did not always produce an improved effect against enamel or dentine erosion.<sup>29,31,32</sup> Rios et al. found that treatments with dentifrices containing 5000 ppm or 1100ppm were not effective in preventing enamel erosion.<sup>32</sup> A recent study comparing the effects of 5000 ppm and 10,000 ppm fluoride indicated that higher concentration of fluoride did not have an improved protective effect against enamel erosion in a cyclic de- and remineralization model.<sup>29</sup>

As fluoride remains to be the main active ingredient in products marketed against dental erosion, it is important to clarify if dentifrices with higher concentrations of fluoride provide better protection against erosion by soft drinks when compared to those with less fluoride. An experiment *in vitro* showed that dentifrices with 5000 ppm fluoride performed better than those with 1450 ppm fluoride on enamel surfaces subjected to erosion by orange juice.<sup>7</sup> As studies *in vitro* did not take into account of the effects of saliva and oral environments, clinical studies are preferable to validate the experimental findings. The purpose of the present study was therefore to conduct a controlled clinical trial *in situ* comparing the preventive effects of dentifrices with different fluoride concentrations against erosion by orange juice.

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## 1. Methods

### 1.1. Study subjects selection and enrollment

This study was a double-blind, randomized, and cross-over design. Male or female subjects who were in good health and willing to sign an informed consent were recruited to participate in this study. Subjects with the following conditions were excluded from the present study: periodontal diseases, two or more untreated caries, impaired salivary flow, immune deficiencies, smoking, and taking oral liquid or chewable medications. The study protocol and the informed consent form were reviewed and approved by the institutional review board of the authors' institution. In the first phase of the study, the subjects were randomly assigned one of the two study dentifrices that were masked and coded. Randomization was conducted with the aid of a computer generated random number table. After a one-week washout period, the subjects were given the second test dentifrice and began the second phase of the study.

### 1.2. Enamel sample preparation

Sample preparation for fluoride treatment followed the same procedures as our previous study *in vitro*.<sup>7</sup> Briefly, freshly extracted human third molars were stored in distilled water at 4°C before use. Enamel slabs of 5 mm × 5 mm × 1.5 mm were cut from the buccal and lingual surfaces of the third molars using a precision low speed diamond saw (SY150, MTI Corp.,

Richmond, CA). Each cut enamel slab was embedded in epoxy resin in a custom Teflon mould to form a 10 mm × 10 mm round disc. Enamel surface was ground under water coolant to achieve a flat surface using 420,800, and 1200 grit silicon carbide paper (Extex Corp, Enfield, CT, USA), followed by polishing with Microlux-R 0.3 micron polishing compound (Adolf Miller Company, Providence, RI, USA) on a polishing cloth (Extex Plano Cloth, Extex Corp, Enfield, USA) to produce a smooth surface. A total of 60 enamel samples were prepared and kept in normal saline. The enamel disks were sterilized with ethylene oxide for 12 h before use in the study.

### 1.3. Study procedures

#### 1.3.1. Intraoral appliance for fluoride treatment *in situ*

The enamel disks were covered partially to expose an approximately 5 mm × 2 mm area and placed on the palatal plate of a custom-made maxillary intra-oral appliance. Enrolled subjects would wear the enamel disc imbedded appliance during the study period. Each intraoral maxillary appliance has space for 2 enamel specimens on the palatal plate. The subjects wore the appliances for three 5-day periods during each treatment phase. After the initial sterilization with ethylene oxide, enamel disks were disinfected in 0.5% chlorhexidine plus 70% ethanol for 30 min before insertion into the mouth. The appliance was worn from 9:00am in the morning to 5:00pm in the afternoon, and stored in normal saline after immersion in 0.2% chlorhexidine for 3 min each night and weekend. The appliances were again immersed into 0.2% chlorhexidine for 3 min before insertion into the mouth each morning. During the treatment period, only water drinking was allowed whilst the appliance was being worn. The appliance were removed during eating and sleeping and stored as described above.

#### 1.3.2. Preparation of fluoride dentifrice slurries

A commercially available product with high fluoride concentration, Prevident 5000<sup>®</sup> (Colgate–Palmolive, New York, New York, USA), was selected as the test dentifrice for the present study. Prevident 5000<sup>®</sup> (PV) contains 5000 ppm fluoride in the form of 1.1% NaF and requires prescription by a dentist in the USA. A commercially available fluoride dentifrice, Sensodyne<sup>™</sup> ProNamel<sup>®</sup> (GlaxoSmithKline, Middlesex, UK), was selected for the reference treatment group for the present study. ProNamel<sup>®</sup> (PN) contains 1450 ppm fluoride in the form of 0.315% NaF and is marketed as dentifrice protective against acidic erosion.

Slurries of the two study products, PV and PN, were prepared as follows: 3 grams of dentifrice were mixed with 10 ml distilled water and shaken thoroughly to produce uniform toothpaste slurries. The slurries were kept at room temperature and new slurries were prepared immediately before use.

#### 1.3.3. Fluoride dentifrice treatment *in situ* and erosion by orange juice *ex vivo*

The erosive challenge and dentifrice treatment protocol was modified from that of Hooper et al.<sup>33</sup> During the course of the treatment and starting at 9:00am each morning, subject was instructed to wear the appliance for 15 min, rinse with the

assigned dentifrice slurry for 1 min and expectorate. At 1 h and 3 h after the treatment, the enamel disc imbedded appliance was immersed in 250 ml of orange juice (Minute Maid<sup>®</sup> Plus, pH 3.8) at room temperature for 10 min each time. The orange juice was not stirred or agitated. These procedures were repeated again in the afternoon. After 5 days of fluoride treatments and erosive challenges by orange juice, the imbedded enamel disks were removed from the appliance and the erosive changes were observed and measured with a focus variation 3D vertical scanning microscope (IFM, InfiniteFocus<sup>®</sup> G4, Alicona Imaging, Grambach/Graz, Austria). After the IFM measurement, the enamel disks were again imbedded into the appliance to start the next 5-day phase of fluoride treatment and erosive challenges by orange juice. The treatment procedure was repeated for 3 phases for each dentifrice during a 3-week period with IFM measurement of erosion between phases.

#### 1.3.4. IFM assessment of enamel surface erosion

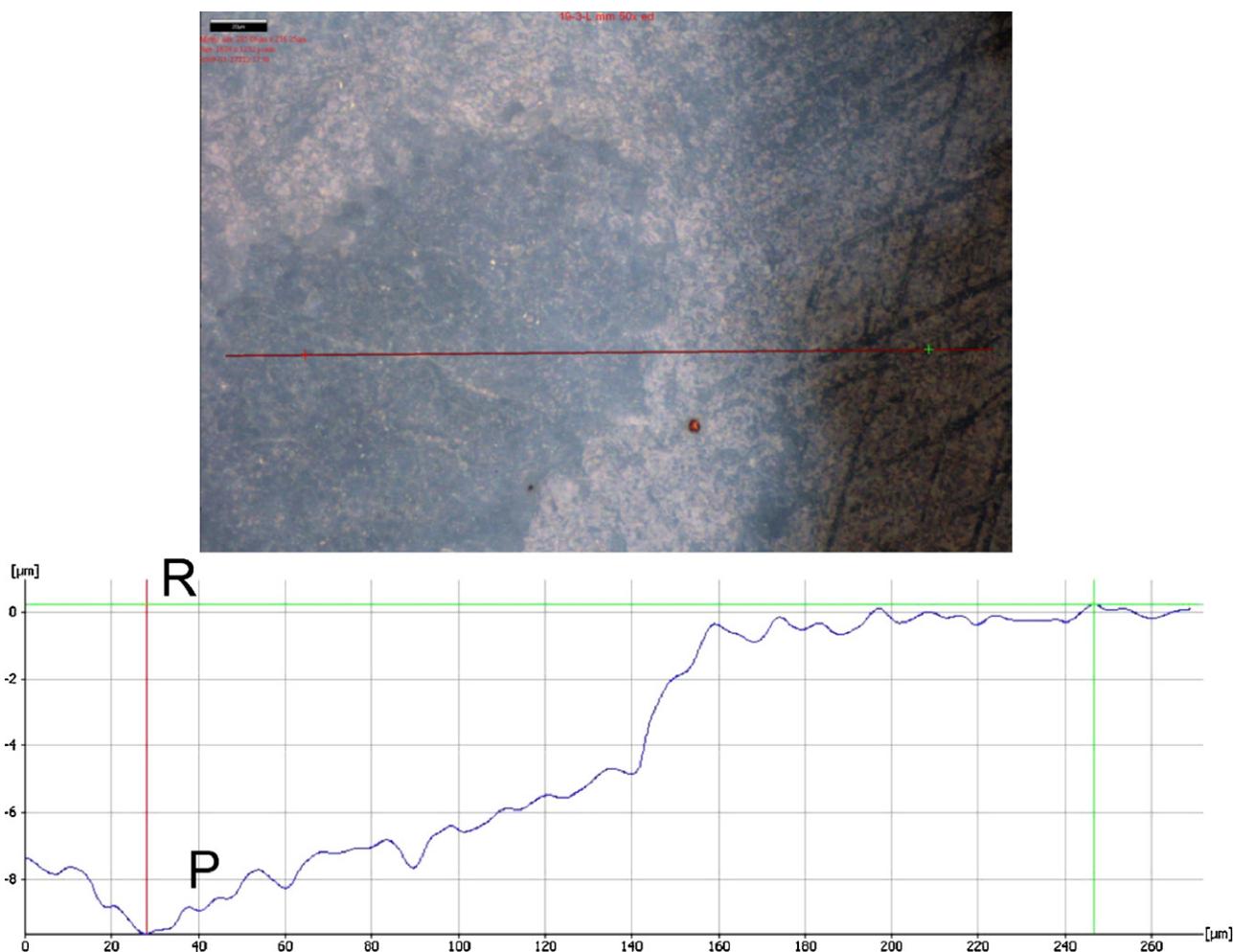
The enamel disks were measured after each 5-day fluoride treatment and erosive challenge cycle and the 3D topography of the eroded enamel surfaces were measured by the IFM as described in our previous studies.<sup>7,34</sup> The images were taken at magnifications of approximately 200× and 1000× with vertical resolutions of 0.1 and 0.02 μm, respectively. The IFM surface profile was measured across the exposed area on the enamel disks and the mean erosion depths were calculated with the IFM 3D measurement software (Fig. 1). The mean erosion depth was measured as a mean of the vertical distances from the reference line to valleys in the measurement field profile.

### 1.4. Statistical analysis

The sample size of the present study was estimated from a pilot *in vitro* experiment with the two study dentifrices, where we found that the mean erosion depth was approximately 5.0 μm in the study group and 8.0 μm in the control group with a standard deviation of 3.0 μm after 15 cycles of 2 min fluoride treatments and 20 min of erosion by orange juice (unpublished data). A sample size of 16 subjects in each group was needed to have 80% power at an alpha level of 0.05. The mean erosion depths (in microns) measured at 3 sites on each disc were averaged to give a subject-wise mean for each 5-day phase of the study. For the data was not normally distributed as indicated by our pilot study, medians and inter-quartile ranges (IQR) were reported for each treatment group. The non-parametric Mann–Whitney test was used to compare the erosion depth between the groups. The difference between groups is considered statistically significant at *p* level <0.05.

## 2. Results

Sixteen subjects, 10 males and 6 females with a mean age of 39.8 (range 26.4–56.8) years, who met the inclusion/exclusion criteria were enrolled in the study. All subjects completed the study. The mean erosion depths of enamel varied greatly amongst the subjects and were not normally distributed (Fig. 2). We chose to report the median and IQR and use non-parametric statistic method based on these distribution



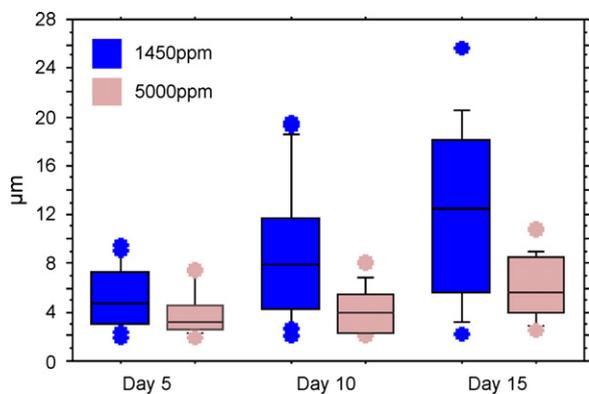
**Fig. 1 – Measurement of erosion depths. Mean erosion depth is measured as the mean distances from the bottom of the eroded surface profile (P) to the reference line (R).**

patterns. At 15 days after orange juice erosive challenge ex vivo and fluoride dentifrice treatment in situ, the median erosion depth of enamel was 5.7 μm (IQR 4.5 μm) in the 5000 ppm group and 12.6 μm (IQR 12.3 μm) in the 1450 ppm

group, indicating treatment with higher concentration fluoride had a better protective effect against enamel erosion by orange juice ( $p < 0.05$ ).

The median erosion depths and the results of Mann-Whitney test between the two treatment groups are listed in Table 1. There were statistically significant differences ( $p < 0.05$ ) between the 5000 ppm and the 1450 ppm groups in mean erosion depths at all 3 phases of the study. With time, erosion depths increased significantly in the 1450 ppm group, but less so in the 5000 ppm group, resulting in an increased difference in erosion depths between the two study groups at the end of the study. As shown in Table 1, the IQR in both groups are large in relation to their respective median values, signifying significant variations in enamel erosion depths amongst the study subjects.

Typical 3D topographic images of the eroded enamel surfaces are presented in Fig. 3. Qualitative changes occurred first on the enamel surfaces at the early stage of the study as indicated by surface texture and colour discrepancies between the eroded and non-eroded surfaces, followed by apparent substance loss at late stages of the erosive challenge and fluoride treatment cycles, especially in the 1450 ppm fluoride group (Fig. 3B).



**Fig. 2 – Mean erosion depth of enamel treated with 5000 ppm and 1450 ppm fluoride dentifrices, respectively. Note non-normal data distribution and outliers.**

**Table 1 – Comparison of mean erosion depths (median and IQR) at Day 5, 10 and 15 between dentifrices containing 5000 ppm and 1450 ppm fluoride.**

|          | Day 5  |      | Day 10 |      | Day 15 |       |
|----------|--------|------|--------|------|--------|-------|
|          | Median | IQR  | Median | IQR  | Median | IQR   |
| 5000 ppm | 3.19   | 1.42 | 3.89   | 2.98 | 5.70   | 4.46  |
| 1450 ppm | 4.73   | 3.28 | 7.94   | 7.50 | 12.59  | 12.33 |
| Z*       | 2.174  |      | 2.609  |      | 2.095  |       |
| p        | 0.0297 |      | 0.0091 |      | 0.0362 |       |

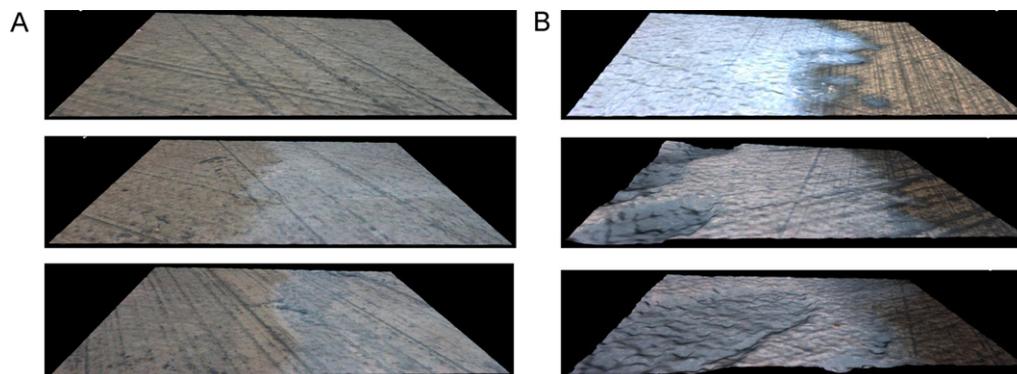
### 3. Discussion

The findings of the present study indicate that treatment using 5000 ppm fluoride dentifrice significantly increased the resistance of dental enamel to erosive challenges by orange juice when compared to treatment with dentifrice with a lower fluoride concentration. The median surface enamel loss, as measured by mean erosion depth, was 4.7  $\mu\text{m}$  at 5 days, 7.9  $\mu\text{m}$  at 10 days and 12.6  $\mu\text{m}$  at 15 days after erosive challenges by orange juice and treatment with dentifrice containing 1450 ppm fluoride, but was only 3.2  $\mu\text{m}$ , 3.9  $\mu\text{m}$  and 5.7  $\mu\text{m}$  at each corresponding stage of evaluation when treated with dentifrice containing 5000 ppm fluoride. The protective effect of 5000 ppm fluoride dentifrice against erosion grew more apparent with time, signified by a 33%, 51%, and 55% reduction in enamel loss as compared to 1450 ppm fluoride at 5, 10, and 15 days after erosive challenges, respectively. These findings are in agreement with previous studies that reported a better protective effects against dental erosion when higher concentrations of fluoride were used for topical applications,<sup>11,24,27</sup> but are in conflict with other studies which showed that increasing the concentrations of fluoride had no added benefits against dental erosion.<sup>29,31,32</sup>

Though frequent application of high concentration fluoride has traditionally been considered the regime of choice for prevention and treatment of dental erosions,<sup>35</sup> some recent studies questioned the usefulness of high concentrations of fluoride for prevention of dental erosion and suggested that patients at risk of enamel erosion should consider preventive measures other than fluoride therapy.<sup>32</sup> However, the findings that high concentrations of fluoride lacked efficacy in prevention of dental erosion are more likely related to the

study design than a true absence of therapeutic effects. In the study in situ conducted by Rios et al., the authors evaluated bovine enamel disks subjected to erosive challenge and fluoride treatments ex vivo for 7 days, and concluded that neither the high (5000 ppm) nor the low (1100 ppm) concentration fluoride treatment was effective against erosion.<sup>32</sup> This study had a small sample size ( $n = 10$ ) and was seriously underpowered (40% power). The perils of underpowered clinical studies are well known, the most serious of which are failing to identify the true effects of the intervention and consequently arriving at a misleading conclusion.<sup>36,37</sup> The authors did find less enamel loss with increasing fluoride concentrations, but such a trend was not recognized as statistically significant due to the lack of statistical power.<sup>32</sup> In another study that found no added benefits of higher fluoride concentrations, the authors identified the brief treatment protocol (3 min total) as the probable cause for the negative finding, implying that too short an application time would not allow the formation of the protective calcium fluoride on the treated enamel surfaces.<sup>29</sup>

The mechanism by which fluoride exerts its protective effects against erosion by soft drinks is not very well understood, and is generally believed to be due to the formation of a calcium fluoride layer on the enamel surface.<sup>21,29</sup> The action of the calcium fluoride layer against acid challenge is believed to be two fold: as a physical barrier isolating the enamel surface from acid attacks, and as a reservoir of fluoride ions for the formation of the more acid resistant fluoroapatite. The amounts of calcium fluoride deposited on enamel surfaces increased with time, concentration of fluoride and calcium availability.<sup>26</sup> Frequent and longer-term applications of higher concentrations of fluoride have resulted in better protective effects against dental erosion.<sup>11,27,38</sup> The results of the present study further corroborate these findings as indicated by an increased benefit of high fluoride concentration with time as compared to the control group (Fig. 3). The protective effects of 5000 ppm fluoride became more evident at 15 days than that at 5 days after application. A recent study showed that brushing teeth with 5000 ppm fluoride dentifrice resulted in a significantly higher concentration of fluoride in dental plaques and saliva than with 1450 ppm dentifrice when evaluated as long as 60 min after brushing under the same condition.<sup>39</sup> This finding signifies that more fluoride ions are available long after



**Fig. 3 – Typical 3D IFM images (1000 $\times$ ) of enamel erosive changes at Day 5, 10 and 15 in 5000 ppm (A) and 1450 ppm (B) groups.**

brushing with 5000 ppm than with 1450 ppm fluoride, which most likely is the underlying mechanism for the better protective effects of high concentrations of fluoride. Availability of fluoride ions was crucial for the effect of fluoride dentifrices against dental erosion.<sup>40</sup> It has been consistently shown that the amount of fluoride uptake by normal or eroded enamel is largely proportional to the fluoride concentrations in the oral hygiene products.<sup>41,42</sup> Dentifrices containing 5000 ppm fluoride provides more fluoride ions for a longer time to allow better fluoride uptake by enamel surfaces, therefore achieving a better protective effect against erosive challenges than those with 1450 ppm fluoride.

We noticed a large variation in enamel loss amongst the study subjects under the same erosive challenge and fluoride treatment protocols (Fig. 2). These variations could not be sufficiently explained by the qualitative characteristics of the enamel disks for the two imbedded disks carried by the same study subject were from different donors. Calcium availability, saliva pH and buffering capacity of the study subjects may partly account for the differences in the resistance of dental enamel to erosive challenges. Subjects wore the appliance for over an hour before the erosive challenges, which allowed the formation of salivary pellicles that may provide substantial protective effects against erosion.<sup>2</sup> Differences in composition and thickness of salivary pellicles may also contribute to the variation in erosion depths.<sup>43,44</sup> It may be worthwhile to consider in future clinical studies the effect of chemical and physiological properties of saliva and salivary pellicles as modifying factors for dental erosion.

In summary, the results of the present study indicate that dentifrice containing high concentrations of fluoride had a better protective effect against enamel erosion by orange juice. Applications of 5000 ppm dentifrices twice daily for a period of 3 weeks will benefit the individuals at risk of acidic erosion associated with soft drink consumptions.

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