In vitro caries lesion rehardening and enamel fluoride uptake from fluoride varnishes as a function of application mode

Frank Lippert, MSc, PhD, **Anderson T. Hara**, DDS, MS, PhD, **Esperanza Angeles Martinez-Mier**, DDS, MSD, PhD & **Domenick T. Zero**, DDS, MS

Abstract: Purpose: To study the laboratory predicted anticaries efficacy of five commercially available fluoride varnishes (FV) by determining their ability to reharden and to deliver fluoride to an early caries lesion when applied directly or in close vicinity to the lesion (halo effect). **Methods:** Early caries lesions were created in 80 polished bovine enamel specimens. Specimens were allocated to five FV groups (n=16) based on Knoop surface microhardness (KHN) after lesion creation. All tested FV claimed to contain 5% sodium fluoride and were: CavityShield, Enamel Pro, MI Varnish, Prevident and Vanish. FV were applied (10 ± 2 mg per lesion) to eight specimens per FV group (direct application); the remaining eight specimens received no FV but were later exposed to fluoride released from specimens which received a FV treatment (indirect application). Specimens were paired again and placed into containers (one per FV). Artificial saliva was added and containers placed into an incubator (27 hours at 37°C). Subsequently, FV was carefully removed using chloroform. Specimens were exposed to fresh artificial saliva again (67 hours at 37°C). KHN was measured and differences to baseline values calculated. Enamel fluoride uptake (EFU) was determined using the acid etch technique. Data were analyzed using two-way ANOVA. **Results:** The two-way ANOVA highlighted significant interactions between FV vs. application mode, for both ΔKHN and EFU ($P < 0.001$). All FV were able to reharden and deliver fluoride to caries lesions, but to different degrees. Furthermore, considerable differences were found for both variables between FV when applied either directly or in close vicinity to the lesion: MI Varnish and Enamel Pro exhibited greater fluoride efficacy when applied in vicinity rather than directly to the lesion, whereas CavityShield and Vanish did not differ. Prevident exhibited a higher EFU when applied directly, but little difference in rehardening. (*Am J Dent* 2013;26:000-000).

Clinical significance: The present laboratory study showed that commercially available fluoride varnishes vary considerably in their ability and mechanism to deliver fluoride to enamel for the prevention of dental caries.

✉: Frank Lippert, Department of Preventive and Community Dentistry, Oral Health Research Institute, Indiana University School of Dentistry, 415 Lansing Street, Indianapolis, IN 46202, USA. E-✉: flippert@iupui.edu

Introduction

In 1964, Schmidt[1] developed the first fluoride varnish (FV) to prolong the contact time of sodium fluoride with the tooth surface as fluoride is cleared rather rapidly from the oral cavity, thereby shortening its ability to interact with the target tissues. FV are relatively simplistic delivery vehicles for cariostatic amounts of fluoride and typically contain 5% sodium fluoride, beeswax or white wax and ethanol or ethyl acetate as organic solvents, to form a gel-type structure to stabilize sodium ions; shellac and mastic, to provide a flexible, permeable hard surface that prevents the varnish from dissolving rapidly in saliva; a flow enhancer, such as colophonium, as well as sweeteners, such as xylitol or sodium saccharin, and flavor compounds.[2] Essentially, FV are non-aqueous suspensions of sodium fluoride which are painted onto all accessible 'toothbrush-clean' tooth surfaces where they remain for several hours until they are abraded by food, through mastication, intra-oral friction with the tongue and soft tissues, or simply flake off. During their contact hours, FV will not only release fluoride to the tooth surface but also into saliva. Fluoride can then migrate to areas not covered by FV during the application phase (e.g. interproximal) and interact with these surfaces.

This is the author's manuscript of the article published in final edited form as:
Lippert, F., Hara, A. T., Martinez-Mier, E. A., & Zero, D. T. (2013). In vitro caries lesion rehardening and enamel fluoride uptake from fluoride varnishes as a function of application mode. American journal of dentistry, 26(2), 81-85.

A previous study[3] highlighted no differences in the in vitro remineralization efficacy when FV was applied over vs. around the lesion. However, this study was limited to only one commercially available FV. In recent years, at least 32 different FV (based on a personal review of fluoride varnishes available through distributors of dental professional products in the USA) are commercially available in the United States, compared to only three in 2000.[4] Some contain added ingredients, such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), ACP, tri-calcium phosphate or xylitol, which have been shown to enhance anticaries efficacy,[5-8] although not yet in a varnish delivery format. Apart from two FV, Duraphat[a] and Fluor Protector,[b,9] none of the currently commercially available FV have been clinically tested for their ability to prevent caries, and the majority has not been tested under laboratory conditions either, highlighting a clear need for further research to establish suitable testing guidelines for their efficacy.

In contrast to other fluoride delivery formats, comparative studies on different FV are relatively sparse and have typically only focused on fluoride release from FV into a surrounding medium.[10-14] Enamel fluoride uptake[15-18] and remineralization studies,[3,19,20] have been conducted, but not in combination and while focusing only on few FV. Furthermore, mechanistic studies on fluoride delivery as a function of application mode have not been conducted yet.

Therefore, the present study investigated the in vitro anticaries efficacy of five commercially available FV. The null hypothesis tested was that FV did not differ in their ability to reharden and to deliver fluoride to an early caries lesion when applied directly or in close vicinity to the lesion (so-called 'halo effect').

Materials and Methods

Specimen preparation - Enamel specimens were obtained from approximately 150 bovine incisors, with one specimen being prepared per tooth. Tooth crowns were cut into 4 × 4 mm specimens using a low-speed saw (Isomet[c]). The teeth were stored in deionized water saturated with thymol during the sample preparation process. Specimens were ground and polished to create flat, planar parallel dentin and enamel surfaces using a Struers Rotopol 31/Rotoforce 4[d] polishing unit. The dentin side of the specimens was ground flat to a uniform thickness with 500-grit silicon carbide grinding paper. The enamel side of the specimen was serially ground using 1,200, 2,400 and 4,000 grit paper. The specimens were then polished using a 1 µm diamond polishing suspension on a polishing cloth. Resulting specimens had a thickness range of 1.7 – 2.2 mm. The specimens were assessed under a Nikon SMZ 1500[e] stereomicroscope at ×20 magnification for cracks, hypomineralized (white spots) areas or other flaws in the enamel surface that would exclude them from use in the study. Prepared specimens were stored at 4°C (walk-in refrigerator) and 100% relative humidity (closed container containing excess deionized water saturated with thymol, specimens stored enamel facing upwards) until use. Sixteen specimens per FV treatment group were used for this study. Specimens were individually mounted onto a 1-inch square acrylic block using sticky wax to facilitate surface

microhardness measurements. Specimens remained on their blocks for the entire study period.

Lesion formation - Artificial caries lesions were formed in the enamel specimens using a modification of the method described by White.[21] Specimens were immersed into a solution of 0.05 M lactic acid and 0.2% Carbopol C907 which was 50% saturated with hydroxyapatite and adjusted to pH 5.0. Initial demineralization was performed at 37°C for 24 hours and at a ratio of 40 ml solution per specimen. Specimens were then rinsed with deionized water and stored at 4°C and approximately 100% relative humidity until further use.

Lesion baseline characterization - Initial hardness of the demineralized specimens was determined using a Knoop microhardness indenter (Wilson 2100 Hardness Tester[e]) at a load of 50 g for 11 seconds. The Knoop surface microhardness test was chosen ahead of other specimen interrogation techniques for various reasons. It is inherently less prone to variability and has offered greater sensitivity than the current "gold standard" technique, transverse micro-radiography, when studying de- and remineralization of early caries lesions similar to those studied presently.[22]

The average specimen surface microhardness (KHN_{base}) was determined from five indentations, spaced 100 μm apart in the center of each specimen. Only specimens with a mean KHN_{base} between 17 and 32 were accepted to minimize variability and to allow for potential treatment effects to be observed. Specimens were then assigned to groups and subgroups following a stratified randomization procedure, based on their KHN_{base}.

Sound enamel hardness measurements were performed in the present study (only specimens with $336 < KHN_{sound} < 445$ were accepted). As these data were not needed in the calculation of rehardening efficiency, these are merely reported for completeness.

Test products - A total of five fluoride varnishes were tested in the present study (Table 1). FV were chosen with the aim to include FV with different added ingredients and those from the most prominent manufacturers of professional dental products. A fluoride-free placebo varnish was not included as these are not commercially available. Fluoride content of FV was not investigated as no reliable method for the analysis of total fluoride content has been reported yet. A method that was proposed recently[23] was later[24] found to be inadequate for FV containing calcium compounds, such as several of those currently marketed.

Fluoride varnish application - All FV showed signs of phase separation which was expected given their composition. FV were applied according to manufacturers' instructions and using the supplied microbrushes. All FV were supplied in single unit doses and were first homogenized after removal of the foil covering by stirring the FV for at least 10 seconds using said brush. Any material adhering to the inside of the foil covering was removed using the microbrush and added to the bulk varnish. Immediately after homogenization, approximately 10 ± 2 mg of FV were applied to each of eight specimens per FV treatment group (direct application). The remaining eight specimens did not receive a direct FV

application but were exposed to fluoride released from the specimens which did receive a FV treatment and thus received an indirect application. The time required to apply the FV was approximately 5 minutes per treatment group.

Lesion rehardening - Immediately after FV application, all 16 specimens per FV treatment group were placed into an airtight container (square-shaped, 1.1 l volume), FV-treated and untreated specimens side-by-side and in alternating order (four rows of four acrylic blocks with one specimen per block). Then, 200 ml of artificial saliva (1.45 mM $CaCl_2 \times 2H_2O$; 5.40 mM KH_2PO_4; 14.90 mM KCl; 28.40 mM NaCl; 2.20 g/l porcine gastric mucin, adjusted to pH 7.0 with KOH) was carefully poured into the container, avoiding direct contact with the FV-treated specimens, but submerging all specimens eventually. The container was then placed into an incubator set at 37°C for 27 hours. The artificial saliva was not replaced during that period. Subsequently, the artificial saliva was decanted and specimens and container rinsed with deionized water. The FV was now carefully removed using chloroform-moistened cotton swabs. An average of two swabs was required to remove the FV from the FV-treated specimens. Specimens were assessed for successful FV removal by eye (mirror-like appearance). Specimens not treated with FV were not cleaned.

All specimens were placed back into their containers and 200 ml of fresh artificial saliva was added. The container was placed into the incubator[k] again but for 67 hours. The artificial saliva was not replaced during this period. After rehardening, specimens were rinsed with deionized water and stored at 4°C and 100% relative humidity until further use.

Post-treatment lesion characterization - The mean KHN_{post} of each specimen was determined, as described above, from five indentations placed in close proximity to the lesion baseline indentations on the surface of each specimen. The change in KHN vs. lesion baseline was calculated as follows: $\Delta KHN = KHN_{post} - KHN_{base}$.

Enamel fluoride uptake - Each enamel specimen was acid-etched using 0.5 ml of 1 M $HClO_4$ for 15 seconds. Throughout this period, the acid-etch solution was continuously agitated using an up and down motion of the specimens. This was immediately followed by rinsing the specimens thoroughly with deionized water. A sample of each solution was then buffered with TISAB II (0.25 ml sample, 0.5 ml TISAB II and 0.25 ml 1 N NaOH) and the fluoride content determined by comparison to a similarly prepared standard curve (1 ml standard + 1 ml TISAB II). In order to calculate the amount of enamel removed by the 15-second acid-etch procedure, the calcium content of the acid-etch solution was determined by atomic absorption (0.05 ml sample, 1 ml 0.18 M LaCl3 and 3.95 ml deionized water). From these fluoride and calcium data, the fluoride concentration of each specimen was calculated and expressed as ppm. The fluoride content in the specimens before FV treatment was not evaluated as previous studies conducted in the present authors' laboratories highlighted comparatively low values (< 50 ppm).

Statistical analysis - The data were tested for normal distribution (Shapiro-Wilk test). The variables ΔKHN and

EFU were calculated for each specimen and analyzed using a two-way ANOVA with factors for 'fluoride varnish' and 'application mode (direct vs. indirect)' and their interaction. ΔKHN was the primary variable. Where significant differences were indicated, the individual means were analyzed by the Fisher's Least Significant Difference test. The significance level for the analyses was set at 5%. Correlation coefficient (Pearson) was calculated to evaluate the association between variables.

Results

KHN_{base} were virtually identical between and within treatment groups (mean range 24-25). The two-way ANOVA highlighted significant interactions between fluoride varnish vs. application mode, for both ΔKHN and EFU (both $P< 0.001$). The results and statistical analyses for both study variables can be found in Table 2. When comparing for ΔKHN and the direct application of FV onto the lesion, the FV's efficacy decreased in the following order: Prevident, Vanish, CavityShield, Enamel Pro, MI Varnish. Almost opposite results were obtained for the indirect application of FV: MI Varnish, Enamel Pro, Prevident, CavityShield, Vanish. While the EFU matched the ΔKHN data well, there were several groups with opposing trends (Prevident, CavityShield). Overall, there was a moderate yet statistically significant correlation between ΔKHN and EFU ($R = 0.34$; $P= 0.003$).

Discussion

The present study has showed that FV differ in their ability to reharden and to deliver fluoride to an early caries lesion when applied directly or in close vicinity to the lesion (Table 2). Therefore, the null hypothesis was rejected.

FV are approved by the Food and Drug Administration (FDA) in the United States as medical devices for the use as cavity liners or for the treatment of dentin hypersensitivity. Since their introduction in 1994, FV have, however, also been used 'off-label' by dental professionals as a topical fluoride agent for the prevention of caries. While the American Dental Association (ADA) Council on Scientific Affairs has concluded that "fluoride varnish applied every 6 months is effective in preventing caries in the primary and permanent dentition of children and adolescents",[25] none of the FV sold in the United States are marketed as such. As caries prevention constitutes a drug claim, FV manufacturers would have to submit substantial scientific evidence (two clinical caries trials are required) for review by the FDA. This has created a grey area for manufacturers as unlike for over-the-counter oral care products, such as fluoride dentifrices and mouthwashes, no efficacy or safety testing is currently required for FV. This highlights a clear need to establish the anticaries efficacy of currently marketed FV. While a comparative clinical evaluation of several FV would appear most appropriate, the cost implications of such an undertaking are prohibitive. Therefore, laboratory studies are used first to determine the FV's predicted anticaries efficacy and mode of action.

The present study showed that the tested, commercially available FV vary considerably in their ability and mechanism to deliver their active ingredient, sodium

fluoride, to early caries lesions. Furthermore, the tested FV exhibited significant differences in their ability to reharden these lesions. Especially the differences in fluoride delivery between FV – approximately two- and four-fold differences were observed depending on the application mode (Table 2) – give rise to concern. While there is still some debate in the literature as to the exact mode of action of FV, it seems logical to assume that the delivery of fluoride to the dental hard tissues rather than the release of fluoride into saliva is the primary mode of action of FV. FV application is rather infrequent (every 3-6 months); and while salivary fluoride concentrations are elevated after FV application, they do reach baseline levels again after approximately 24 hours.[26] Such short, infrequent periods of elevated fluoride concentrations in the oral cavity do not necessarily explain the rates in caries reduction observed in many clinical trials.[9] Thus, it seems all the more likely that fluoridation of incipient subclinical lesions is the primary mode of action. In fact, substantial increases in enamel fluoride content were observed in many clinical studies, not only shortly after a single FV application but also several weeks later.[27-32]

Fluoride can be taken up by enamel in various forms, which can be, albeit crudely, divided into loosely- (e.g. as calcium fluoride) and structurally-bound fluoride (e.g. as fluoridated hydroxyapatite). The former is the primary mode of fluoride uptake by enamel and dentin, acts as a labile reservoir of fluoride ions, and can serve as a source for the latter.[34] Indeed, such observations were made after clinical FV application.[16] Fluoride applied at high concentrations, such as from FV, will primarily form non-stoichiometric calcium fluoride,[21] which is considerably more stable in situ than under laboratory conditions due to the protection by pellicle proteins.[35] Thus, it is likely that in vitro investigations underestimate the anticaries efficacy of FV and especially when FV are compared to other fluoride delivery formats, which may explain the results of some previous studies.[36,37]

The purpose of added ingredients, such as those of the FV tested presently, can be questioned in light of the application frequency and mode of action of FV. Considering the direct application mode, the addition of CPP-ACP, ACP or tri-calcium phosphate failed to enhance EFU and lesion rehardening compared to those FV containing xylitol (Table 2). Inherent formulation differences may have overcome any potential benefits, but unless an added ingredient can directly enhance the delivery of fluoride to the dental hard tissues, its addition is questionable. In contrast, however, are the results of the indirect application mode which highlighted beneficial effects of the FV containing CPP-ACP or ACP. These can potentially be attributed to the added ingredients, although it is more likely that inherent formulation differences between FV, resulting in different fluoride release characteristics and interaction with the enamel surface, may have accounted for the present observations. Thus, the present results for both direct and indirect application modes can be best explained by (a) the ability of the FV to interact with the enamel surface and to deliver fluoride directly to the lesion, and (b) the ability of the FV to release fluoride into the surrounding medium to fluoridate inaccessible areas not covered by FV during the application phase. Ideally, FV should accomplish

both. However, this did not appear to be the case as, for example, MI Varnish was somewhat inferior in its direct interaction with the enamel surface, but was able to effectively release fluoride into saliva. Likewise, it could be argued that Vanish was inferior to all other tested FV due to its poor fluoride delivery in general. Consequently, one of the major findings of the present study was that FV are far more complex fluoride delivery vehicles which deserve considerably more attention from manufacturers and researchers alike.

Manufacturers of FV recommend painting all accessible tooth surfaces with FV, regardless of the presence of early or established caries lesions. The present study showed that, albeit its limitations (see below), three of the tested five FV differed greatly in their ability to deliver fluoride between direct and indirect application modes; however with no clear indication which mode would be favorable (Table 2). Previous clinical[38] and in situ[17] studies highlighted that fluoride migration is rather limited in the oral cavity and dependent on the particular FV being applied. However, both studies investigated Duraphat which has been shown in several studies[11,13,39] to be inferior in its fluoride release into the surrounding medium compared to many other FV, including those investigated presently. The results of one recent study[40] somewhat mirrored those of the indirect application mode (Table 2). It can therefore be speculated that FV exhibiting superior fluoride ion release may be able to effectively fluoridate more remote sites, although this would have to be confirmed in situ/in vivo.

Fluoride can contribute to lesion arrest through hypermineralization of the surface layer which would be a warranted side effect of a FV application. The indirect application mode is perhaps also a suitable way to determine FV efficacy for inaccessible areas, such as interproximal spaces. Unless the FV can migrate on its own into these spaces, fluoride released from FV will have to migrate there and effectively interact with these vulnerable surfaces.

Naturally, laboratory models are only surrogates for clinical caries by not completely mimicking every facet of the de- and remineralization processes. The present study is no exception as, (a) saliva was not continuously replenished which would occur clinically; (b) the FV/saliva ratio was lower than it would have been clinically; and (c) bovine enamel was used which was recently shown to exaggerate fluoride effects to some degree,[40] to name only a few of the shortcomings which can, however, be applied to many other FV laboratory studies. Likewise, surface microhardness measurements do not allow for a complete understanding of mineral changes within caries lesions. Any rehardening observed in the present study may also be indicative of hypermineralization, although this could be considered a clinically meaningful outcome as it is representative of lesion arrest. Nonetheless, the results of the present study have to be seen with caution until they are confirmed clinically. Regardless, the observed differences in fluoride delivery and predicted efficacy highlight that FV deserve greater attention not only from the research community but also from legislative bodies.

In summary, the present laboratory study showed that commercially available fluoride varnishes vary greatly in

their predicted anticaries efficacy as evidenced by the differences in enamel fluoride uptake and the FV's ability to reharden early caries lesions.

a. Duraphat, Colgate Oral Pharmaceuticals, New York, NY., USAManufacturer, CITY, STATE, COUNTRY.
b. FluorProtector, Ivoclar Vivadent, Schaan, LiechtensteinManufacturer, CITY, STATE, COUNTRY.
c. Buehler, Lake Bluff, IL, USA.
d. Struers Inc., Cleveland, OH, USA.
e. Nikon, Tokyo, Japan.
f. Wilson, Norwood, MA, USA.
g. 3M ESPE Dental Products, St. Paul, MN, USA.
h. Premier Dental Products Co., Plymouth Meeting, PA., USA.
i. GC America Inc., Alsip, IL, USA.
j. Colgate Oral Pharmaceuticals, Inc., New York, NY., USA.
k. Precision Scientific Group, Chicago, IL, USA.

Disclosure statement: The authors declared no conflict of interest. The present study was solely funded by the Oral Health Research Institute Remineralization Research Program.

Dr. Lippert is Assistant Research Professor, Dr. Hara is Assistant Professor, Dr. Martinez-Mier is Associate Professor, and Dr. Zero is Professor and Department Chair, Director, Oral Health Research Institute, Department of Preventive and Community Dentistry, Oral Health Research Institute, Indiana University School of Dentistry, Indianapolis, Indiana, USA.

References

1. Schmidt HFM. Ein neues Tauchierungsmittel mit besonders lang anhaltendem intensiven Fluoridierungseffekt. *Stoma* 1964;17:14-20 (In German). **Translate into English A new varnish with extremely long lasting fluoridation effect.**
2. Lam A, Chu CH. Caries management with fluoride varnish of children in U.S. *NY State Dent J* 2011;77:38-42.
3. Castellano JB, Donly KJ. Potential remineralization of demineralized enamel after application of fluoride varnish. *Am J Dent* 2004;17:462-464.
4. Beltran-Aguilar ED, Goldstein JW, Lockwood SA. Fluoride varnishes - A review of their clinical use, cariostatic mechanism, efficacy and safety. *J Am Dent Assoc* 2000;131:589-596.
5. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. *J Dent Res* 2012;89:1187-1197.
6. Papas A, Russell D, Singh M, Kent R, Triol C, Winston A. Caries clinical trial of a remineralising toothpaste in radiation patients. *Gerodontology* 2008;25:76-88.
7. Mensinkai PK, Ccahuana-Vasquez RA, Chedjieu I, Amaechi BT, Mackey AC, Walker TJ, Blanken DD, Karlinsey RL. In situ remineralization of white-spot enamel lesions by 500 and 1,100 ppm F dentifrices. *Clin Oral Invest* 2012;16:1007-1014.
8. Zhan L, Cheng J, Chang P, Ngo M, DenBesten PK, Hoover CI, Featherstone JDB. Effects of xylitol wipes on cariogenic bacteria and caries in young children. *J Dent Res* 2012;91:S85-S90.
9. Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride varnishes for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev* 2002(3):CD002279.
10. Castillo JL, Milgrom P, Kharasch E, Izutsu K, Fey M. Evaluation of fluoride release from commercially available fluoride varnishes. *J Am Dent Assoc* 2001;132:1389-1392.
11. Shen C, Autio-Gold J. Assessing fluoride concentration uniformity and fluoride release from three varnishes. *Am J Dent* 2002;133:176-182.
12. Castillo JL, Milgrom P. Fluoride release from varnishes in two in vitro protocols. *J Am Dent Assoc* 2004;135:1696-1699.
13. Jablonowski BL, Bartoloni JA, Hensley DM, Vandewalle KS. Fluoride release from newly marketed fluoride varnishes. *Quintessence Int* 2012;43:221-228.
14. Ritwik P, Aubel JD, Xu X, Fan Y, Hagan J. Evaluation of short term fluoride release from fluoride varnishes. *J Clin Pediatr Dent* 2012;36:275-278.
15. Eronat C, Eronat N, Alpoz AR. Fluoride uptake by enamel in vitro following application of various topical fluoride preparations. *J Clin Pediatr Dent* 1993;17:227-230.

16. Attin T, Grieme R, Paque F, Hannig C, Buchalla W, Attin R. Enamel fluoride uptake of a novel water-based fluoride varnish. *Arch Oral Biol* 2005;50:317-322.
17. Attin T, Lennon AM, Yakin M, Becker K, Buchalla W, Attin R, Wiegand A. Deposition of fluoride on enamel surfaces released from varnishes is limited to vicinity of fluoridation site. *Clin Oral Invest* 2007;11:83-88.
18. Schemehorn BR, Wood GD, McHale W, Winston AE. Comparison of fluoride uptake into tooth enamel from two fluoride varnishes containing different calcium phosphate sources. *J Clin Dent* 2011;22:51-54.
19. Hazelrigg CO, Dean JA, Fontana M. Fluoride varnish concentration gradient and its effect on enamel demineralization. *Pediatr Dent* 2003;25:119-126.
20. Lin R, Hildebrand T, Donly KJ. In vitro remineralization associated with a bioerodible fluoridated resin and a fluoride varnish. *Am J Dent* 2009;22:203-205.
21. White DJ. Use of synthetic polymer gels for artificial carious lesion preparation. *Caries Res* 1987;21:228–242.
22. Churchley D, Lynch RJM, Lippert F, Eder JSO, Alton J, Gonzalez-Cabezas C. Terahertz pulsed imaging study to assess remineralization of artificial caries lesions. *J Biomed Opt* 2011;16:026001.
23. McCracken JM, Schmuck BD, Carey CM. Assessing fluoride concentration and leachability in dental varnishes. *J Dent Res* 2009 (Abstr 1096).
24. Flanigan P, Vang F, Klaiber PR, Fitch JA, Miyazaki CL. Evaluation of proposed total fluoride method for varnishes. *J Dent Res* 2012 (Abstr 2956).
25. American Dental Association Council on Scientific Affairs. Professionally applied topical fluoride. *J Am Dent Assoc* 2006; 137:1151-1159.
26. Eakle WS, Featherstone JDB, Weintraub JA, Shain SG, Gansky SA. Salivary fluoride levels following application of fluoride varnish or fluoride rinse. *Community Dent Oral Epidemiol* 2004;32:462-469.
27. Bang S, Kim YJ. Electron micro probe analysis of human tooth enamel coated in-vivo with fluoride varnish. *Helv Odont Acta* 1973;17:84-88.
28. Bruun C, Givskov H, Stoltze K. In vivo uptake and retention of fluoride in human surface enamel after application of a fluoride-containing lacquer (fluor protector). *Caries Res* 1980;14:103-109.
29. Ogaard B, Rolla G, Helgeland K. Fluoride retention in sound and demineralized enamel in vivo after treatment with a fluoride varnish (Duraphat). *Scand J Dent Res* 1984;92:190-197.
30. Petersson LG. In vivo fluorine uptake in human enamel following treatment with a varnish containing sodium fluoride. *Odontol Revy* 1975;26:253-266.
31. Petersson LG. Fluorine gradients in outermost surface enamel after various forms of topical application of fluorides in vivo. *Odontol Revy* 1976;27:25-50.
32. Stamm JW. Fluoride uptake from topical sodium fluoride varnish measured by an in vivo enamel biopsy. *J Can Dent Assoc* 1974;40:501-505.
33. White DJ, Nelson DG, Faller RV. Mode of action of fluoride: Application of new techniques and test methods to the examination of the mechanism of action of topical fluoride. *Adv Dent Res* 1994;8:166-174.
34. Ogaard B, Seppa L, Rolla G. Professional topical fluoride applications. Clinical efficacy and mechanism of action. *Adv Dent Res* 1994;8:190-201.
35. Ganss C, Schlueter N, Klimek J. Retention of KOH-soluble fluoride on enamel and dentine under erosive conditions - A comparison of in vitro and in situ results. *Arch Oral Biol* 2007;52:9-14.
36. Maia LC, de Souza IPR, Cury JA. Effect of a combination of fluoride dentifrice and varnish on enamel surface rehardening and fluoride uptake in vitro. *Eur J Oral Sci* 2003;111:68-72.
37. Zhou SL, Zhou J, Watanabe S, Watanabe K, Wen LY, Xuan K. In vitro study of the effects of fluoride-releasing dental materials on remineralization in an enamel erosion model. *J Dent* 2012;40:255-263.
38. Dijkman AG, de Boer P, Arends J. In vivo investigation on the fluoride content in and on human enamel after topical applications. *Caries Res* 1983;17:392-402.
39. Cochrane NJ, Shen P, Yuan Y, Reynolds EC. Ion release from calcium-containing fluoride dental varnishes. *J Dent Res* 2012 (Abstr 1166).
40. Lippert F, Hara AT. Fluoride dose-response of human and bovine enamel caries lesions under remineralizing conditions. *Am J Dent* 2012;25:205-209.

Table 1. Tested fluoride varnishes.

Name	Noteworthy ingredients*
CavityShield	Xylitol
Enamel Pro	ACP
MI Varnish	CPP-ACP
Prevident	Xylitol
Vanish	Tri-calcium phosphate, Xylitol

* All FV claimed to contain 5% sodium fluoride.

Table 2. Least square means ± standard error of the least square means and results of the statistical analyses for both study variables.

	ΔKHN		EFU	
Varnish	Indirect	Direct	Indirect	Direct
MI Varnish	146±9 a*	66±9 b*	6707±618 b*	3291±618 b*
Enamel Pro	132±9 ab*	89±9 ab*	8858±660 a*	4590±660 ab*
Prevident	124±9 abc	110±9 a	3320±618 c*	5116±618 a*
CavityShield	110±9 bc	98±9 a	1801±618 c	3450±660 ab
Vanish	103±9 c	103±9 a	2157±660 c	2838±660 b

Different letters indicate statistically significant differences between groups, in columns ($P < 0.05$).
* Statistically significant differences ($P < 0.05$) between application modes, for both ΔKHN and EFU.