ACTA ODONTOLOGICA SCANDINAVICA
2019, VOL. 77, NO. 6, 419–425
https://doi.org/10.1080/00016357.2019.1582796

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Adjunctive use of fluoride rinsing and brush-on gel increased incipient caries-like lesion remineralization compared with fluoride toothpaste alone *in situ*

Phan Bhongsatiern[a], Ploypailin Manovilas[a], Methaphon Songvejkasem[a], Siriporn Songsiripradubboon[b], Thipawan Tharapiwattananon[b], Paiboon Techalertpaisarn[c], Hidenori Hamba[d], Junji Tagami[d], Dowen Birkhed[e] and Chutima Trairatvorakul[b]

[a]Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand; [b]Department of Pediatric Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand; [c]Department of Orthodontics, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand; [d]Department of Cariology and Operative Dentistry, Tokyo Medical and Dental University, Tokyo, Japan; [e]Fersens väg, Malmö, Sweden

ABSTRACT

Objective: The objective of this study was to compare the remineralizing effect of sodium fluoride (NaF) mouth rinse or NaF gel as an adjunct to NaF dentifrice on incipient caries-like lesions in an *in situ* cross-over design study, with three sessions of 30 days each.

Materials and methods: Orthodontic brackets with artificial demineralized enamel slabs were attached to the upper first molars of 12 participants. A set of 3 test specimens from the same tooth was randomly assigned to each participant and allocated into three 30-day sessions: 1) brushing with 0.22% NaF dentifrice 2 times/day (F dentifrice), 2) brushing with 0.22% NaF dentifrice 2 times/day + rinsing with 0.05% NaF before bedtime (F mouth rinse), 3) brushing with 0.22% NaF dentifrice 2 times/day + brushing with 1.1% NaF gel before bedtime (F brush-on gel). The mineral gain and lesion depth of the specimens were evaluated by micro-computed tomography.

Results: The mean mineral gain from the NaF mouth rinse and the NaF brush-on gel was similar, but greater than that from the NaF dentifrice ($p < .05$). The NaF brush-on gel yielded the greatest mean depth of remineralization (168 μm), followed by the NaF mouth rinse (144 μm). Both depths were significantly greater than that of the NaF dentifrice (84 μm) ($p < .05$).

Conclusions: Both 0.05% NaF mouth rinse and 1.1% NaF brush-on gel, used at bedtime, increased incipient caries-like lesion remineralization *in situ* in combination with brushing with NaF dentifrice twice a day.

ARTICLE HISTORY
Received 30 July 2018
Revised 7 February 2019
Accepted 8 February 2019

KEYWORDS
Artificial caries; fluoride gel; fluoride mouth rinse; *in situ* model; Micro-CT; mineral density; remineralization

Introduction

Dental caries continues to be a major health concern worldwide [1–4] and is the most wide-spread disease in the world [5]. However, the prevalence of caries has declined in most industrialized countries since the mid-60s, mainly because of the widespread use of fluoride (F) toothpaste [6,7]. Other F products, such as mouth rinse solutions, gels, and varnishes, may have contributed to this decline [8]. Moreover, oral hygiene (e.g. tooth brushing habits) and dietary factors (e.g. sugar intake frequency) play an important role in caries development [9].

It has been found around the world, including in European [10] and Asian countries [11] that for both children and adults with low/moderate caries risk, brushing with F toothpaste twice a day reduces caries. An example of the proper technique is found in the recommendations by the Swedish National Board of Health and Welfare [12]. Although tooth brushing frequency has increased in most European countries during 2004–2010 [10], in Asia, tooth brushing with F toothpaste remains the most effective public health preventive measure for reducing the burden of oral disease, according to Anggraeni [13].

For patients with an increased risk of developing caries, or having signs of active caries, it is important to recommend the use of other F products in addition to the twice daily use of toothpaste. There are also indications that tooth brushing with F toothpaste 3 times/day or using a high F toothpaste (5000 ppm F) may have an additional effect on F availability in the oral cavity [14,15]. For these patients, self-applied F products, such as F mouth rinse solutions and F gel may play an important role in caries reduction. However, according to these authors, there is a scientific knowledge gap on the benefit and optimal use of these F adjuncts in combination with daily tooth brushing with F toothpaste [16].

There have been three extensive Cochrane reviews on: i) ‘combinations of topical fluoride (toothpastes, mouth rinses, gels, varnishes) versus single topical fluoride for preventing dental caries in children and adolescents’ [17], ii) ‘fluoride gels for preventing dental caries in children and adolescents’ [18] and iii) ‘fluoride mouth rinses for preventing dental

CONTACT Chutima Trairatvorakul ctrairat@gmail.com Department of Pediatric Dentistry, Faculty of Dentistry, Chulalongkorn University, 34 Henry Dunant Road, Pathumwan, Bangkok 10330, Thailand

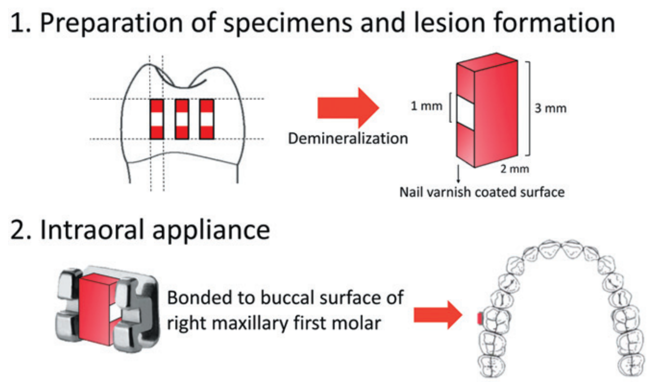

Figure 1. Specimen attached to an orthodontic bracket.

caries in children and adolescents' [19]. The main conclusions from these three reviews are that: i) The preventive fraction increases from fluoride gel or mouth rinse combined with toothpaste versus toothpaste alone were higher in the combined regimens, however, the differences were not statistically significant [17], ii) there is moderate quality evidence of a large caries-inhibiting effect of fluoride gel in the permanent dentition compared with placebo gel, non-placebo control and no treatment group [18] and iii) supervised regular use of fluoride mouth rinse by children and adolescents is associated with a large reduction in caries increment in permanent teeth [19].

Mouth rinse solution and brush-on gel, two home care F products for high-caries risk patients, are recommended to be used as an adjunct to F toothpaste [20]. Fluoride mouth rinse solutions are available over-the-counter for daily or weekly rinsing in many countries as 0.05% NaF (225 ppm F) or 0.2% NaF (900 ppm F). From 35 trials (15,305 participants), the pooled prevented fraction (PF) of F mouth rinsing was 27% [19]. Due to its high flowability, a F solution can easily penetrate into the inaccessible areas of the dentition, such as the interproximal space [21].

Self-applied topical F gels, containing 1–2% NaF (4500–9000 ppm F), used either in individual trays or as a brush-on gel, have similar indications as F mouth rinse. However, the F gel may be more difficult for patients to acquire, because it must be prescribed by a dentist. It is also more time consuming to use the gel in a tray than to brush with the gel as a toothpaste. From 25 trials (8479 participants), the pooled PF of NaF gel was 28%, similar to using NaF mouth rinse solutions [18]. An advantage of the gel is, however, its high viscosity, which provides longer F oral retention compared with a mouth rinse [22,23].

Various *in situ* interproximal models have been used for studying the effect of F products on enamel remineralization [24,25]. The mineral changes are often determined using transverse microradiography (TMR), which is considered the best method for determining enamel mineral density (MD) *in vitro*. Recently, we have developed an *in situ* model and analyzed the MD with micro-computed tomography (Micro-CT; μCT), which has the advantage of non-destructively measuring enamel sample MD [26]. A methodological study of white spot lesions found that there is a close correlation between Micro-CT and TMR evaluation of enamel lesion parameters [27]. TMR requires sample embedding, drying and precise sectioning, followed by development and digitization of the micrographs, while μCT can provide 3D images and MD profiles without these time-consuming procedures.

The objective of this study was to compare the spatial and quantitative remineralizing effects of NaF mouth rinse or NaF brush-on gel used as an adjunct to NaF dentifrice in an *in situ* model by analysing the MD using Micro-CT. We hypothesized that these two NaF products would increase enamel remineralization compared with NaF toothpaste alone and the remineralizing ability of these two fluoride products would be significantly different.

Materials and methods

Design, ethical approval and sample size

The design of this investigation was similar to our prior study [26]. The present randomized, cross-over, single-blinded *in situ* study was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Thailand (HREC-DCU 2014-011).

Fifteen post-orthodontic treatment volunteers, aged 20–23 years, in good general and oral health, were recruited. They had normal salivary flow rate, no active caries and moderate oral hygiene. The volunteers were considered to have high caries risk because they consumed sugary snacks or beverage more than 3 times/day. After they had been given verbal and written explanations of the experimental protocol, informed consent was obtained.

Specimen preparation and lesion formation

The enamel specimens were prepared from the proximal surfaces of 15 sound human premolars extracted for orthodontic purposes (without white spot lesions or other defects). Each surface was polished using an automatic polishing machine (DPS 3200, IMPTECH, Boksburg, South Africa) at 100 rpm for 45 s with water lubrication to remove the outer F-rich layer. Three specimens ($3 \times 2 \times 1$ mm; length $\times$ width $\times$ height) were obtained from each proximal surface with a low-speed precision sectioning saw (ISOMET 1000, Buehler, Lake Bluff, IL). All specimen surfaces were covered with nail varnish (L04, Sephora Nail Lacquer, San Francisco, CA), except for a middle 1×1 mm window (Figure 1).

The specimens were then immersed in a demineralizing solution containing 0.1 M lactic acid (Sigma-Aldrich, Saint Louis, MO), 0.2% (g/100 ml) polyacrylic acid (Carbopol® C907, BF Goodrich, Cleveland, OH), and 50% saturated with hydroxyapatite (Sigma-Aldrich, Saint Louis, MO), pH 4.8 [28], at 37 °C for 7 d to create artificial caries-like lesions that were ≍150 μm in depth. The demineralizing solution was replaced every day. An indented reference line was made on the lateral surface above the window margin of each slab. This reference mark was used for the subsequent Micro-CT evaluations.

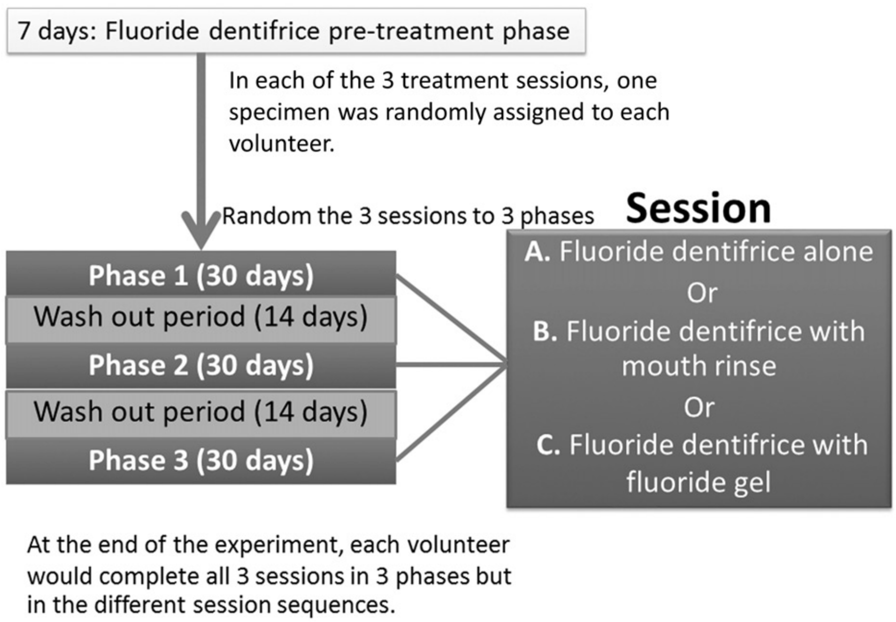

Figure 2. Experimental protocol.

Intraoral appliance preparation

Each specimen was attached to an orthodontic bracket with flowable composite resin (Filtek, 3M-ESPE, ST. Paul, MN) (Figure 1). The lesion was placed against the mesial wing of the bracket to simulate proximal caries. The specimens were then sterilized with ethylene oxide for 12 h and stored in deionized water. A set of 3 specimens from the same proximal surface of a tooth was randomly assigned to each volunteer, who was allocated into 3 experimental sessions by simple randomization. The brackets were bonded using Transbond XT (3M Unitek, Monrovia, CA) to the buccal surface of the right upper first molars of the volunteers.

Experimental protocol and test products

The study was conducted in 3 sessions of 30 days each, A, B and C (Figure 2). At each phase, one specimen was randomly assigned to one of the 3 sessions. During session A, the volunteers were instructed to brush their teeth for 2 min with 1.5 cm (≍1 g) 0.22% NaF toothpaste (1000 ppm F) (Colgate Total 12 Advanced Fresh Gel, Colgate-Palmolive Company, Bangkok, Thailand), 2 times/day, after breakfast and before bedtime, as a control group. After brushing, the subjects were not allowed to eat, drink or rinse for 30 min. Session B was the same as A + rinsing for 1 min with 10 ml 0.05% NaF mouth rinse (225 ppm F; Oral-B Pro-Health Tooth and Gum Care Mouth Rinse, Procter & Gamble, Thailand); the rinsing was performed 30 min after brushing with the toothpaste, just before bedtime. The subjects were advised to expectorate the solution after use, and not to eat, drink or rinse for 30 min. Session C was the same as A + brushing with a thin ribbon (≍0.5 g) 1.1% NaF brush-on gel (5000 ppm F; Prevident Gel, Colgate-Palmolive Company, New York, NY) for 2 min; the brushing with the gel was done 30 min after brushing with the toothpaste, just before bedtime. The subjects were, as per the manufacturer's instructions, advised to expectorate the brush-on gel after use, and not to eat, drink or rinse for 30 min.

One week before the first phase (pre-treatment phase) and during the 2-week washout periods between each phase, the volunteers brushed their teeth with the 0.22% NaF toothpaste as used in the 3 sessions (Figure 2). After each phase, the orthodontic brackets were removed, and the enamel samples were analyzed by Micro-CT again. A new bracket with another specimen from the same tooth was attached following the washout period. Throughout all sessions, the volunteers were instructed to maintain their normal dietary habits and write daily diet records. Daily diet records would help the volunteers in maintaining a similar frequency and amount of food or snack intake during each session.

Two of the test products contained antimicrobial agents; Colgate Total toothpaste contains triclosan and Oral-B Pro-Health contains cetylpyridinium chloride (CPC), the dentifrice and fluoride mouth rinse were bought from a supermarket. The fluoride brush-on gel was ordered from a supplier because it is not available to the public, only through dentists under their instruction.

Mineral density measurement

The MD of the 1 × 1 mm window of each specimen was measured using a desktop cone-beam Micro-CT system (μCT 35, Scanco Medical AG, Bassersdorf, Switzerland). The samples were scanned at 70 kVp, 114 μA, and under standard resolution (1024 × 1024). The samples were rotated at 180° with an integration time of 407 milliseconds and an isometric voxel size of 12 μm. A 0.5-mm thick aluminium filter was placed in the beam path to reduce beam-hardening effects. A 1200 mg HA/cm^3 hydroxyapatite phantom was used to calibrate the beam hardening correction. To calibrate the mineral density, a series of hydroxyapatite phantom

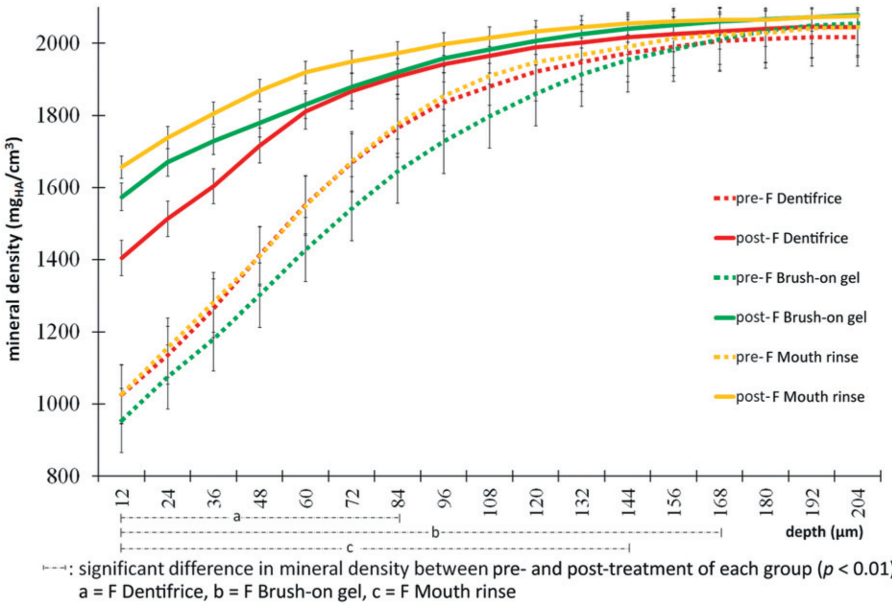

Figure 3. Mean mineral density (mgHA/cm3) through the depth of the lesion (µm) of the experimental groups.

standards with densities of 0, 100, 200, 400 and 800 mg HA/cm^3 were scanned. All Micro-CT measurements were analyzed by the same blinded investigator (PB).

Data analysis

The grey scale value of the Micro-CT scan was converted to MD (mg_{HA}/cm^3). Mean values were calculated from each specimen every 12 µm depth through the entire lesion. To calculate the lesion depth and MD, a mineral density profile was created by plotting MD against lesion depth. The mean MD gain was calculated by subtracting the pre-treatment area under the curve (AUC) with the post-treatment AUC of each control and experimental group. The lesion depth was marked at the depth where the MD was equivalent to 95% of the maximum density [29]. The percent remineralization (%R) indicated the ratio of the MD gain and the MD of the original lesion, which was obtained from the equation:

$$\%R = \left(AUC_{post} - AUC_{pre}/AUC_{pre}\right) \times 100)$$

The percent increased MD compared with dentifrice was calculated from the equation [30]:

$$\%\text{ increased MD compared with dentifrice} = [(\Delta Zd - \Delta Zr)/\Delta Zd] \times 100$$

(ΔZr = the difference in AUC between pre- and post-treatment of the brush-on gel or fluoride mouth rinse phase. ΔZd = the difference in AUC between pre- and post-treatment of the dentifrice phase).

Statistical analysis

Prior to the study, and based on Songsiripradubboon et al, [26], a sample size calculation determined that 12 samples were required to demonstrate an absolute difference of 5% in mean MD change between the two F products (mouth rinse and gel) with 90% power ($\alpha = .05$, $\beta = .10$).

The SPSS program version 17.0 (SPSS, Chicago, IL) was used to analyze the data. The difference between the mineral gain within each experimental treatment was evaluated by the paired *t*-test with a *p* level of .01. The difference in initial MD, initial lesion depth, mineral gain, decrease in lesion depth and depth of remineralization between the three treatments (dentifrice, mouth rinse and brush-on gel) were analyzed using one-way ANOVA with Bonferroni correction, at *p* value of .05.

Results

Twelve out of the 15 volunteers completed all three experimental phases. This is because three specimens from three volunteers broke during orthodontic bracket removal. Thus, 36 (12×3) out of 45 (15×3) specimens (80%) were included in the final analyses.

MD increased through the entire lesion depth after all three treatments and became constant when approaching the maximum density, which was assumed to be sound enamel (Figure 3). A significant difference between the pre- and post-treatment MD was observed from 0 to 168 microns in depth for the F brush-on gel, 0 to 144 microns for the F mouth rinse and 0 to 84 microns for the F dentifrice ($p < .01$, Figure 3).

The mean MD gain between the brush-on gel and mouth rinse was not significantly different. However, both experimental sessions gained significantly more MD compared with F dentifrice alone ($p < .05$) (Table 1).

In addition to the mean MD gain, the remineralizing effect of the two F products was evaluated by % remineralization (%R) (Table 1). The mean MD and %R showed the same trend. The adjunctive use of brush-on F gel and F mouth rinse showed an additional remineralizing effect of approximately 78% and 41%, respectively, compared with using F dentifrice alone.

Table 1. Mineral density, mineral gain, % remineralization, and % increased mineral density (Mean ± S.D.).

Treatment	Mineral density (mg_{HA}/cm^3) Pre	Post	MD gain[A] (mg_{HA}/cm^3)	%R[B]	% Increase compared to dentifrice[C]	Baseline lesion depth (μm)	Decrease in lesion depth (μm)
Dentifrice	1367.8 ± 60.7	1480.6 ± 102.5	112.8 ± 65.9[a]	6.9 ± 4.0[a]	0	128.37 ± 18.83[d]	30.76 ± 21.39[e]
Dentifrice + Gel	1323.9 ± 100.1	1514.4 ± 128.1	190.5 ± 50.3[b]	12.0 ± 3.0[b]	78	138.87 ± 19.28[d]	48.06 ± 23.86[e]
Dentifrice + Mouth rinse	1380.3 ± 80.3	1545.5 ± 112.7	165.3 ± 74.4[b]	10.0 ± 4.6[b]	41	128.93 ± 23.82[d]	46.04 ± 24.17[e]

[A]MD Gain: Mineral gain.
[B]%R: % Remineralization.
[C]% Increase compared to dentifrice: % increase mineral density compared to dentifrice.
Different letters indicate significant difference ($p > .05$) in the same column.

The brush-on gel treatment yielded the greatest mean depth of remineralization (168 μm), followed by the rinse (144 μm), and the dentifrice (84 μm) treatments. Although the mean depth of remineralization between brush-on gel and rinse treatment was not significantly different, both additional F products yielded a significant difference in depth of remineralization compared with F dentifrice treatment ($p < .05$).

Discussion

The present study found that the use of the two topical F agents, 1.1% NaF brush-on gel and 0.05% NaF mouth rinse, as an adjunct to 0.22% NaF dentifrice resulted in increased incipient caries-like enamel lesion mineralization. Although the brush-on gel had a somewhat greater effect compared with the mouth rinse solution, the difference was not significant.

In the present study, we used Micro-CT to evaluate the remineralization effect of the F agents on demineralized enamel. This method is as a non-destructive alternative to TMR, which is considered as the optimum technique for evaluating enamel lesions [27].

The reason we used a dentifrice containing triclosan in our study is that this toothpaste is widely available in Thailand. Triclosan does not increase or reduce the anticaries efficacy of sodium fluoride toothpaste [31]. The mouth rinse used contained CPC because there is no pure sodium fluoride mouth rinse commercially available. Moi et al. demonstrated that a NaF mouth rinse containing CPC had a similar effect on enamel remineralization to that of NaF mouth rinse alone [32]. Thus, these substances in the two products tested presumably had no confounding effects on the results in our study.

Topical F efficacy can be evaluated by the amount of available F ions and their accessibility to cariogenic sites [33]. Fluoride bioavailability is influenced by many so-called individual-related factors (e.g. oral clearance and plaque amount) and product-related factors (e.g. F concentration and formulation). The individual-related factors played only a minimal role in the present investigation because the study was performed using a cross-over design. An important factor to take into consideration is how well the subjects followed the instructions given. To monitor the compliance of the volunteers, we tracked the product amount used and replenished to the volunteers according to the estimated 30-day use.

Fluoride bioavailability is also influenced by many product-related factors, such as the F dose, product formulation and the F concentration. The total F dose was approximately the same for the two regimens, 0.5 g of 5000 ppm gel = 2.5 mg F and 10 ml of 225 ppm solution = 2.25 mg F. The two products formulations differ in many respects, including contact time (1 vs. 2 min), viscosity, taste, product handling. The mouth rinse is highly flowable and can easily access a proximal lesion, bringing more F ions to the lesion compared with a brush-on gel. However, as a liquid, it is not well retained in areas it reaches. Thus, the F ions rapidly wash out before reaching the inner part of the caries lesion. The product use is easy, with 10 ml swished around in the mouth for 1 min.

In contrast to mouth rinse solutions, fluoride gels have a high viscosity and thereby a longer salivary retention time [22,23]. Thus, the brush-on gel used in the present investigation may be retained in the dental plaque, on the oral mucosa and on the tooth surfaces. The brush-on gel then gradually dissolves into the saliva, releasing F ions, which can penetrate into the enamel, resulting in a cariostatic effect. Thus, the brush-on gel acts as an F reservoir, maintaining sufficient amounts of F ions to penetrate into the deeper lesion area. A brush-on gel is somewhat more complicated to handle compared with a rinsing solution, because it is applied on a toothbrush and used for 2 min.

For an F containing product to have a cariostatic effect, F has to dissolve in the saliva and other oral fluids, and then diffuse into the enamel [34]. The difference between the pre- and post-treatment MD values (Figure 3) may partly result from the respective penetrating ability of the three products evaluated in the present study. The greater difference between the pre- and post-treatment MDs at the superficial lesion depths compared with the deeper lesion depths of the fluoride mouth rinse group indicate a greater mineral gain was achieved in the superficial lesion layer. However, progressing further through the lesion, the mineral gain of the fluoride brush-on gel group was higher than that of the mouth rinse group. These results indicate that at the deeper lesion layer, fluoride brush-on gel was more effective.

Topical agents with higher F concentrations result in a greater increase in salivary F levels immediately after application and are retained longer in the saliva compared with the lower F concentration agents [35,36]. Because the brush-on gel has a high F concentration (5000 ppm), it will result in a high initial oral F ion retention, which provides a greater driving force and F ion penetration through the dental plaque and into the enamel fluids compared with the F rinse. Based on this condition, a brush-on gel is likely to produce a more favourable outcome compared with a mouth rinse solution with lower (225 ppm) F concentration [22,23]. For a 900-ppm F rinse, which is used for daily rinsing in the Nordic countries including Sweden [12,35,36], the results may be different.

The overall results of our study indicate a similar remineralizing ability of the brush-on F gel and the F mouth rinse with a trend to more remineralization for the gel. In the present study, we focussed on interproximal caries lesions and the results may have been different if we used buccal lesions. The F concentration in the plaque at the proximal area is approximately 50% of that at the smooth surface area [37]. The gel viscosity may account for limited accessibility to the proximal lesion site [21]. However, in the present study, we used the brush-on gel according to the instructions given by the manufacturer; thus, it was applied by a toothbrush. If the gel had been applied directly to the interproximal lesion with a finger, a higher MD gain might occur. This hypothesis is supported by a study by Nordström & Birkhed [14], showing that a 5000- or 1450-ppm F toothpaste applied as a 'lotion' and massaged into the buccal surfaces with a fingertip increased F retention in the plaque and saliva. To extrapolate these data into the clinical situation and to caries risk patients, it is important to perform further clinical studies by extending the experimental period and using subtraction bitewing radiography to evaluate lesion remineralization.

According to the experimental protocol, these two fluoride products could be used as adjunct to fluoride toothpaste in daily routine. However, our results may represent only short-term efficacy of these products, owing to the limited experimental time. Therefore, long-term clinical trial on the practicality and ease of use should be further studied.

In conclusion, both the 226-ppm F mouth rinse solution and the 5000-ppm F brush-on gel resulted in increased MD throughout the lesions and deeper remineralization compared with those of the 1000-ppm F dentifrice. Thus, using these two topical F agents for 30 d resulted in a significantly greater remineralizing effect on artificial caries lesion lesions in enamel compared with the F dentifrice alone.

Acknowledgements

The authors thank Dr. Tewarit Somkotra for statistical advice, Dr. Thanit Charoenratana for orthodontic bracket application, Dr. Kevin Tompkins for manuscript revision, and the Faculty of Dentistry, Chulalongkorn University for research funding.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390:1211–1259.

[2] Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. Am J Dent. 2009;22:3–8.

[3] da Silveira Moreira R. Epidemiology of dental caries in the world. Oral health care – Pediatric, research, epidemiology and clinical practices. London: InTech; 2012.

[4] Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. Community Dent Oral Epidemiol. 2004;32:319–321.

[5] Lagerweij MD, van Loveren C. Declining caries trends: are we satisfied? Curr Oral Health Rep. 2015;2:212–217.

[6] Petersson GH, Bratthall D. The caries decline: a review of reviews. Eur J Oral Sci. 1996;104:436–443.

[7] Bratthall D, Hänsel-Petersson G, Sundberg H. Reasons for the caries decline: what do the experts believe? Eur J Oral Sci. 1996;104:416–422, 423–425, 430–432.

[8] Bansal A, Ingle NA, Kaur N, et al. Recent advancements in fluoride: a systematic review. J Int Soc Prev Community Dent. 2015;5:341–346.

[9] Pitts NB, Zero DT, Marsh PD, et al. Dental caries. Nat Rev Dis Primers. 2017;3:17030.

[10] Honkala S, Vereecken C, Niclasen B, et al. Trends in toothbrushing in 20 countries/regions from 1994 to 2010. Eur J Public Health. 2015;25:20–23.

[11] Petersen PE, Phantumvanit P. Perspectives in the effective use of fluoride in Asia. J Dent Res. 2012;9:119–121.

[12] National Guidelines for Adult Dental Care. Socialstyrelsen; 2011.Available from: http://www.socialstyrelsen.se/nationalguidelines

[13] Angraenni ZR. Tooth brushing behaviour in Asia: understanding the barriers that impact oral health status. Int Dent J. 2009;59:229–236.

[14] Nordström A, Birkhed D. Effect of a third application of toothpastes (1450 and 5000 ppm F), including a 'massage' method on fluoride retention and pH drop in plaque. Acta Odontol Scand. 2013;71:50–56.

[15] Ekstrand KR, Ekstrand ML, Lykkeaa J, et al. Whole-saliva fluoride levels and saturation indices in 65+ elderly during use of four different toothpaste regimens. Caries Res. 2015;49:489–498.

[16] Twetman S, Keller MK. Fluoride rinses, gels and foams: an update of controlled clinical trials. Caries Res. 2016;50(Suppl 1):38–44.

[17] Marinho VC, Higgins JP, Sheiham A, et al. Combinations of topical fluoride (toothpastes, mouthrinses, gels, varnishes) versus single topical fluoride for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2004;CD002781.

[18] Marinho VC, Worthington HV, Walsh T, et al. Fluoride gels for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2015;CD002280.

[19] Marinho VC, Chong LY, Worthington HV, et al. Fluoride mouthrinses for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2016;CD002284.

[20] American Dental Association. Topical fluoride for caries prevention: executive summary of the updated clinical recommendations and supporting systematic review. J Am Dent Assoc. 2013;144:1279–1291.

[21] Särner B, Birkhed D, Lingström P. Approximal fluoride concentration using different fluoridated products alone or in combination. Caries Res. 2008;42:73–78.

[22] Zero DT, Raubertas RF, Pedersen AM, et al. Studies of fluoride retention by oral soft tissues after the application of home-use topical fluorides. J Dent Res. 1992;7:1546–1552.

[23] Zero DT, Raubertas RF, Fu J, et al. Fluoride concentrations in plaque, whole saliva, and ductal saliva after application of home-use topical fluorides. J Dent Res. 1992;71:1768–1775.

[24] Dunipace AJ, Hall AF, Kelly SA, et al. An in situ interproximal model for studying the effect of fluoride on enamel. Caries Res. 1997;31:60–70.

[25] Kashani H, Birkhed D, Petersson LG. Fluoride concentration in the approximal area after using toothpicks and other fluoride-containing products. Eur J Oral Sci. 1998;106:564–570.

[26] Songsiripradubboon S, Hamba H, Trairatvorakul C, et al. Sodium fluoride mouthrinse used twice daily increased incipient caries lesion remineralization in an in situ model. J Dent. 2014;42: 271–278.

[27] Hamba H, Nikaido T, Sadr A, et al. Enamel lesion parameter correlations between polychromatic micro-CT and TMR. J Dent Res. 2012;91:586–591.

[28] White D. Use of synthetic polymer gels for artificial carious lesion preparation. Caries Res. 1987;21:228–242.

[29] Arends J, Dijkman T, Christoffersen J. Average mineral loss in dental enamel during demineralization. Caries Res. 1987;21: 249–254.

[30] Walker GD, Cai F, Shen P, et al. Consumption of milk with added casein phosphopeptide-amorphous calcium phosphate remineralizes enamel subsurface lesions in situ. Aust Dent J. 2009;54: 245–249.

[31] Hawley GM, Hamilton FA, Worthington HV, et al. A 30-month study investigating the effect of adding triclosan/copolymer to a fluoride dentifrice. Caries Res. 1995;29:163–167.

[32] Moi GP, Tenuta LM, Cury JA. Anticaries potential of a fluoride mouthrinse evaluated in vitro by validated protocols. Braz Dent J. 2008;19:91–96.

[33] Mellberg JR. Evaluation of topical fluoride preparations. J Dent Res. 1990;69:771–779.

[34] Ekstrand J. Pharmacokinetic aspects of topical fluorides. J Dent Res. 1987;66:1061–1065.

[35] Aasenden R, Brudevold F, Richardson B. Clearance of fluoride from the mouth after topical treatment or the use of a fluoride mouthrinse. Arch Oral Biol. 1968;13:625–636.

[36] Seppä L, Salmenkivi S, Hausen H. Salivary fluoride concentration in adults after different fluoride procedures. Acta Odontol Scand. 1997;55:84–87.

[37] Wilson RF, Ashley FP. Collection and biochemical analysis of human dental plaque from the approximal tooth surfaces and comparison with plaque from free smooth surfaces. Arch Oral Biol. 1988;33:473–478.