Final Report:
Short-Term Enamel Fluoride Uptake Potential of Fluoride Varnishes Utilizing an *in-Vitro* Flow Model and Aqueous Fluoride Release Rate
Study Number 19-81

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Dental Product Testing Number 19-81

Conducting Agency

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Purpose

The purpose of this *in vitro* study was to determine the effect of fluoride containing varnishes on promoting fluoride uptake into artificially lesioned enamel specimens following a 1-hr. and 4-hr. treatment. This "flow" model has shown to be a predictive *in vitro* model for determining enamel fluoride uptake potential of fluoride containing varnishes observed clinically (based on independent clinical *in situ* trials conducted at Therametric Technologies, Inc.).[1,2] The fluoride assay procedure is similar to the one identified as Procedure #40 in the FDA Monograph. In addition, the aqueous fluoride release rate of the fluoride varnishes was determined at 1-hr., 4-hr, and 24 hr.

Procedure

Flow Model Enamel Fluoride Uptake

Sound, bovine teeth were selected and cleaned of all adhering soft tissue. Several cores of enamel 3mm in diameter were prepared from each tooth by cutting perpendicularly to the labial surface with a hollow-core diamond drill bit. This was performed under water to prevent overheating of the specimens. Each specimen was embedded in the end of a plexiglass rod (1/4" diameter x 2" long) using methylmethacrylate. The excess acrylic was cut away exposing the enamel surface. The enamel specimens were polished with 600 grit wet/dry paper and then with micro-fine Gamma Alumina. The resulting specimens were 3mm disks of enamel with all but the exposed surface covered with acrylic. Artificial lesions were formed in the enamel specimens by a 24hr. exposure at 37°C to a 0.1M lactic acid and 0.2% Carbopol 907 solution 50% saturated with HAP at a pH of 5.0. Following demineralization, the specimens were removed from the lesion forming solution and rinsed well with DI water. The specimens were kept hydrated and stored at 4 °C.

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Following demineralization, the lesion surface microhardness (SMH) of each specimen was determined using a LECO LM 247AT microhardness tester equipped with a Vickers diamond indenter. Four (4) indentions were made on the surface of each specimen (Vickers Hardness Number (VHN), 200 gF, 15s dwell time) and the mean VHN of each specimen was determined. The specimens were balanced into treatment groups (N=8) based on their VHN values.

Eight (8) specimens per group were utilized for this study. The N=8 specimens were numbered and placed into a neoprene stopper with the enamel surface of the specimens being flush with the stopper. The stoppers have been specifically designed to evenly distribute the 8 enamel specimens around the outer edge of the stopper. A single layer of test varnish (0.0050g ± 0.001 g) was applied to the surface of each individual specimen on a tared analytical balance. Following application of the test varnish, the stopper was place in a specimen cup, enamel surfaces facing up, and the "flow" solution was initiated within 90-seconds. Tubing from the solution container (Artificial Saliva, see Appendix) passed through a multi-channel peristaltic pump and was affixed to a hole in the lid of the specimen cup. The multi-channel pump was set to provide a slow drip of solution (approximately 1.0 ml/min) centrally over the stopper (drip of solution did not fall directly onto any of the 8 specimens). The solution collecting on the surface (evenly covering all 8 specimens) eventually broke the tension holding it on the stopper and ran off into the bottom of the specimen cup. The specimen cup was equipped with a drain to ensure the solution level never reached the surface of the stopper. Therefore, the solution in contact with the varnish treated enamel specimens was slowly replaced by fresh solution, mimicking intra-oral salivary flow.

Following a 1-hr or 4-hr. treatment time, the specimens were removed from the stopper and excess varnish was carefully removed (physical removal using a spatula and subsequent removal using a cotton swab saturated with reagent grade ethyl alcohol). The specimens were then rinsed well under running DI water for 30 seconds.

One layer of enamel was removed from each specimen by immersion in 0.5 ml of 1.0 N $HClO_4$ for 15 seconds. A sample of each solution was buffered with TISABII to a pH of 5.2 (0.25 ml sample, 0.5 ml TISABII and 0.25 ml 1N NaOH) and the fluoride concentration determined using a fluoride ion specific electrode (ISE, Orion™) by comparison to a similarly prepared standard curve. A second sample was analyzed for calcium concentration via atomic absorption spectroscopy (Perkin Elmer AAnalyst 200) for use in depth of etch determination (0.05 ml sample diluted to 5.0 ml). The resultant calculation provided fluoride concentration in ppm F for each enamel specimen.

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Aqueous Fluoride Release Rate

The ends of ¼ inch Plexiglas rods were coated with the designated varnishes. The varnishes (0.0075 ± 0.001 g) were applied to the ends of the rods in a uniform manner and allowed to cure for twenty minutes. This resulted in relatively uniformly-sized samples of the varnishes (area = ~31.7 mm^2). Twelve (12) replicate samples for each test varnish were prepared. The specimens were individually placed into 2.0 ml aliquots of deionized water with constant stirring of 130 rpm at 23°C. After 1-hour the specimens were removed and placed into a fresh 2.0 ml aliquot of deionized water. After a subsequent 3-hours (4 hr. total) the specimens were removed and placed into another fresh 2.0 ml aliquot of deionized water. After a subsequent 20 hours (24-hr. total) the specimens were removed and the treatment period was complete. All individual aliquots were analyzed for fluoride concentration by adding 2.0 ml of TISABII and measuring the mV potential using a fluoride ion specific electrode (ISE, Orion™). The mV readings were compared to a similarly prepared fluoride standard curve and the fluoride concentration of each individual aliquot was determined. The µg F / g of applied varnish was then calculated for each aliquot.

Test Products

The treatment groups utilized in this study are listed in the table below. The test varnishes were blinded and color coded prior to initiation.

Group #	Sample ID	Treatment Time
A	**3M Vanish White Varnish** (5% NaF; Lot # N999480, Exp: 2020-08-28)	1 Hour
B	**Colgate PreviDent Varnish** (5% NaF; Lot # 81140BFT16; Exp: 2020-03-31)	1 Hour
C	**Elevate Oral Care FluoriMax Varnish** (2.5% NaF; Lot # 0319-03)	1 Hour
D	**3M Vanish White Varnish** (5% NaF; Lot # N999480, Exp: 2020-08-28)	4 Hour
E	**Colgate PreviDent Varnish** (5% NaF; Lot # 81140BFT16; Exp: 2020-03-31)	4 Hour
F	**Elevate Oral Care FluoriMax Varnish** (2.5% NaF; Lot # 0319-03)	4 Hour

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Statistical Analyses

All individual specimen EFU values (ppm F) and were reported and the Mean (N=8), std. deviation (SD) and standard error (SEM) for each group were calculated. In addition, all individual µg F / g of applied varnish were reported and the Mean (N=12), SD and SEM of each time period for each group were calculated. Statistical analyses were performed with a one-way analysis of variance (ANOVA) model using Sigma Plot Software (13.0). Since the ANOVA indicated significant differences, the individual means were analyzed by Student-Newman-Keuls (SNK) pairwise analysis. Analyses were performed between treatment groups within each treatment time period. Significance of all analyses was determined at $P \leq 0.05$.

Results

The results are summarized in Tables I-III below.

Flow Model Enamel Fluoride Uptake

1-hr. treatment timepoint (Table I): The FluoriMax varnish treated specimens, Group C, exhibited a mean (N=8) ± SEM enamel fluoride concentration of 4902 ± 86 ppm F following the 1-hr. *in vitro* treatment period. The FluoriMax varnish was significantly (P = <0.001) more effective at promoting fluoride uptake into incipient lesioned enamel specimens following the 1-hr. *in vitro* treatment period compared to both the 3M Vanish White varnish, Group A (1639 ± 134 ppm F) and the Colgate PreviDent varnish, Group B (1120 ± 148 ppm F). The 3M Vanish White varnish was significantly (P = 0.009) more effective at promoting fluoride uptake into incipient lesioned enamel compared to the Colgate PreviDent varnish following the 1-hr. *in vitro* treatment period.

4-hr. treatment timepoint (Table II): The FluoriMax varnish treated specimens, Group F, exhibited a mean (N=8) ± SEM enamel fluoride concentration of 4954 ± 85 ppm F following the 4-hr. *in vitro* treatment period. The FluoriMax varnish was significantly (P = <0.001) more effective at promoting fluoride uptake into incipient lesioned enamel specimens following the 4-hr. *in vitro* treatment period compared to both the Colgate PreviDent varnish, Group E (2555 ± 372 ppm F) and the 3M Vanish White varnish, Group D (2547 ± 315 ppm F). The Colgate PreviDent varnish and 3M Vanish White varnish did not exhibit statistically significant (P = 0.984) differences in incipient lesioned enamel fluoride uptake potential following the 4-hr. treatment period.

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Aqueous Fluoride Release Rate

At the 1-hr. timepoint assessment all three test varnishes exhibited statistically significant (P = <0.001) differences in aqueous fluoride released. The FluoriMax varnish exhibited an aqueous fluoride release of 6,233 ± 365 µg F / g of applied varnish (Mean (N=12) ± SEM), the Colgate PreviDent varnish (4,013 ± 192 µg F / g of applied varnish) and the 3M Vanish White varnish (119 ± 4 µg F / g of applied varnish).

Following 4-hr. of total time (1-hr. + additional 3-hr. time period) all three test varnishes again exhibited statistically significant (P = < 0.001) differences in aqueous fluoride released. The FluoriMax varnish exhibited an aqueous fluoride release of 13,843 ± 254 µg F / g of applied varnish, the Colgate PreviDent varnish (12,259 ± 373 µg F / g of applied varnish) and the 3M Vanish White varnish (296 ± 19 µg F / g of applied varnish).

Following 24-hr. of total time (1-hr. + additional 3-hr. and 20-hr. time periods) the three test varnishes once again all exhibited statistically significant (P = <0.001) differences in aqueous fluoride released. The Colgate PreviDent varnish exhibited an aqueous fluoride release of 22,928 ± 450 µg F / g of applied varnish, the FluoriMax varnish (14,345 ± 255 µg F / g of applied varnish) and the 3M Vanish White varnish (891 ± 89 µg F / g of applied varnish).

Conclusions

Following the first hour of treatment, the FluoriMax varnish exhibited 98.95% of the enamel fluoride concentration observed following the 4-hr. treatment period, while the 3M Vanish White varnish and Colgate PreviDent varnish exhibited 64.35% and 43.84% respectively. The FluoriMax varnish was significantly (P = <0.001) more effective at promoting fluoride uptake into incipient lesioned enamel following 1-hr. of treatment compared to both the Colgate PreviDent and 3M Vanish White following 1-hr. and 4-hr. of treatment.

During the first 4-hr. of aqueous fluoride release testing, the FluoriMax varnished released approx. 100% of the total theoretical fluoride present in the formula (2.5% NaF = ~11,300 ppm F), while the Colgate PreviDent varnish and 3M Vanish White varnish released approx. 54% and 1.3% of total theoretical fluoride (5% NaF = ~22,600 ppm F) respectively. Following 24-hr. both the FluoriMax and Colgate PreviDent released approx. 100% of total theoretical fluoride, while the 3M Vanish varnish only released approx. 3.9% of total theoretical fluoride.

The 3M Vanish White varnish exhibited only 3.0% and 2.4% aqueous fluoride release of what was observed for the Colgate PreviDent varnish following 1-hr. and 4-hr. respectively. However, the 3M Vanish White varnish exhibited

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significantly greater enamel fluoride uptake following 1-hr. of treatment compared to the Colgate PreviDent varnish and did not differ significantly following 4-hr. of treatment. These findings are consistent with previously conducted *in vitro* flow model and clinical *in situ* enamel fluoride uptake studies.[1,2] From these findings, the aqueous fluoride release rate of a varnish is not solely predictive of the ability of that varnish to promote fluoride uptake into incipient lesioned enamel and other properties of the varnishes should be considered.

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12-Apr-2019

This study has been conducted and reviewed according to the FDA Monograph on Anticaries Drug Products for Over the Counter Human Use and the FDA Good Laboratory Practices to the best of our knowledge.

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12-Apr-2019
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Table I:

Fluoride Uptake into Incipient Lesioned Bovine Enamel Provided by Fluoride Varnishes

1-Hr. Treatment Period

Test Group	Test Varnish	Enamel Fluoride Concentration (ppm F)
B	Colgate Prevident (5% NaF)	1120 ± 148 * **
A	3M Vanish White (5% NaF)	1639 ± 134[1]
C	Elevate FluoriMax (2.5% NaF)	4902 ± 86

* Mean ± SEM (N=8)

** All values differ significantly (P < 0.05) as determined by SNK pairwise analysis

1- Mean ± SEM (N=7); outlier value discarded from data set based on Dixon Q-test (95% CI)

Comparisons for Factor

Comparison	Diff of Means	p	q	P	P<0.050
Group C vs. Group B	3782.000	3	30.912	<0.001	Yes
Group C vs. Group A	3263.000	2	25.766	<0.001	Yes
Group A vs. Group B	519.000	2	4.098	0.009	Yes

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Table II:
Fluoride Uptake into Incipient Lesioned Bovine Enamel Provided by Fluoride Varnishes

4-Hr. Treatment Period

Test Group	Test Varnish	Enamel Fluoride Concentration (ppm F)	
D	3M Vanish White (5% NaF)	2547 ± 315 *	**
E	Colgate Prevident (5% NaF)	2555 ± 372	
F	Elevate FluoriMax (2.5% NaF)	4954 ± 85	

* Mean ± SEM (N=8)

** Values connected by line do not differ significantly (P = 0.984) as determined by SNK pairwise analysis

Comparisons for Factor

Comparison	Diff of Means	p	q	P	P<0.050
Group F vs. Group D	2407.000	3	8.426	<0.001	Yes
Group F vs. Group E	2399.000	2	8.398	<0.001	Yes
Group E vs. Group D	519.000	2	0.0280	0.984	No

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Table III:

Aqueous Fluoride Release Rate of Fluoride Containing Varnishes

Test Group	µg F / g of Applied Varnish		
	1-Hr.	4-Hr. (Total)	24-Hr. (Total)
3M Vanish White (5% NaF)	119 ± 4 *	296 ± 19	891 ± 89
Colgate PreviDent (5% NaF)	4013 ± 192	12,259 ± 373	22,928 ± 450
Elevate FluoriMax (2.5% NaF)	6,223 ± 365	13,843 ± 254	14,345 ± 255

* Mean ± SEM (N=12)

** Values within each column all differ significantly (P = <0.001) as determined by SNK pairwise analysis

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Appendix

Artificial Synthetic Saliva

1)	Gastric mucin	2.200 g/liter
2)	NaCl	0.381 g/liter
3)	$CaCl_2$-$2H_2O$	0.213 g/liter
4)	KH_2PO_4	0.738 g/liter
5)	KCl	1.114 g/liter

*Adjust to a pH of 7.0 after all ingredients are dissolved completely

References

1. A Validated In-Vitro Model for Assessing EFU From Fluoride Varnishes. T.J. Keefer, H.C. McClure, G.K. Stookey, B.R. Schemehorn, G.D. Wood, J Dent Res J Dent Res Vol #97>(A): 0909, (www.iadr.org).

2. An In-Situ Enamel Fluoride Uptake Model for Fluoride Varnishes. G.K. Stookey, B.R. Schemehorn, A.J. Nunez, W.B. Carr, J.A. McClure, J Dent Res J Dent Res Vol #97>(A): 1542, (www.iadr.org).