Acta Odontologica Scandinavica, 2010; 68: 323–328

informa healthcare

ORIGINAL ARTICLE

Combination of high-fluoride toothpaste and no post-brushing water rinsing on enamel demineralization using an *in-situ* caries model with orthodontic bands

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Abstract

Objective. To compare the effects on enamel demineralization and fluoride (F) retention of two different brushing–rinsing regimens. ***Material and methods.*** An *in-situ* caries model with orthodontic bands was used for 8–9 weeks. A total of 20 orthodontic patients participated. They were randomized into two groups: (1) a test group using 5000 ppm F ($n = 10$) with no post-brushing water rinsing; and (2) a control group using 1450 ppm F ($n = 10$) with three sessions of post-brushing water rinsing. Orthodontic stainless-steel bands were applied to the two upper first premolars, leaving 2–3 mm of space away from the exposed buccal surface in order to accumulate plaque and provoke initial caries development. The teeth were extracted after 8 and 9 weeks, then analysed using quantitative light-induced fluorescence (QLF). Additionally, oral F retention was compared for the two groups. ***Results.*** In comparison to the control group, the test regimen resulted in a non-significant smaller QLF lesion area and a significantly lower average QLF loss of fluorescence ($P < 0.05$). The highest F retention concentration under the band was found in the test group ($P < 0.001$). ***Conclusions.*** The combination of using a 5000 ppm F toothpaste and no post-brushing water rinsing had a greater anti-caries potential and resulted in elevated oral F retention compared to a 1450 ppm F toothpaste with three sessions of post-brushing water rinsing.

Key Words: *Caries model, orthodontic bands, quantitative light-induced fluorescence, tooth brushing, toothpaste, water rinsing*

Introduction

The use of fluoride (F) toothpaste is generally recognized as the main reason for the decline in caries in industrialized countries over the last four decades [1–3]. Numerous studies have shown that even low levels of F, resulting from the regular use of toothpaste, have a profound effect on enamel demineralization and remineralization [4,5]. As F is the key component of oral health promotion, a coordinated approach on a community level seems to be needed to maximize the cost–benefit ratio of caries prevention using F toothpaste [6].

Two factors play an important role in the anti-caries effect of F toothpaste: (1) the concentration of F in the toothpaste; and (2) the post-brushing water-rinsing behaviour. There seems to be a correlation between the F concentration of dentifrices ranging between 0 and 5000 ppm F and caries prevention [7]. Treatment of demineralized dentin with a toothpaste containing 5000 ppm F reduces mineral loss and lesion depth on exposed dentin [8]. In a randomized clinical trial comparing 5000 and 1450 ppm F, the high F toothpaste reversed non-cavitated fissure caries lesions. Moreover, the group using 5000 ppm F showed a significantly higher decrease in laser fluorescence of enamel than the 1450 ppm F group [9]. Furthermore, a dentifrice containing 5000 ppm F was significantly better at remineralizing root caries lesions than a dentifrice with 1100 ppm F [10].

The rinsing method after tooth brushing has been found to correlate with caries experience and caries increment [11]. Salivary F concentration measured

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(Received 25 May 2010; accepted 28 July 2010)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016357.2010.512863

after dentifrice application decreases significantly with increasing water volume, rinse duration and frequency of rinsing [12,13]. A toothpaste technique where a slurry rinse with the toothpaste was carried out after brushing increased the efficacy of F toothpaste, reducing approximal caries in preschool children by an average of 26% [14]. Furthermore, eating immediately after brushing reduced the salivary F level ≈12–15-fold compared with brushing alone [15]. An increase in F in both proximal saliva and plaque was observed using a dentifrice with 5000 ppm F without post-brushing water rinsing compared to a with-rinsing regimen [16].

Since the introduction of the *in-situ* caries model by Koulourides et al. [17], many researchers have used various models to test caries-preventive products and methods. By using *in-situ* models it may be possible to study both fundamental aspects of the caries process and the role of F in caries prevention [18–20]. The aim of the present study was to compare the anti-caries potential and oral F retention of a combination of a high F dentifrice (containing 5000 ppm F) without post-brushing water rinsing versus a standard F dentifrice (containing 1450 ppm F) followed by three sessions of post-brushing water rinsing using an *in-situ* caries model with orthodontic bands.

Material and methods

Subjects

During a period of 12 months, ≈200 patients were screened at the Department of Orthodontics in Göteborg. For each patient their paraffin-stimulated whole saliva was cultured on selective agar medium to determine the mutans *Streptococci* count (MS). A total of 24 healthy orthodontic patients were recruited during the screening. They had to fulfil the following three inclusion criteria: (1) medium to high scores of MS in saliva [≥10^4 colony-forming units (CFUs)/ml]; (2) two upper premolars scheduled for extraction; and (3) willingness to participate. The 24 patients were randomized into two groups: (1) a test group (six males, six females, mean ± SD age 16.4 ± 1.8 years); and (2) a control group (six males, six females, mean ± SD age 16.2 ± 2.1 years) (Table I). They were all in good general health and had sound upper first premolars with no evidence of initial caries. The Ethics Committee of the Sahlgrenska Academy at the University of Gotebörg approved the study.

In-situ *caries model*

The model used in this study was first described by Øgaard et al. [21]. The teeth to be extracted for orthodontic reasons were carefully cleaned with a rubber cup on a hand-piece and a non F-containing pumice prior to banding (in order to remove the outer F-rich surface). Orthodontic stainless-steel bands were then applied to the first premolars. An orthodontic separator holder was divided into two pieces and ligated to the buccal surface of the band before banding, leaving 2–3 mm of space adjacent to the exposed buccal surface of the tooth upon subsequent removal (Figure 1). The bands were cemented using a non-F-containing material (zinc phosphate cement). The teeth were extracted at two intervals, i.e. 8 and 9 weeks after banding.

Table I. Main brushing/rinsing characteristics of the test and control groups.

Characteristic	Test group	Control group
Toothpaste		
Fluoride concentration (ppm)	5000	1450
Type of fluoride	NaF	NaF + MFP
Toothpaste slurry rinsing?	Yes	No
Post-brushing water rinsing?	No	Yes

MFP = monofluorophosphate.

Toothpaste technique

During the 8/9-week period, each of the two groups were instructed to use one of two different procedures: (1) the test group used a high F dentifrice containing 5000 ppm F, as NaF (Duraphat; Colgate-Palmolive AB, Danderyd, Sweden), with toothpaste slurry rinsing for 30 s and no post-brushing water rinsing; (2) the control group used a normal F dentifrice containing 1450 ppm F (1000 ppm as NaF and 450 ppm as monofluorophosphate) (Maximum Cavity Protection; Colgate) with three sessions of post-brushing water rinsing, each lasting for ≈10 s. All subjects were instructed to brush their teeth with a reasonable amount of toothpaste (≈1 g) three times a day, i.e. after breakfast, after lunch and just before bedtime. They were asked not to brush the banded teeth, thereby avoiding the brush bristles coming close to or reaching the exposed buccal surface of the banded premolars. Both groups were carefully instructed how to brush and were trained in the clinic. They were also given a pamphlet detailing the exact procedure to be followed. The patients were instructed not to use any F products, apart from the test toothpaste, not to use any approximal cleaning and not to use chewing gum or snuff products during the test period.

Intra-oral fluoride retention

Between weeks 8 and 9, the patients were asked to come back to the clinic, when unstimulated whole saliva was sampled by asking the patient to spit twice

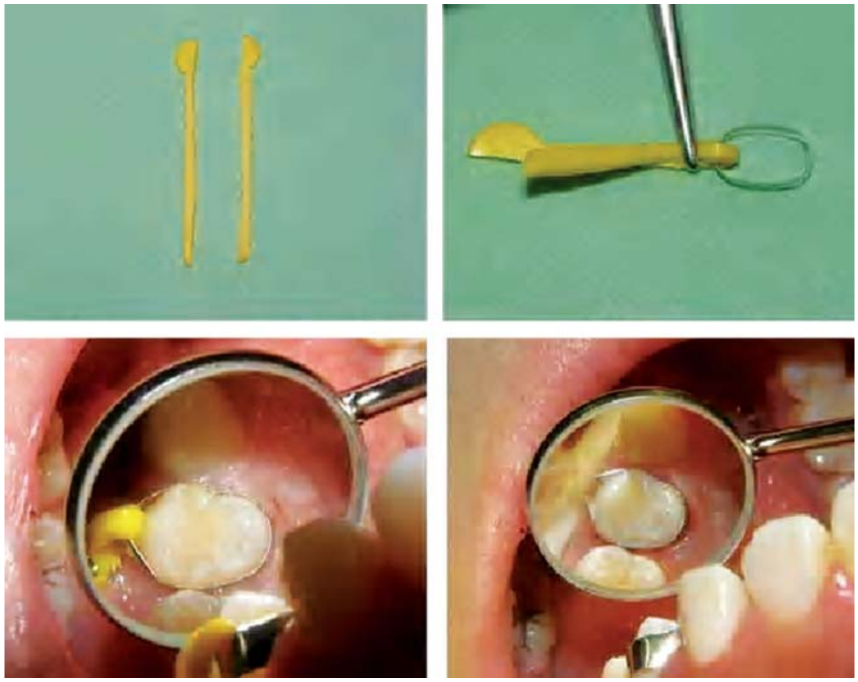

Figure 1. Aspects of the *in-situ* caries experimental model used in the study. Upper left: The orthodontic separator holder is divided into two pieces. Upper right: One half of the separator is wrapped around the buccal surface of the stainless-steel band. Lower left: Non-F-containing cement is used to cement the band (with a 1–2 mm distance from the separator holder). Lower right: After removal of the separator, a 2–3 mm space is present between the buccal surface of the premolar and the band.

into a beaker. The solution under the band was sampled using standardized, triangular-shaped paper points. The points were punched from filter paper (Munktell Filter; Laboratorieläsk, Grycksbo, Sweden) to a size of 5×2 mm^2 using a punch instrument. Each paper point absorbs ≈4 µl (± 10%) of fluid when kept in place for 20 s using a pair of forceps. Immediately after removal, the paper points were transferred to a 0.5-ml Eppendorf tube [15,22]. After collection of the baseline samples, the subjects brushed their teeth with the same toothpaste and used the same post-brushing water-rinsing procedure as during the 8/9-week test period. Sampling of the two band sites was then repeated after 1, 3, 5, 10, 20 and 30 min. The samples were frozen at –20°C until analysed. The patients were also observed during the brushing regarding water-rinsing volume, amount of toothpaste and brushing time.

Fluoride analysis

Analysis of samples was carried out in blinded fashion with regard to subjects and brushing–rinsing method. A 200-µl volume of liquid, consisting of distilled water and TISAB III (10:1; Thermo Electron, Waltham, MA), was added to the samples. The filter paper samples were kept in a refrigerator at +4°C overnight in order to release F from the paper. The tubes were then vibrated in a Minishaker MS1 (IKA, Wilmington, NC) for 20 s. A 100-µl drop of the solution was placed on a Petri dish. The F concentration was measured by an ion-specific electrode (Model 96-09; Orion Research, Beverly, MA) by carefully lowering the electrode into the fluid. The surface tension of the drop ensured that the liquid enclosed the entire membrane surface of the electrode. In order to calibrate the electrode, three standard solutions were used (0.1, 1.0 and 10 ppm F). The F concentration in the sample was expressed as parts per million. The sample F concentrations were plotted versus time and the area under the curve ($AUC_{0–30}$ min) was calculated for each individual and each brushing method using a computer program (KaleidaGraph 3.01; Synergy Software, Reading, PA).

Quantitative light-induced fluorescence

After the 8/9-week period, the banded premolars were extracted and sent to the Divisions of Cariology at the Department of Dental Medicine in Huddinge for quantitative light-induced fluorescence (QLF) measurements. The QLF method has previously been described in detail [23] and has been reviewed by Angmar-Månsson and ten Bosch [24]. The buccal test surfaces were illuminated by blue light from a xenon arc lamp with a blue filter (peak intensity wavelength of 370 nm with a full-width at half-maximum of 80 nm). The lesions were recorded with a micro-charge-coupled device video camera

(WV-KS 152; Panasonic, Tokyo, Japan) equipped with a yellow high-pass filter (Hoya Y-50; Haya Corporation, Fremont, CA) to exclude excitation and ambient light with wavelengths < 520 nm. A computer program (QLF 1.97e; Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to display, store, browse and analyse the images. Two quantities were obtained: (1) lesion area (mm^2); and (2) average change in fluorescence (△F; %).

Statistical analysis

The unpaired *t*-test was used to compare the two groups regarding water–rinsing volume, amount of toothpaste, brushing time, retention of F in the band fluid sample (based on AUC_{0-30}) and QLF data. For each subject, the mean of the data for the two extracted teeth was used for QLF analysis. The paired *t*-test was also used to check intra-examiner reliability of 20 randomly selected teeth for repeat QLF measurements. $P < 0.05$ was considered statistically significant.

Results

During the 8/9-week test period four subjects were lost to the study, leaving 20 subjects, 10 in each group. The reason for subject dropouts was the development of loose bands on the premolars. The mean ± SD MS score was 5.1 ± 1.0 log CFU/ml in the test group and 4.9 ± 0.8 log CFU/ml in the control group, with no significant difference between the groups.

The rinsing volume, amount of toothpaste used and brushing time in the two groups are shown in Table II. There were no significant differences in the last two parameters; for rinsing volume there were significant differences, as per the protocol design. The QLF variables are shown in Table III. In comparison to the test group, the control group had a non-significantly larger average lesion area and increased average loss in fluorescence ($P < 0.05$) compared to the surrounding sound enamel. The 20 randomly selected teeth were checked after 1 month. The *t*-test showed no significant difference between the repeat QLF measurements, indicating good reproducibility for the QLF readings.

Table II. Water rinsing, amount of toothpaste and brushing time for the two groups. Significant differences are also shown.

Characteristic	Test group (n = 10)	Control group (n = 10)	P
Water rinsing (ml)	0.0 ± 0.0	28.0 ± 16.9	< 0.001
Amount of toothpaste (g)	1.3 ± 0.5	1.3 ± 0.5	NS
Brushing time (min)	1.9 ± 0.7	2.1 ± 0.8	NS

Table III. Lesion area and ΔF based on QLF for the two groups. Significant differences are also shown.

Characteristic	Test group (n = 10)	Control group (n = 10)	P
Lesion area (mm^2)	1.2 ± 1.7	3.2 ± 2.7	NS
ΔF (%)	–10 ± 3.9	–15.8 ± 6.3	< 0.05

The mean F concentration of the fluid under the band at the various time points and the AUC values are shown in Figure 2. The test group showed the highest F concentration under the band compared to the control group ($P < 0.001$). The difference between the groups was most pronounced within the first 20 min. The F concentration results for the whole-saliva test were very similar to those for the fluid under the band (data not shown).

Discussion

The application of the *in-situ* model used in this study was slightly challenging. The four subjects who were lost at the beginning of the study played an important role in improving the *in-situ* model since the bands became loose due to practical reasons relating to insertion and cementation. Although both groups exhibited loss of enamel fluorescence, the changes over the study period were on the whole relatively small. The reasons could be that the study period was short and/or that both groups used F toothpastes, and that they brushed three times a day. The space between the band and the tooth (2–3 mm) was adequate for the present study, leaving a large volume for plaque accumulation and initial caries development. The study period of 8/9 weeks might be considered too short, but extending it would have been difficult as the subjects were eager to start the orthodontic treatment as soon as possible. On the other hand, the 8/9-week period has advantages, since it is relatively easy for the subjects to follow the brushing–rinsing protocol and to keep frequent contact with the principal investigator throughout the study. Moreover, earlier studies have shown that QLF can disclose remineralization of white spot lesions after an interval of only 6 weeks [23].

During the 12-month screening period, only 24 patients were recruited. This was mainly due to two reasons: (1) not all orthodontic patients require extraction of two upper premolars; and (2) many of the patients had saliva MS counts that were too low. It

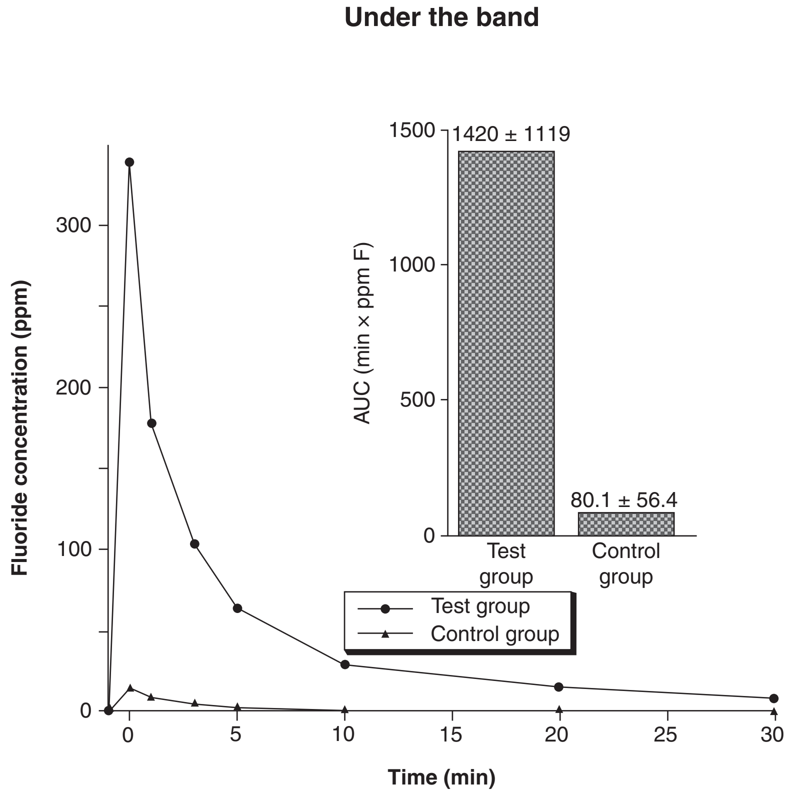

Figure 2. Mean values of F concentration under the band (expressed as parts per million) at various time points up to 30 min in the two groups (5000 and 1450 ppm F). The inset shows the AUC values (0–30 min) expressed as the mean ± SD. The bars indicate statistically significant differences between the two groups ($P < 0.001$).

would be interesting to determine whether the F toothpaste concentration or the post-brushing rinsing behaviour is more important. It may be that it is the combination of the two factors which produces the observed differences. We decided to focus on this combination, i.e. 5000 ppm F with no post-brushing water rinsing and 1450 ppm F with three sessions of water rinsing, based on data from a recent study at our department by Nordström and Birkhed [16] which concluded that the group using high F toothpaste and no post-brushing water rinsing had the highest F concentration in plaque and saliva. In the present study we aimed to confirm these findings clinically in relation to enamel demineralization. Many countries do not have 5000 ppm F toothpaste available in their markets. We believe, however, that using a 1450 ppm F toothpaste with no post-brushing water rinsing would be a good compromise for caries prevention, which is in agreement with Nordström and Birkhed [16].

In the fluoride retention curve (Figure 2), the first 20 min showed high F values under the band, especially in the test group. There were, however, large SD values, which is in accordance with other studies [16,25]. This variation could be due to individual behaviour factors, for example the amount of toothpaste used, amount of rinsing water, number of rinsing sessions or motor activity by the lips, tongue and cheeks.

Groups with high caries risk, e.g. patients with dry mouth or orthodontic appliances/extensive restorations or periodontal disease patients with exposed root surfaces, should be targeted to use 5000 ppm F toothpaste. Orthodontic patients are usually teenagers with multiple newly erupted permanent teeth. Compared with other dental practitioners, orthodontists have a great opportunity to place the emphasis on F toothpaste regimens in their clinics, as they usually visit the clinic every 8–12 weeks. In a recent long-term clinical study we showed that improved F toothpaste technique results in a caries-preventive fraction of ≈80% over a 2-year period in orthodontic patients [26]. A recent study by Zero et al. [27] has shown that both brushing time and dentifrice quantity may be important for F retention in the oral cavity and for the extent of enamel remineralization.

Even if the use of 5000 ppm F with toothpaste slurry rinsing and no post-brushing water rinsing is interesting from a cariological point of view, patients must be aware of the possible side-effects. Slurry rinsing with toothpaste can cause some oral discomfort and irritation of the oral mucosa. However, our experience is that very few patients experienced any side-effects. Another problem is that a 5000 ppm F toothpaste delivers more F to the oral cavity than standard toothpastes containing 1000–1500 ppm F and that some of this will be retained and swallowed when no post-brushing water rinsing is used.

However, only 5–10% is normally swallowed using the slurry-rinsing technique [28]. If 1 g of a 5000 ppm F toothpaste containing 5 mg F/ml is used and 10% is swallowed each time, the result is an intake of $0.10 \times 5 = 0.5$ mg F, which is negligible from a toxicological point of view for an adult. Children < 16 years old should not use 5000 ppm F toothpaste, according to instructions given by the manufacturer. We believe, however, that this age could be lowered to 12 years (when many permanent teeth erupt) if the child has increased caries risk and is carefully instructed to spit out the toothpaste.

Based on the findings of the present study, one may conclude that the combination of using a 5000 ppm F toothpaste and no post-brushing water rinsing will have a greater anti-caries potential and give higher oral F retention compared to a 1450 ppm F toothpaste with three sessions of post-brushing water rinsing.

Acknowledgements

The authors express their appreciation to Ann-Britt Lundberg for her great support in the laboratory.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Marinho VC, Higgins JP, Sheiham A, Logan S. Fluoride toothpastes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2003(1); CD002278.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Kallestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: a systematic review. Acta Odontol Scand 2003;61: 347–55.

[3] Twetman S. Caries prevention with fluoride toothpaste in children: an update. Eur Arch Paediatr Dent 2009;10: 162–7.

[4] Lynch RJ, Navada R, Walia R. Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. Int Dent J 2004;54(5 Suppl 1):304–9.

[5] Bowen WH. The role of fluoride toothpastes in the prevention of dental caries. J R Soc Med 1995;88:505–7.

[6] Brambilla E. Fluoride—is it capable of fighting old and new dental diseases? An overview of existing fluoride compounds and their clinical applications. Caries Res 2001;35(Suppl 1): 6–9.

[7] Tavss EA, Mellberg JR, Joziak M, Gambogi RJ, Fisher SW. Relationship between dentifrice fluoride concentration and clinical caries reduction. Am J Dent 2003;16:369–74.

[8] Bizhang M, Chun YH, Winterfeld MT, Altenburger MJ, Raab WH, Zimmer S. Effect of a 5000 ppm fluoride toothpaste and a 250 ppm fluoride mouth rinse on the demineralisation of dentin surfaces. BMC Res Notes 2009;2:147.

[9] Schirrmeister JF, Gebrande JP, Altenburger MJ, Monting JS, Hellwig E. Effect of dentifrice containing 5000 ppm fluoride on non-cavitated fissure carious lesions in vivo after 2 weeks. Am J Dent 2007;20:212–6.

[10] Baysan A, Lynch E, Ellwood R, Davies R, Petersson L, Borsboom P. Reversal of primary root caries using dentifrices containing 5,000 and 1,100 ppm fluoride. Caries Res 2001; 35:41–6.

[11] Chestnutt IG, Schafer F, Jacobson AP, Stephen KW. The influence of toothbrushing frequency and post-brushing rinsing on caries experience in a caries clinical trial. Commun Dent Oral Epidemiol 1998;26:406–11.

[12] Duckworth RM, Knoop DT, Stephen KW. Effect of mouthrinsing after toothbrushing with a fluoride dentifrice on human salivary fluoride levels. Caries Res 1991;25:287–91.

[13] Attin T, Hellwig E. Salivary fluoride content after toothbrushing with a sodium fluoride and an amine fluoride dentifrice followed by different mouthrinsing procedures. J Clin Dent 1996;7:6–8.

[14] Sjögren K, Birkhed D, Rangmar B. Effect of a modified toothpaste technique on approximal caries in preschool children. Caries Res 1995;29:435–41.

[15] Sjögren K, Birkhed D. Effect of various post-brushing activities on salivary fluoride concentration after toothbrushing with a sodium fluoride dentifrice. Caries Res 1994;28:127–31.

[16] Nordström A, Birkhed D. Fluoride retention in proximal plaque and saliva using two NaF dentifrices containing 5,000 and 1,450 ppm F with and without water rinsing. Caries Res 2009;43:64–9.

[17] Koulourides T, Phantumvanit P, Munksgaard EC, Housch T. An intraoral model used for studies of fluoride incorporation in enamel. J Oral Pathol 1974;3:185–96.

[18] Zero DT. In situ caries models. Adv Dent Res 1995;9:214–30.

[19] Øgaard B, Rølla G. The in vivo orthodontic banding model for vital teeth and the in situ orthodontic banding model for hard-tissue slabs. J Dent Res 1992;71(Spec No.):832–5.

[20] Featherstone JD, Zero DT. An in situ model for simultaneous assessment of inhibition of demineralization and enhancement of remineralization. J Dent Res 1992;71(Spec No.):804–10.

[21] Øgaard B, Rølla G, Helgeland K. Fluoride retention in sound and demineralized enamel in vivo after treatment with a fluoride varnish (Duraphat). Scand J Dent Res 1984;92: 190–7.

[22] Särner B, Lingström P, Birkhed D. Fluoride release from NaF- and AmF-impregnated toothpicks and dental flosses in vitro and in vivo. Acta Odontol Scand 2003;61: 289–96.

[23] Tranaeus S, Al-Khateeb S, Björkman S, Twetman S, Angmar-Månsson B. Application of quantitative light-induced fluorescence to monitor incipient lesions in caries-active children. A comparative study of remineralisation by fluoride varnish and professional cleaning. Eur J Oral Sci 2001;109:71–5.

[24] Angmar-Månsson B, ten Bosch JJ. Quantitative light-induced fluorescence (QLF): a method for assessment of incipient caries lesions. Dentomaxillofac Radiol 2001;30:298–307.

[25] Duckworth RM, Maguire A, Omid N, Steen IN, McCracken GI, Zohoori FV. Effect of rinsing with mouthwashes after brushing with a fluoridated toothpaste on salivary fluoride concentration. Caries Res 2009;43:391–6.

[26] Al Mulla A, Birkhed D. Modified fluoride toothpaste technique reduces caries in orthodontic patients—a longitudinal, randomized clinical trial. Am J Orthod Dentofacial Orthop 2010; In press.

[27] Zero DT, Creeth JE, Bosma ML, Butler A, Guibert RG, Karwal R, et al. The effect of brushing time and dentifrice quantity on fluoride delivery in vivo and enamel surface microhardness in situ. Caries Res 2010;44:90–100.

[28] Sjögren K, Ekstrand J, Birkhed D. Effect of water rinsing after toothbrushing on fluoride ingestion and absorption. Caries Res 1994;28:455–9.