Remineralizing Potential of Clinpro® and Tooth Mousse Plus® on Artificial Carious Lesions

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Background: *Calcium phosphate and fluoride (F) delivery systems claim to facilitate enamel remineralization.* **Aim:** *To evaluate and compare (i) the remineralizing potential of Clinpro® Tooth Crème(CTC) and Tooth Mousse Plus®(TMP) on artificial carious lesions, and (ii) the benefit of 1000ppm F dentifrice prior to the application of CTC and TMP.* **Study design:** *Carious lesions, 200-300μm deep were produced by placing molars in demineralizing solution for 96h, sections 100-150μm thick were then randomly assigned to six groups(n=150). Specimens were treated thrice daily with a non-fluoridated(Group A), or fluoridated dentifrice[1000ppm, (Group B)], or CTC(Group C), TMP(Group D), fluoridated dentifrice followed by CTC(Group E), or a fluoridated dentifrice followed by TMP(Group F), and then subjected to a 10-day pH cycling model. Lesion evaluation involved polarizing light microscopy and microradiography.* **Results:** *Post-treatment maximum mineral content at the surface zone(V_{max}) was significantly increased in Groups B, C, and D compared to the other groups. The lesion depth(LD) decreased in Group D>Group C>Group E, and the net mineral content gain(ΔZ) in Group C>Group D, which did not reach statistical significance.* **Conclusions:** *CTC and TMP exhibited similar efficacy in remineralizing artificial carious lesions. Nevertheless, the net mineral gain or lesion consolidation following CTC use was higher than TMP.*

Keywords: remineralization, Tooth Mousse Plus, Clinpro,

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INTRODUCTION

Several calcium phosphate and fluoride (F) delivery systems are currently available, of which Tooth Mousse Plus™ [TMP (MI Paste Plus™), 10% CPP-ACP with 900ppm F, GC Corp, Tokyo, Japan] and Clinpro™ Tooth Crème [CTC, 950ppm sodium F with 500ppm functionalized tri-calcium phosphate, 3M ESPE Dental, Minnesota, USA] are commonly prescribed by clinicians. In TMP, a milk-derived protein, calcium phosphopeptide (CPP), facilitates bonding of calcium and phosphate ions to stabilize them as amorphous calcium phosphate (ACP). This CPP-ACP enables enamel remineralization by adhering to the plaque pellicle, hydroxyapatite as well as soft tissues, and supplying bioavailable calcium and phosphate into saliva fluids and plaque pellicle[1].

CTC contains functionalized tri-calcium phosphate (fTCP) and sodium F, where the tri-calcium phosphate particles have been ball milled with sodium lauryl sulphate to produce particles within the size range of 1μm to 15μm[2]. fTCP has been reported to exhibit remineralizing effects in both *in vitro* and *in situ* studies[3-5]. fTCP prevents calcium from prematurely interacting with ionic F and forming calcium F, thus providing more F and calcium ions to the enamel surface[2,6]. Similarly, several *in vitro* studies[7-9] report that when CPP-ACP is placed on the tooth surface, it interacts with hydrogen ions and can diffuse into enamel to produce subsurface mineral gains[2]. Recently, Shen and co-workers[10] reported that TMP exhibited the highest level of enamel remineralization compared to CTC and 5000ppm F paste. They stated that CTC lower solubility,

larger particle size and lower amount of calcium phosphate as the reason for its inability to significantly increase salivary calcium and inorganic phosphate levels to enable remineralization. Conversely, Vanichvatana and Auychai[11] reported that CTC provided similar benefits as the F toothpaste; however, no additional benefit of TMP was observed when used in conjunction with the F toothpaste. Furthermore, a latest systematic review[12] reported that there were no significant benefits of using Tooth Mousse® [TM, (MI Paste®)]. In addition, they highlighted the lack of evidence to support the use of TMP over TM[12].

Given the limited scientific evidence to support the efficacy of these calcium phosphate and F delivery systems, clinicians are constantly faced with the dilemma as to which product to recommend for their patients as part of their oral health care advise. To date, only two studies have performed a direct comparison of TMP and CTC and have reported dissimilar findings. Therefore, this *in vitro* study aimed to evaluate and compare (i) the remineralizing potential of TMP and CTC on artificial carious lesions, and (ii) the benefit of 1000ppm F dentifrice prior to the application of TMP and CTC.

MATERIALS AND METHOD

The sample size for paired studies comparing the values before and after the intervention was computed based on the formula proposed by Snedecor & Cochran[13] $n = 2 + C (s/d)^2$; where s is the standard deviation, d is the difference to be detected, and C is the constant based on the significance level (α) and the power of the experiment (1- β).

Based on our series of published studies[14-16], s was set at 4, d at 3, α at 0.05, and 1- β at 0.9 then $n = 2 + 10.51 (4/3)^2 = 20.68$ indicating that there should be 21 specimens in each group. To accommodate for loss or damage of the specimen's during the various phases of the study it was decided to include 25 specimens per group so as to ensure an adequate sample size for the final analysis.

De / remineralizing solution preparation

The buffered remineralizing and demineralizing solutions were prepared using analytical grade chemicals and deionized water. The demineralizing solution, which contained 2.2mM $CaCl_2$, 2.2mM KH_2PO_4 and 0.05M acetic acid had the pH adjusted to 4.4 using 1M KOH. The remineralizing solution, which contained 1.5mM $CaCl_2$, 0.9mM NaH_2PO_4, 0.15M KCL, was similarly adjusted to a neutral pH using 5M KOH. These solutions approximated to the super saturation of apatite minerals found in saliva and replicated those utilized by ten Cate and Duijsters[17].

Artificial enamel carious lesion formation

Soft tissues were debrided from the surfaces of extracted human third molars and the crowns were inspected for cracks, hypoplasia and white spot lesions. The teeth were then coated with acid-resistant nail varnish (Revlon®, New York, USA) leaving a narrow window approximately 1mm wide on the intact buccal and lingual enamel surfaces. Subsequently, the teeth were immersed in the demineralizing solution (10ml/specimen) for 96h to produce artificial carious lesions in the enamel that were 200µm to 300µm deep.

The roots of the teeth were sectioned horizontally at the cement-enamel junction using a saw microtome, and later discarded. The

tooth crowns were mounted on the microtome (Leica® 1600 saw microtome, Wetzlar, Germany) using sticky wax (Model cement® Dentsply Sirona Pty Ltd, Victoria, Australia) and sectioned longitudinally through the lesions to produce specimens approximately 100µm to 150µm thick. Each specimen was measured using a micrometer.

Polarizing light microscopy (PLM) [Nikon Eclipse LV100POL, Nikon, Tokyo, Japan] and microradiography (MRG) [Softex ISR-20, Jira, Tokyo, Japan] were performed for each specimen before and after the 10-day pH cycling. Prior to subjecting the specimens to pH cycling, each specimen was carefully coated, under a stereomicroscope (Zeiss, Jena, Germany), with an acid resistant nail varnish (Revlon®, New York, USA), leaving only the lesion surface exposed. The enamel sections (n=150) were then stored in 100% humidity until required for use. This was achieved by suspending the specimens, using dental floss, in a beaker containing deionized water that was sealed with paraffin wax (Parafilm®, Wisconsin, USA).

One hundred and fifty sections were randomly assigned to six treatment groups (n=25) and subjected to the following treatment protocols:

Group A: Non-fluoridated dentifrice (Vicco® Laboratories, Goa, India) as a supernatant for 60s (negative control).

Group B: Fluoridated dentifrice with 1000ppm F (Colgate Total, Bangkok, Thailand) as a supernatant for 60s (positive control).

Group C: fTCP+950ppm F (Clinpro™ Tooth Crème, 3M ESPE, Minnesota USA) as a topically applied cream for 180s.

Group D: CPP-ACP+900ppm F (Tooth Mousse Plus™ GC Corp, Tokyo, Japan) as a topically applied cream for 180s.

Group E: Fluoridated dentifrice with 1000ppm F (Colgate Total, Bangkok, Thailand) as supernatant for 60s followed by a topical coating of fTCP+950ppm F (Clinpro™ Tooth Crème, 3M ESPE, USA) for a further 180s.

Group F: Fluoridated dentifrice with 1000ppm F (Colgate Total, Bangkok, Thailand) as supernatant for 60s followed by a topical coating of CPP-ACP+900ppm F (Tooth Mousse Plus™, GC Corp, Tokyo, Japan) for a further 180s.

Agent preparation

Dentifrice supernatants were prepared by adding 15g of the respective dentifrice to 45ml of deionized water to obtain a ratio of 1:3. The suspensions were stirred thoroughly for 60s by mechanical agitation using a vortex mixer (Super Mixer®, Lab Line Instruments Inc, Illinois, USA) and later centrifuged for 20min at 4000rpm at room temperature (Beckman, Avanti J-251, California, USA). Subsequently, the higher density sediment layer was discarded and only the dentifrice supernatants were used for treating the specimens. TMP and CTC were dispensed directly (as a paste) onto the damp surface of the lesions in Groups C, D, E, and F.

The pH cycling model

All the tooth specimens were subjected to a 10-day pH cycling system on an orbital shaker (Labnet, Woodbridge, USA). The daily cycle involved 3h of demineralization twice a day with 2h of remineralization in between. The tooth specimens were placed in the remineralizing solutions overnight. Specimens received treatment thrice a day, before the first demineralization, and before and after the second demineralization cycle respectively. Treatment for Group A and Group B involved immersion of the specimens in the respective dentifrice supernatants (5ml/specimen) for 60s. While specimens belonging to Group C and Group D were placed directly in contact with TMP and CTC for 180s, specimens in Group E and Group F were treated with the dentifrice supernatant for 60s before being subjected to treatment with CTC and TMP, for a further 180s respectively. After the 10-day pH cycle the nail varnish was carefully removed from the tooth specimens using acetone (Advanced Technology & Instruments Co. Ltd, Hong Kong), to allow lesion evaluation.

The specimens were evaluated both before and after the 10-day pH cycling. Qualitative evaluation was performed using digital PLM while MRG was employed to provide quantitative evaluation of the tooth specimens.

Each tooth specimen was imbibed in water and subjected to digital PLM using a Nikon Eclipse LV100POL microscope (Nikon, Tokyo, Japan, LV-UEPI) with a rotating stage; polarizer and analyzer at a magnification of 5X to qualitatively evaluate the body of the lesion. Following computer capture using NIS-Elements AR 3.0 software; at the same magnification for all of the specimens, before and after the 10-day pH cycling experiment, they were analyzed for changes in lesion depth.

Each tooth specimen was exposed to Cu (Kα) x-rays (Softex IRS-20, Jira, Japan) at 15Kv and 3mA for 60s. The images were recorded on high-resolution Kodak 4489 film (Kodak, New York, USA), which was subsequently developed using the standard Kodak chemistry. All MRG's were exposed to the same developing process (60s in the developer, rinsed for 60s with water followed by 60s in the fixer). Furthermore, all tooth specimens were subjected to MRGs before and after the 10-day pH cycle. Finally, each MRG was mounted on a glass slide and the image captured using a flatbed color image scanner (Epson Perfection 3200 Photo, Tokyo, Japan). An image analysis system, Image J, version 1.37 (Bethesda, Maryland, USA) was used to plot the mineralization profile which enabled the evaluation of lesion depths, mineral changes and mineral distribution before and after the 10-day pH cycling, within the same tooth specimen, and among all the different test groups. Three parameters (i) maximum mineral content at the surface zone (V_{max}) and (ii) the depth of the lesion (LD), and (iii) the differences between the mineral content compared with sound enamel (ΔZ) were then calculated from the profile for each specimen. The mineral content profile and lesion parameters were determined for 3 windows on each specimen section. These values were averaged to give the mean ΔZ for each section. The mean ΔZ values from each section were again averaged to give the mean ΔZ for the group. The changes in ΔZ (ΔZ diff) for a given window before and after treatment were calculated as follows: ΔZ diff = ΔZa – ΔZb, where ΔZa and ΔZb refer to the ΔZ values of the same window area of a single specimen section before and after the treatment, respectively. The changes in the lesion depth were similarly calculated. The difference between the V_{max} before and after the 10-day pH cycling was calculated as a percentage increase/decrease in the V_{max}, and changes in the LD were calculated in a similar manner.

Statistical analysis

The paired t test was used to compare LD, V_{max}, and ΔZ before and after the 10-day pH cycling within the different groups, while One-way ANOVA and Tukey-Kramer comparison tests were employed to the differences between the groups (α=0.05). Statistical analysis was performed using SPSS version 16.0.

RESULTS

There was no statistical difference in the V_{max} (Group A, p = 0.56; Group B, p = 0.21; Group C, p = 0.10, Group D, p = 0.45, Group E, p = 0.58, Group F, p = 0.67; ANOVA), and LD (Group A, p = 0.78; Group B, p = 0.36; Group C, p = 0.43; Group D, p = 0.24; Group E, p = 0.12; Group F, p = 0.24; ANOVA) values within and between each of the six groups before the 10-day pH cycle.

Post-treatment analysis

The paired t test showed significant increase in the V_{max} values in Groups B (1000ppm F), C (CTC), and D (TMP) [Group B > Group D > Group C] while Group A (No F) exhibited a significant decrease in the V_{max} value. Furthermore, Groups E (1000ppm F + CTC) and F (1000ppm F + TMP) exhibited an increase in their V_{max} values however; this did not reach statistical significance. The LD scores in Group C (CTC), Group D (TMP), and Group E (1000ppm F + CTC) were significantly (p<0.05) decreased [Group D > Group C > Group E], while Group A (Non-F) exhibited a significant increase in the LD scores (p<0.05). Specimens in Group B and Group F exhibited a slight increase in their LD scores but this did not reach statistical significance [Table 1].

The percentage increase in V_{max} was the highest for Group B at approximately 70%, compared to 61% and 66% for Group C (Figure 1a) and Group D (Figure 1b) respectively. Furthermore, both Group E and Group F exhibited a mild increase in their V_{max} values at 5% and 3% respectively. Group A exhibited a 5% decrease in V_{max} and a 30% increase in LD while Group B exhibited a mild increase (4%) in the LD score.

One-way ANOVA was used to compare the post-treatment changes in V_{max} and LD. Further multiple comparison tests (Tukey Kramer) were performed which revealed that there was no significant difference in the V_{max} between Group B (1000ppm F), Group C (CTC) and Group D (TMP), but significantly higher when compared to Groups A, E, and F. No significant differences (p>0.05) in post-treatment LD values were observed in specimens treated with CTC for 180s (Group C), TMP applied for 180s (Group D), or a combination of CTC and fluoridated dentifrice (Group E). Furthermore, Groups C and D exhibited a significant decrease in the LD scores compared to the non-fluoridated dentifrice (Group A) and 1000ppm F dentifrice (Group B).

Figures 1c and 1d illustrate examples of the lesions in Group A (non-fluoridated dentifrice) and Group B (1000ppm F) respectively, observed using PLM before and after pH cycling. A significant increase in LD was evident in the Group A (non-fluoridated dentifrice) specimen while a slight increase in size of lesion was observed in Group B (1000ppm F).

The net mineral gain/loss (ΔZ values) between the six treatment groups did not reach statistical significance (p=0.71). Nevertheless, Groups C, E and F exhibited a trend favoring a higher net mineral gain.

Table 1: Mean values ± SD of lesion depth [LD (μm)], maximum mineral content in the surface zone (V$_{max}$), and differences of the mineral content (ΔZ) of the samples in the six treatment Groups.

Test agent/ Group	Sample (n)	V$_{max}$			LD			ΔZ
		pre ± SD	post ± SD	% change ± SD	pre ± SD	post ± SD	% change ± SD	(% vol mineral x μm) ± SD
Group A Non-fluoridated dentifrice	25	35.3±12.1	33.5±12.8*	-4.9±11.6 [B,C,D]	221.6±60.1	288.8±41.9*	30.3±30.2 [B,C,D,E,F]	-4.53 ± 17.41
Group B Fluoridated dentifrice (1000ppm F)	25	17.8±10.2	30.2±9.7*	69.6±4.9 [A,E,F]	209.6±49.9	218.4±48.2	4.1±19.8 [A,C,D]	-2.90 ± 18.68
Group C Clinpro™ Tooth Crème (fTCP+900ppmF)	25	16.5±6.7	26.5±8.1*	60.6±20.8 [A,E,F]	268.5±45.8	227.6±45.4*	-15.3±19.3 [A,B,F]	1.64 ± 17.31
Group D Tooth Mousse Plus™ (CPP-ACP+900ppmF)	25	17.7±9.8	29.3±10.8*	65.5±10.2 [A,E,F]	273.9±59.0	230.3±48.1*	-15.9±17.1 [A,B,F]	-0.05 ± 14.07
Group E Fluoridated dentifrice (1000ppm F) + Clinpro™ Tooth Crème (fTCP+900ppmF)	25	28.8±9.7	30.4±10.3	5.5±6.1 [B,C,D]	258.5±49.0	240.0±45.6*	-7.1±9.6 [A]	1.52 ± 16.04
Group F Fluoridated dentifrice (1000ppm F) + Tooth Mousse Plus™ (CPP-ACP+900ppmF)	25	29.3±11.7	30.3±9.1	3.4±22.1 [B,C,D]	236.2±43.1	238.6±41.7	1.0±13.1 [A,C,D]	0.72 ± 16.35

* Significant change (*p*<0.01 paired *t* test) in V$_{max}$ and lesion depth, post-treatment, within each group.

[A,B,C,D,E,F] represent Groups A, B, C, D, E, and F respectively. The superscript letters indicate statistically significant differences in the V$_{max}$, LD, and ΔZ changes between the indicated groups (p<0.05, ANOVA, Tukey-Kramer). For e.g. In the LD Changes, Group C exhibited statistical significance when compared with Groups A. B and F.

- ΔZ values indicate the net mineral loss while positive values represent the net mineral gain.

DISCUSSION

To our knowledge this is the first *in vitro* study to conduct a direct comparison of CTC and TMP both in isolation, and in combination with F dentifrice. The remineralization potential of the products evident in the present study were in the following order from highest to lowest: TMP = CTC = CTC + 1000ppm F > 1000ppm F > TMP+ 1000ppm F > non-F dentifrice. Nevertheless, the study findings could not be directly compared to previous studies as they were based on *in situ* models[10,11].

It is well accepted that reduction in LD is by no means concomitant with net remineralization, as significant mineral re-distribution is possible in a way that reduced depth can coincide with greater net mineral-loss, and vice versa. Therefore, changes in integrated mineral loss/gain (ΔZ) after treatment were calculated. CTC (Group C), Group E (1000ppm F + CTC) and Group F (1000ppm F + TMP) exhibited a trend favoring net mineral gain while Group A, B, and D demonstrated a net mineral loss. However, no significant differences were evident in the ΔZ values between the six treatment groups, which could have been due to the overly aggressive nature of the artificial caries system used in this study and/or the relative short cycle used in the pH cycling model.

CTC and TMP exhibited similar efficacy in remineralizing the artificial carious lesions, which is contrary to the findings of Shen, and co-workers[10] who reported that TMP were superior to CTC.

However, the finding that CTC and 1000ppm F exhibited similar remineralizing efficacy agreed with Vanichvatana and Auychai[11] who reported similar remineralizing efficacies for CTC and fluoride toothpaste and no additional benefit of TMP.

Although it is logical that the lack of saliva, plaque and pellicle in the present study is a limitation, as calcium phosphate and F delivery systems acts through immuno-localization within the plaque to access the surface of bacterial cells and intercellular plaque matrix[18-20]. This is not supported by the findings of the two *in situ* studies[10,11] that have conducted a direct comparison of both CTC and TMP. A possible explanation for this, could be that although *in situ* studies have the advantage of an oral environment with the presence of saliva and plaque, a potential drawback is the lack of exposure to acid and other demineralizing environments as the participants were instructed to remove the appliances while eating and drinking[10]. Nevertheless, it is possible that the calcium phosphate and F delivery systems were underestimated in the present study and that CTC and TMP may have a greater efficacy in the oral cavity. Furthermore, even within the constraints of this model, there was a trend to increase remineralization in both CTC and TMP groups.

A lack of an additional benefit was noted when the fluoridated dentifrice was combined with TMP in Group F (1000ppm F + TMP) which is consistent with previous studies[11,21]. Numerous

Figure 1a: Graph showing the relationship between the lesion depth in x-axis (µm) and relative % mineral content in y-axis, before and after 10-day pH cycling, from Group C [Clinpro™ Tooth Crème, (fTCP + 950ppm F)]; 1b: Graph showing the relationship between the lesion depth in x-axis (µm) and relative % mineral content in y-axis, before and after 10-day pH cycling, from Group D [Tooth Mousse Plus®, (CPP-ACP +900 ppm F)]; 1c: Polarized light photomicrographs of an enamel lesion from Group A (non-fluoridated dentifrice), (i) before and (ii) after treatment. Evidence of post-treatment increase in lesion depth; 1d: Polarized light photomicrographs of an enamel lesion from Group B (1000ppm F) (i) before and (ii) after treatment. Evidence of slight post-treatment increase in lesion depth
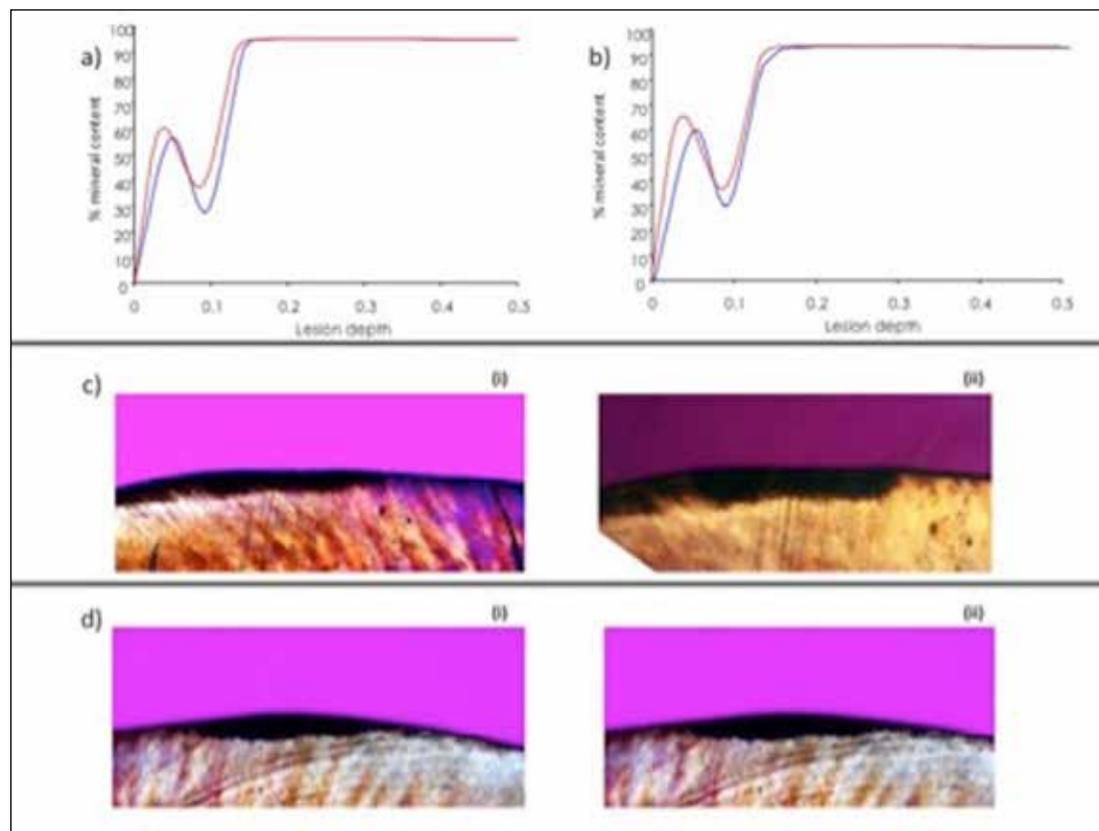
authors[18-20,22,23] have supported the need for a constant low level of F for remineralization rather than an acute exposure to a high level of F. It has been postulated that the F deposition occurs primarily in the surface layer, leading to blockage of the surface layer pores. This may explain why the high dose of F delivered to the enamel of the specimens in this study was unable to significantly remineralize the deeper layers. However, combining fluoridated dentifrice with CTC in Group E (1000ppm F + fTCP) exhibited a higher remineralizing potential than the combination of fluoridated dentifrice with TMP (1000ppm F + TMP), but this did not reach statistical significance.

With regards to the changes in LD, a significant decrease between the pre- and post-treatment scores were noted for Groups C, D and E. Furthermore, multiple comparisons for percentage changes between Groups C, D, and E did not reach statistical significance. These findings reflect those of Iijima and co-workers[24], which support the hypothesis that the fluorapatite that is formed during the first remineralization cycle appears to resist the demineralization of subsequent acid challenges leading to bulk of demineralization beneath this layer. Furthermore, 1000ppm F (Group B) slightly increased the lesion depth compared to the remaining groups, which might be due to the shorter application time 60s compared to 180s for the calcium phosphate and F delivery systems.

Protective factors are indicators of preventive activities that may reduce a child's risk for the onset extension of dental caries. These factors include optimal exposure to fluoride, access to regular dental care, consistent daily brushing with fluoride toothpaste, and use of appropriate remineralizing agents. factors are indicators of preventive activities that may reduce a child's risk for the onset extension of dental caries. These factors include optimal exposure to fluoride, access to regular dental care, consistent daily brushing with fluoride toothpaste, and use of appropriate remineralizing agents. Furthermore, with the increasing focus on early detection and non-invasive management of dental caries, the findings of this present study support the use of CTC and TMP especially in children with a high-caries risk. These calcium and F-systems can be a useful tool in the clinician's armamentarium while employing the "age- and risk-specific" approach when treating high caries-risk individuals.

In conclusion, CTC and TMP when used in isolation remineralized artificial enamel lesions by significantly increasing the V_{max} and decreasing the LD when applied for 180s. The net mineral gain for CTC was higher than TMP, which did not reach statistical significance. Addition of a 60s treatment with 1000ppm F dentifrice prior to the CTC application significantly decreased the LD, increased the V_{max}, and demonstrated increased net mineral gain, but not to statistically significant levels.

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