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http://dx.doi.org/10.1590/1678-775720130574

Evaluation of fluoride release from experimental TiF_4 and NaF varnishes *in vitro*

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Submitted: October 31, 2013 - Modification: January 10, 2014 - Accepted: January 16, 2014

ABSTRACT

Fluoride varnishes play an important role in the prevention of dental caries, promoting the inhibition of demineralization and the increase of remineralization. Objective: This study aimed to analyze the amount of fluoride released into water and artificial saliva from experimental TiF_4 and NaF varnishes, with different concentrations, for 12 h. Material and Methods: Fluoride varnishes were applied on acrylic blocks and then immersed in 10 ml of deionized water and artificial saliva in polystyrene bottles. The acrylic blocks were divided in seven groups (n=10): 1.55% TiF_4 varnish (0.95% F, pH 1.0); 3.10% TiF_4 varnish (1.90% F, pH 1.0); 3.10% and 4% TiF_4 varnish (2.45% F, pH 1.0); 2.10% NaF varnish (0.95% F, pH 5.0); 4.20% NaF varnish (1.90% F, pH 5.0); 5.42% NaF varnish (2.45% F, pH 5.0) and control (no treatment, n=5). The fluoride release was analyzed after ½, 1, 3, 6, 9 and 12 h of exposure. The analysis was performed using an ion-specific electrode coupled to a potentiometer. Two-way ANOVA and Bonferroni's test were applied for the statistical analysis ($p<0.05$). Results: TiF_4 varnishes released larger amounts of fluoride than NaF varnishes during the first ½ h, regardless of their concentration; 4% TiF_4 varnish released more fluoride than NaF varnishes for the first 6 h. The peak of fluoride release occurred at 3 h. There was a better dose-response relationship among the varnishes exposed to water than to artificial saliva. Conclusions: The 3.10% and 4% TiF_4 -based varnishes have greater ability to release fluoride into water and artificial saliva compared to NaF varnish; however, more studies must be conducted to elucidate the mechanism of action of TiF_4 varnish on tooth surface.

Keywords: Fluoride varnishes. Sodium fluoride. Titanium tetrafluoride.

INTRODUCTION

Fluoride products play an important role in the control of dental caries lesions, by reducing demineralization and promoting remineralization. Professional application of topical fluoride leads to a considerably high fluoride acquisition by enamel and dentin, which might favor its effect against dental caries[2,3,27].

Fluoride varnishes were developed more than 60 years ago to prolong the contact time between fluoride and tooth surface, increasing fluoride uptake by enamel and the effect on caries control[17,26]. Clinical evidence has shown that biannual application of fluoride varnishes is effective in the control of caries progression, with a preventive fraction of 30% compared with placebo or non-treatment[5,16,19,25]. The application of high concentrated NaF, as varnish, is able to promote a CaF_2-like layer precipitation on enamel, which can serve as a mineral reservoir, releasing fluoride during the cariogenic challenges[22,24].

More recently, another type of fluoride containing a polyvalent metal, titanium tetrafluoride (TiF_4), was incorporated into an experimental varnish to improve its effect on enamel de-remineralization[13]. The effect of TiF_4 is related to both fluoride and titanium contents. It is hypothesized that titanium might complex with the phosphate groups producing an acid-resistant surface coating[11,20].

The experimental TiF_4 varnish has shown to have a superior effect on enamel remineralization *in vitro*[13] and on the reduction of enamel demineralization *in situ* compared to NaF varnish[8].

Despite studies having shown promising results with the use of TiF_4 varnish, there are still no sufficient and insightful data about its mechanism of action from the chemical point of view. Therefore, it would be interesting to test if fluoride release may be different between NaF and TiF_4 varnishes using a simple model, without interference of their reaction with tooth, to check if the higher efficacy of TiF_4 may be related to the titanium content only or it could be explained by differences in the fluoride release pattern.

Therefore, the aim of the present study was to analyze the amount of fluoride released from experimental TiF_4 and NaF varnishes in deionized water and artificial saliva, for a period of 12 h. The null hypothesis was that there is no difference in the amount of fluoride released between the TiF_4 and NaF varnishes.

MATERIAL AND METHODS

Sample preparation and treatments

One hundred and thirty acrylic blocks (10x10x2 mm, Emporium Acrílicos, Bauru, SP, Brazil) were manufactured for this protocol. Sixty-five blocks were used for the fluoride release analysis in deionized water and 65 for the fluoride release analysis in artificial saliva. The acrylic blocks were then distributed into 6 treatment groups (n=10) and one control (n=5):

1.55% TiF_4 varnish (0.95% F, pH 1.0);
3.10% TiF_4 varnish (1.90% F, pH 1.0);
4% TiF_4 varnish (2.45% F, pH 1.0);
2.10% NaF varnish (0.95% F, pH 5.0);
4.20% NaF varnish (1.90% F, pH 5.0);
5.42% NaF varnish (2.45% F, pH 5.0);
Control (no treatment).

The varnishes were manufactured by the Brazilian Company FGM (FGM Produtos Odontológicos, Joinville, SC, Brazil). Their pH was measured using pH indicator strips (Whatman® International Ltd., Maidstone, England).

Each acrylic block was fixed with a nylon string to the underside of the plastic lid of a 15 ml polystyrene bottle (Injeplast®, São Paulo, SP, Brazil). The amount of 30 mg of each varnish was painted on a side of the acrylic block using a microbrush (KG Sorensen®, Cotia, SP, Brazil). The weight was checked using an analytical balance (Mettler Toledo®, Greifensee, Switzerland).

The blocks were then immersed into 10 ml of deionized water (pH 7.5) or artificial saliva (0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2.2H_2O$, 3 mM NH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4 and 11 µM ascorbic acid; pH 6.8)[12], inside a polystyrene bottle. All reagents were from Merck® (Darmstadt, Germany), except NaCl (Synth®, LabSynth, Diadema, SP, Brazil), NaSCN (Sigma-Aldrich®, St. Louis, MO, USA) and ascorbic acid (Quimibrás, Rio de Janeiro, RJ, Brazil).

During the course of the experiment, the bottles lids containing the fixed acrylic blocks were transferred to new bottles with equal volume of deionized water or artificial saliva, at room temperature, after ½, 1, 3, 6, 9 and 12 h of the exposure. This procedure was done to check the F release pattern along the time, trying to simulate the saliva clearance *in vivo*. After the experiment, all bottles were kept at 4°C for analysis of the fluoride content.

Fluoride release analysis

The fluoride release analysis was performed using a fluoride ion-sensitive electrode (Thermo ORION 9609, Beverly, MA, USA) coupled to a potentiometer (Thermo ORION 720A+, Beverly, MA, USA). The electrode was calibrated with standard solutions of sequential fluoride concentrations ranging from 0.10 to 25.6 ppm F^-. All standard and experimental bottles were buffered with an equal volume of standard Total Ionic Strength Adjustment Buffer solution (TISAB II) and the readings were performed in volume of 1 ml of each sample bottle, in duplicate.

Each sample bottle was evaluated and the fluoride concentration for each sample was determined by the average of the two readings (mV transformed to µg/ml, standard curve r^2=0.99). The duplicate readings agreement was in a range of 98–100%. The average of the 10 samples/group was calculated to obtain the concentration of the fluoride released by the corresponding varnish, in a given time period. Both punctual (in a given time) and cumulative (the sum of the punctual) fluoride release were considered in the statistical analysis.

Statistical analysis

GraphPad InStat and Prism softwares (GraphPad Software, San Diego, CA, USA) were used. All data passed the normality and homogeneity tests (Kolmogorov–Smirnov and Bartlett, respectively). The data from the deionized water and artificial saliva protocols were analyzed individually using two-way ANOVA followed by Bonferroni *post hoc* test. The treatments and the time points were considered as criteria. The significance level of 0.05 was set for all tests.

RESULTS

Two-way ANOVA revealed significant differences among the treatments, the time points and interaction between the factors for both deionized water and artificial saliva.

In respect to the cumulative fluoride release,

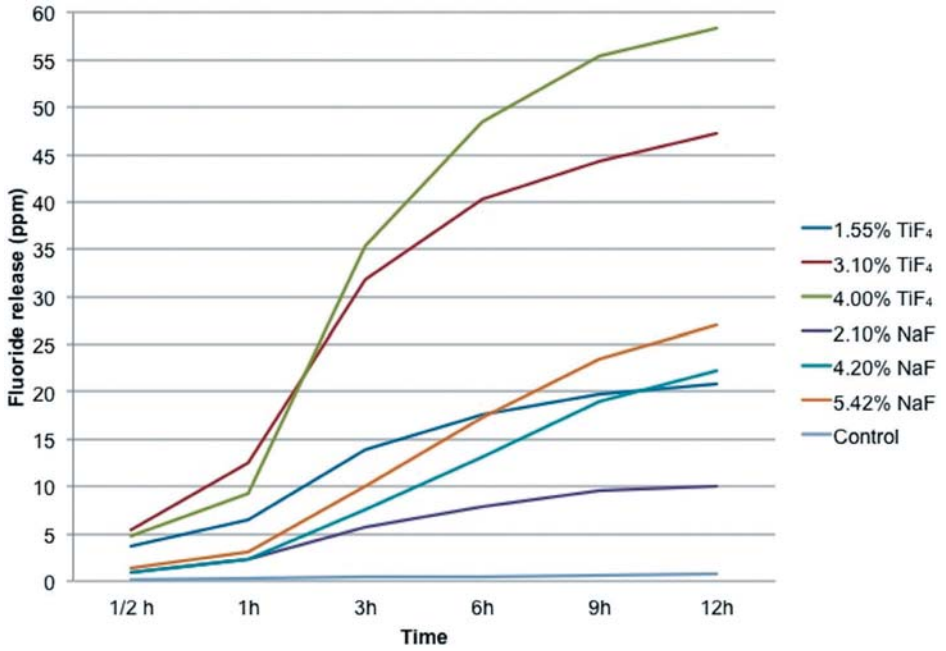

Figure 1- Mean of the cumulative fluoride release (ppm) from the experimental varnishes in deionized water according to the different periods

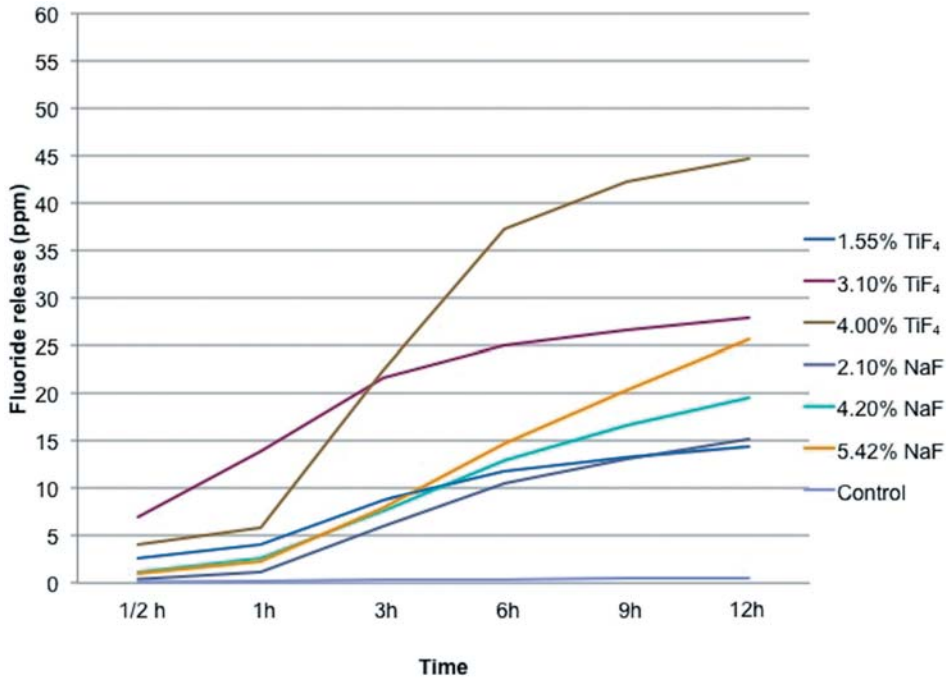

Figure 2- Mean of the cumulative fluoride release (ppm) from the experimental varnishes in artificial saliva according to the different periods

there was some dose–dependency relationship between the fluoride concentration of the experimental varnishes and the amounts of released fluoride, which were better defined for deionized water compared to artificial saliva, especially after 6 h. The TiF_4 varnishes released more fluoride into the water than NaF varnishes for all tested concentrations for 12 h (Figure 1). In the artificial saliva, the TiF_4 varnishes released more fluoride than the respective NaF varnishes for 6, 9 and 12 h, respectively (Figure 2).

Tables 1 and 2 show the punctual fluoride release into the water and saliva, respectively. The peak of the fluoride release occurred in the first 3 h of contact, but the varnishes were still releasing fluoride up to 12 h of exposure. The TiF_4 (except for 1.55%) releasing pattern was significantly higher than the NaF varnishes along 6 h in water and 1/2, 3 and 6 h in artificial saliva.

Table 1- Mean ± standard deviation (s.d.) of fluoride release (ppm) from the experimental varnishes in deionized water at each time point

	½ h	1h	3h	6h	9h	12h
1.55% TiF_4	3.68(0.55)^{bB}	2.73(0.62)^{aAB}	7.59(1.85)^{cC}	3.74(1.35)^{aB}	2.16(0.27)^{aAB}	1.37(0.21)^{aA}
3.10% TiF_4	5.42(1.52)^{bBC}	7.05(2.72)^{bCD}	20.12(3.94)^{dE}	8.58(1.20)^{bD}	4.09(0.70)^{bAB}	2.92(1.04)^{aA}
4.00% TiF_4	4.75(0.87)^{bA}	4.48(2.00)^{bA}	26.85(5.26)^{eD}	13.12(2.22)^{cC}	6.95(1.17)^{cB}	3.33(0.57)^{bA}
2.10% NaF	0.93(0.47)^{aA}	1.32(0.83)^{aA}	3.60(1.88)^{aB}	2.20(0.78)^{aAB}	1.65(0.71)^{aAB}	1.01(0.54)^{aA}
4.20% NaF	0.92(0.30)^{aA}	1.39(0.27)^{aA}	5.42(1.73)^{abBC}	5.59(1.63)^{aBC}	5.95(1.58)^{bcC}	3.77(0.99)^{bB}
5.42% NaF	1.36(0.35)^{aA}	1.79(0.65)^{aAB}	7.20(1.50)^{bcC}	7.55(1.23)^{bC}	6.21(1.06)^{bcC}	3.63(0.83)^{bB}
Control	0.16(0.01)	0.15(0.02)	0.11(0.00)	0.09(0.00)	0.14(0.00)	0.15(0.01)

Different lowercase letters show significant differences among the treatments for each time point (comparison *per* column), whereas capital letters show significant differences among the different time points for each treatment (comparison *per* line). Two-way ANOVA ($p<0.0001$). The control group was included to test any possible contamination.

Table 2- Mean ± standard deviation (s.d.) of fluoride release (ppm) from the experimental varnishes in artificial saliva at each time point

	½ h	1h	3h	6h	9h	12h
1.55% TiF_4	2.55(0.55)^{bAB}	1.45(0.53)^{aA}	4.72(0.64)^{aC}	3.08(0.41)^{aB}	1.40(0.14)^{aA}	1.26(0.28)^{aA}
3.10% TiF_4	6.97(1.73)^{dC}	6.92(1.51)^{bC}	7.69(1.22)^{bC}	3.54(0.40)^{aB}	1.58(0.16)^{aA}	1.17(0.08)^{aA}
4.00% TiF_4	4.03(0.55)^{cB}	1.84(0.32)^{aA}	16.63(4.79)^{cD}	14.78(4.46)^{dC}	5.06(1.56)^{bcB}	2.42(0.58)^{abA}
2.10% NaF	0.40(0.13)^{aA}	0.74(0.17)^{aAB}	4.93(1.55)^{aC}	4.43(0.53)^{abC}	2.64(0.29)^{abB}	1.99(0.19)^{abB}
4.20% NaF	1.09(0.39)^{abA}	1.51(0.36)^{aAB}	5.04(1.11)^{aC}	5.36(0.94)^{bcC}	3.71(0.43)^{bB}	2.83(0.44)^{bB}
5.42% NaF	0.92(0.27)^{aA}	1.34(0.45)^{aA}	5.69(0.58)^{aB}	6.80(1.19)^{cB}	5.56(0.82)^{cB}	5.44(0.50)^{cB}
Control	0.10(0.00)	0.10(0.0)	0.10(0.00)	0.08(0.00)	0.08(0.00)	0.08(0.00)

Different lowercase letters show significant differences among the treatments for each time point (comparison *per* column), whereas capital letters show significant differences among the different time points for each treatment (comparison *per* line). Two-way ANOVA ($p<0.0001$). The control group was included to test any possible contamination.

DISCUSSION

It is acknowledged that the formation of fluoride reservoirs on the tooth is related to the F amount released from the dental product[9,22,24]. This study showed a higher amount of fluoride released from TiF_4 varnish compared to NaF varnish, which might induce greater fluoride uptake by enamel. This finding might help explaining the best anti-caries effect found for TiF_4 compared to NaF in previous studies[8,13]. Therefore, the null hypothesis of this study was rejected.

The fact that TiF_4-based varnishes released more fluoride than NaF varnishes— considering that both were adjusted to contain the same fluoride concentration and the same resin base—might be related to its low pH allowing the dissociation of the fluoride from the resin base.

It is suggested that the maintenance of low levels of fluoride in saliva for long-term periods can control the carious lesions progression[4,24]. The increase of salivary levels after the application of a topical fluoride agent may be an indicative of the fluoride available for interaction with the teeth surfaces[18]. Low salivary fluoride level, around 0.04 ppm, has been shown to be correlated with a significant protective effect on dental caries[10]. Therefore, salivary fluoride levels are considered important parameters to predict the effectiveness of fluoride agents[15,24]. Accordingly, the results of the present study demonstrate that all the experimental fluoride varnishes might have a good effect on the prevention of dental caries (>0.40 ppm F release), and this effect may be even better for the most concentrated TiF_4 varnish (>2.42 ppm F release).

It is important to discuss that the present study was designed to evaluate the amount of fluoride released into water (in the absence of any ionic interaction) and artificial saliva (simulating the ions level in saliva) *in vitro*. For both media, we could see some relationship between F varnish concentration and F release among the tested products, which were more consistent in water due to the absence of ionic interaction.

The periods of exposition of 12 h were chosen based on previous clinical studies. A previous study showed significant levels of fluoride in saliva within 1 h after application of two fluoride (6% and 2.26% F, respectively) varnishes, which lasted up to 6 h *in vivo*[23]. However, the fluoride level returns to the baseline after 24 h *in vivo*[9].

In the present study, the peak of fluoride release occurred in the first 3 h. TiF_4 releasing pattern was significantly higher than NaF for the first 6 h. These results are similar to a recent study[21], in which the short-term fluoride release from four different 5% NaF varnishes was evaluated. The authors observed that over a 48 h period the fluoride release from three NaF varnishes reached a plateau at the first 4 h. One of these varnishes (containing both amorphous calcium phosphate and fluoride) was still showing the highest rate of fluoride release for 8 h.

In a clinical scenario, it is important to determine the fluoride release plateau after an application of fluoride varnish since it can be a parameter to be applied to compare the efficacy of different products. Furthermore, it is also important to establish critical time point considering the instructions that should be given for the patients after a professional fluoride application[21].

We have to keep in mind that there are differences between the laboratory study and clinical trials. Oral functions-such as salivation, swallowing and chewing-in addition to brushing and flossing are not present *in vitro*; thus, the fluoride release and the return to the baseline levels may be faster in a clinical setting[6,7]. An *in situ* model showed that, within the first day after fluoride varnish application, KOH-soluble fluoride uptake occurred in enamel samples located only in the vicinity close to the fluoridation site, so the fluoride transfer via saliva was strictly limited to the close neighborhood[1]. Clinically, it may mean that all sites in the oral cavity that require fluoridation have to be directly treated with the varnish.

Therefore, fluoride release pattern from the experimental varnishes should be confirmed *in vivo*. Considering that the lowest F concentrated varnish was unable to point out differences between both fluoride salts, it would be suggested to compare only 3% and 4% TiF_4 varnishes with the correspondent NaF varnishes in a further clinical trial. There are two hypotheses that could explain this result: TiF_4 varnish is only effective in releasing fluoride from the resin when the concentration is higher than 1.55% or NaF at high concentration (>5%, as 5.42%) reaches the fluoride solubility into the varnish, leading to a reduction in fluoride bioavailability. Further studies should be conducted on this field.

Finally, the present results help to explain previous findings showing that 4% TiF_4 varnish presents higher efficacy than NaF to reduce demineralization and increase remineralization of carious enamel lesions *in vitro* and *in situ*[8,13], in addition to being effective in dental erosion control[14]. However, further studies should focus on the analysis of weak fluoride and fluoride structurally bound enamel after treatment with the experimental varnishes, in order to better answer this issue.

CONCLUSIONS

The 3.10% and 4% TiF_4-based varnishes have greater ability to release fluoride into water and artificial saliva compared to the correspondent NaF varnishes.

ACKNOWLEDGMENTS

The present study was supported by scholarships (LPC #2011/11263-7 and BMS #2010/07001-4) from the São Paulo Research Foundation (FAPESP) and grant(s) from FAPESP (#2012/20698-0) and the National Council for Scientific and Technological Development (CNPq) (#305035/2011-8).

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