

Fluoride varnishes with calcium glycerophosphate: fluoride release and effect on *in vitro* enamel demineralization

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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DOI: 10.1590/1807-3107BOR-2015.vol29.0092

Submitted: Dec 11, 2014
Accepted for publication: Feb 27, 2015
Last revision: Jun 18, 2015

Abstract: The aims of this study were (1) to assess the amount of fluoride (F) released from varnishes containing calcium glycerophosphate (CaGP) and (2) to assess the effect of the experimental varnishes on *in vitro* demineralization. Six test groups using 5 varnishes: base varnish (no active ingredients); Duraphat® (2.26% NaF); Duofluorid® (5.63% NaF/CaF₂); experimental varnish 1 (1% CaGP/5.63% NaF/CaF₂); experimental varnish 2 (5% CaGP/5.63% NaF/CaF₂); and no varnish were set up. In stage 1, 60 acrylic blocks were randomly distributed into 6 groups (n = 10). Then 300 µg of each varnish was applied to each block. The blocks were immersed in deionized water, which was changed after 1, 8, 12, 24, 48 and 72 hours. Fluoride concentration in the water was analyzed using a fluoride electrode. In stage 2, 60 bovine enamel samples were distributed into 6 groups (n = 10), and treated with 300 µg of the respective varnish. After 6 h the varnish was removed and the samples were subjected to a 7-day *in vitro* pH cycle (6 h demineralization/18 h remineralization per day). The demineralization was measured using surface hardness. The results showed that both experimental varnishes released more fluoride than Duofluorid® and Duraphat® (p < 0.05), but Duraphat® showed the best preventive effect by decreasing enamel hardness loss (p < 0.05). Therefore, we conclude that even though (1) the experimental varnishes containing CaGP released greater amounts of F, (2) they did not increase in the preventive effect against enamel demineralization.

Keywords: Fluorides, Topical; Glycerophosphates; Dental Enamel; Dental Caries.

Introduction

Dental caries occurs when the presence of acids on the tooth-plaque interface leads to a shift in the demineralization/remineralization equilibrium favoring a net demineralization of the enamel.^{1,2} The decline seen in the prevalence of dental caries is mostly related to the use of topical fluoride (F) present in dental products.³ Several dental products are available for the prevention and treatment of initial caries lesions.

It is generally accepted that, when they come into contact with the dental enamel, F agents promote the precipitation of a calcium fluoride-like

(CaF₂-like) layer, which serves as a mineral reservoir of F and calcium in the mouth. The F and calcium released from the CaF₂-like layer during cariogenic challenges can reduce demineralization.⁴ Agents containing calcium glycerophosphate (CaGP) have also shown protective effects against cariogenic demineralization,^{5,6,7} since CaGP interacts with hydroxyapatite increasing its resistance,⁸ exhibiting a cumulative effect together with F.⁹ When present in dentifrices, CaGP has been shown to increase the caries-preventive effect,^{5,6,7,10} but conflicting results have also been reported.¹¹

The high F concentration in varnishes also has a positive effect on caries prevention.¹² because substantial amounts of CaF₂-like material can form on enamel.^{13,14,15,16,17} On the other hand, conflicting results have been reported with respect to the application of F varnish for caries control as part of routine clinical practice.^{18,19} Further studies on the improvements resulting from F varnishes are therefore needed. This study aimed at (1) assessing the amount of F released from the F varnishes containing CaGP, and (2) assessing the effect of experimental varnishes on enamel demineralization.

Methodology

This study was divided into two stages. Both stages involved 5 varnishes:

- i. base varnish (no active ingredients); FGM, Joinville, Brazil;
- ii. Duraphat® (2.26% F⁻ as NaF); Colgate-Palmolive GmbH, Hamburg, Germany;
- iii. Duofluorid® (2.71% F⁻ as NaF and 2.92% F⁻ as CaF₂ [total 5.63% F⁻]); FGM, Joinville, Brazil;
- iv. experimental varnish 1 (1% CaGP and 5.63% F⁻); FGM, Joinville, Brazil;
- v. experimental varnish 2 (5% CaGP and 5.63% F⁻); FGM, Joinville, Brazil.

The base varnish, Duofluorid® and experimental varnishes 1 and 2 were provided by the same manufacturer. These whitish varnishes contain synthetic resins and they are less viscous than Duraphat®. Duraphat® contains colophonium, shellac, mastic resin and white wax, and it is a yellowish-orange viscous resin.

Fluoride release from the varnishes

Sixty acrylic blocks (10 × 10 × 1 mm) were distributed into 6 groups (n=10): group 1 – base varnish, group 2 – Duraphat®, group 3 – Duofluorid®, group 4 – experimental varnish 1, group 5 – experimental varnish 2 and group 6 – control group with no varnish application. Using a precision scale, 300 µg of the respective varnish was applied to each acrylic block in groups 1–5. No varnish was applied to the blocks in group 6, which served as the control. The blocks were then placed into plastic vials containing 10 ml of distilled deionized water (DDW). The blocks remained immersed in the DDW for 1 h under still conditions, at room temperature. After this time, the acrylic blocks were removed from the first vial and placed into a new vial containing a fresh 10-mL aliquot of DDW. The acrylic blocks were again placed into new vials containing fresh DDW after 1, 8, 12, 24, 48 and 72 hours. The amount of F released to the DDW was analyzed using a fluoride-sensitive electrode (F electrode, Thermo Orion), adapted with a reference electrode and connected to a millivoltage reader (Thermo Orion). The electrode had been calibrated with standard solutions of known F concentrations: 0.15, 0.3, 0.6, 1.3, 2.5, 5.0, 10.0, 20.0, 40.0 and 80.0 µg/mL, containing standard total ionic strength adjustment buffer solution (TISAB III). A new calibration was performed before the reading of each group of 10 samples and the coefficient of variation of the electrode throughout the whole experiment was 5.39%. The samples were read in duplicate, and the mean of the two readings was calculated and recorded as the concentration of F in the sample (µg/mL).

Effect of the experimental varnishes on enamel demineralization

Sixty bovine incisors were cut (Isomet, Buehler Ltd., Evanston, USA) to obtain 60 enamel samples (4 × 4 × 2 mm). The enamel samples were ground and serially polished using 600, 1200 and 2400 grit papers (ANSI grit; Buehler, Lake Bluff, USA), to obtain flat polished surfaces. Initial enamel surface microhardness was measured using a microhardness tester with a Knoop diamond indenter (HMV-2000; Shimadzu Corp., Tokyo, Japan). Five sequential indentations were made, 100 µm apart, using a 50 g

load for 5 seconds. The initial Knoop hardness number ($KNH_{initial}$) for each enamel sample corresponded to the average of the five indentations. The enamel samples were randomly divided into 6 groups ($n = 10$), for treatment with the abovementioned varnishes. Half of each enamel sample surface was covered with a layer of nail varnish to maintain a control surface (untreated surface). The other half was covered with 300 μg of the test varnish. The samples were then individually placed in 30 mL demineralizing solution (2.0 mM CaCl_2 , 2.0 mM NaH_2PO_4 ; 0.075 mM acetate buffer, 0.02 ppm F, pH 4.7) for 6 h at 37°C.²⁰ The varnish layer was then carefully removed from the enamel surface using a cotton pellet soaked in acetone (1:1 dilution with DDW) and a scalpel blade, taking care not to touch the enamel surface and leaving the other half covered with a layer of nail varnish. Each sample were then individually placed in 15 mL remineralizing solution (1.5 mM CaCl_2 , 0.9 mM NaH_2PO_4 , 150 mM KCl, 0.1 M Tris buffer, 0.03 ppm F, pH 7.0)²⁰ for 18 h at 37°C.

In total, the samples underwent a 7-day pH cycle of 6 h demineralization and 18 h remineralization per day, without stirring. Between each step, the samples were washed in running DDW. The solutions were changed daily, and during the last 2 days of the experiment the samples were immersed in remineralizing solution only. At the end of the experiment, final enamel surface hardness was again measured following the same process as previously described. The percentage of surface hardness change for each sample was calculated using the formula: $\%SHC = 100 * (KNH_{final} - KNH_{initial}) / KNH_{initial}$.

Statistical analyses

In stage 1, the F measurements were taken after different lengths of time; therefore, the area under the curve (AUC) was calculated for each time interval ($[(t_2 - t_1) * (F_2 + F_1)] / 2$; where t is the time and F is the fluoride measurement), as well as the total AUC for the whole experiment. Normality and homogeneity of variance were analyzed using the Shapiro-Wilk and Levine tests, respectively. Repeated measures ANOVA analysis and Tukey's post-hoc test were then carried out using the AUC values as the dependent variable to verify differences between the groups.

For stage 2, the effects of the different varnishes on artificial caries were checked using ANOVA and post-hoc Tukey's tests using the mean values for %SHC.

Results

Figure 1 shows the concentration of F released by each varnish to the DDW, and the respective AUC is presented in Table 1. The base varnish and the negative control group released similar amounts of F and their lines on the graph trace the same trajectory (Figure 1). The base varnish and negative control released significantly less F than the other test materials ($p < 0.05$). In respect to the F varnishes, Duraphat® released the least amount of F than the other F varnishes (e.g. Duraphat® released about 70% less F than Duofluorid®). However, Duofluorid® released significantly less F than the experimental varnishes containing CaGP ($p < 0.05$). Both experimental varnishes released significantly more F than the commercially available varnishes. Moreover, experimental varnish 1, which contained 1% CaGP, released the greatest amount of F ($p < 0.05$).

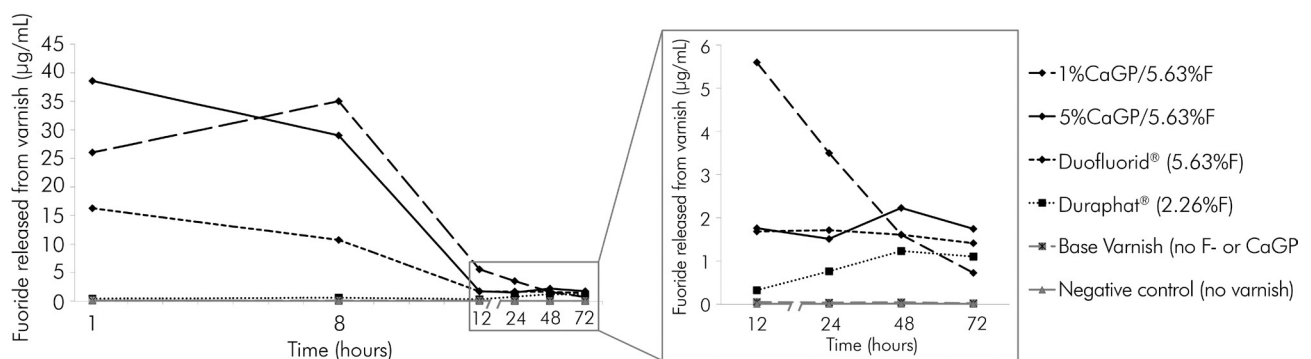


Figure 1. The amount of fluoride released from each varnish throughout the 72 hours of the experiment.

Table 1. Mean cumulative \pm SD values for the area under the curve (AUC) for fluoride release from each group.

Varnish	Mean (\pm SD) AUC values for the time periods between					AUC total
	1 and 8 h	8 and 12 h	12 and 24 h	24 and 48 h	48 and 72 h	
Base varnish (no F ⁻ or CaGP)	0.55(\pm 0.05)	0.46(\pm 0.02)	0.35(\pm 0.04)	0.28(\pm 0.06)	0.24(\pm 0.04)	1.98(\pm 0.18) ^a
Duraphat® (2.26% F)	3.68(\pm 0.62)	1.77(\pm 0.27)	6.06(\pm 0.86)	23.63(\pm 3.15)	27.99(\pm 4.82)	64.16(\pm 8.11) ^b
Duofluorid® (5.63% F)	90.56(\pm 15.89)	21.37(\pm 4.08)	18.24(\pm 2.75)	38.98(\pm 2.26)	39.22(\pm 2.60)	204.14(\pm 23.4) ^c
1% CaGP/5.63% F ¹	215.74(\pm 26.02)	84.09(\pm 10.72)	58.18(\pm 10.16)	64.69(\pm 16.06)	28.59(\pm 3.67)	438.86(\pm 65.53) ^d
5% CaGP/5.63% F ²	233.76(\pm 11.75)	61.46(\pm 4.36)	19.35(\pm 0.65)	44.14(\pm 2.73)	46.82(\pm 3.38)	410.38(\pm 24.19) ^e
Negative control (no varnish)	0.39(\pm 0.01)	0.19(\pm 0.02)	0.05(\pm 0.00)	0.07(\pm 0.01)	0.07(\pm 0.02)	0.85(\pm 0.12) ^a

^aDifferent letters mean statistical differences between the varnishes.

¹ Experimental varnish 1.

² Experimental varnish 2.

The analysis of the effect of the varnishes on enamel demineralization, showed that Duraphat®, Duofluorid® and experimental varnish 2 significantly reduced enamel demineralization compared to the negative control and base varnish groups ($p < 0.05$, Table 2). The experimental varnish 1, however, showed similar results to Duofluorid® and experimental varnish 2, but also similar to the negative control.

Discussion

Previous studies had shown positive effects of CaGP in F dentifrices against dental caries *in vitro* and *in vivo*.^{5,6,7,21} In the present study, we added CaGP to F varnish, at concentrations of 1% and 5%, with the aim of measuring the amount of F released from these varnishes and their effect on *in vitro* demineralization. The concentrations of CaGP were chosen based on the results of previous studies testing CaGP in F dentifrice.^{7,21} The protective effect of F varnish is related to the formation of a CaF₂-like layer on the enamel surface.²² We therefore speculated that the addition of CaGP would increase the amount of F released from

the varnish and, consequently, enhance CaF₂ formation and the protective effect against demineralization.

The mechanism of action of CaGP on caries prevention is still not well defined, but CaGP has been shown to protect enamel from cariogenic demineralization,^{5,6,7} probably owing to its interaction with the tooth mineral⁸ or its action on dental plaque (buffering effect).^{23,24} In addition, CaGP and F have a synergic effect, promoting greater protection for enamel.⁹ In this study, we measured the release of F from the experimental varnishes following immersion in DDW for up to 72 hours. Other studies have reported such analyses,^{25,26} where F release was observed for periods of up to 20 weeks and over. Since, clinically, F varnish only remains in contact with enamel for a few hours, we decided to study clinically relevant periods of time, with intervals of 1, 8 and 12 hours. We did, however, include other time-points (24, 48 and 72 h) to determine whether F varnish could release F for longer periods.

In general, we observed that the highest F release occurred during the first 8 h after application, and the amount of F released from varnishes containing CaGP was significantly greater than that from the commercial F varnishes. After the first 8-h period, the amount of F released from these varnishes substantially decreased. From these results, and taking other studies of the protective effect of F varnish against demineralization into consideration,^{25,26} a 6-hour exposure to F varnish seemed reasonable for the second stage of this study.

Considering both stages of the present study together, it is striking that the varnish with the lowest F release (Duraphat®) was the one that exhibited the

Table 2. Mean (\pm SD) of the percentage of the surface hardness change (%SHC).

Varnish	Mean % SHC (\pm SD)
Base varnish (no F ⁻ or CaGP)	-49.2(\pm 5.1) ^a
Duraphat® (2.26% F)	-14.1(\pm 7.5) ^b
Duofluorid® (5.63% F)	-21.9(\pm 8.2) ^{b, c}
1% CaGP/5.63% F ¹	-29.6(\pm 7.1) ^{c, d}
5% CaGP/5.63% F ²	-23.1(\pm 4.1) ^{b, c}
Negative Control (no varnish)	-36.1(\pm 10.1) ^d

^aDifferent letters mean statistical differences between the varnishes.

¹ Experimental varnish 1.

² Experimental varnish 2.

greatest preventive effect against demineralization. This was unforeseen, as one would expect that greater F release would generate more CaF₂-like material, which would, in turn, lead to a greater preventive effect. Greater F release, however, was observed from the experimental varnishes.

The greater amounts of F released from the experimental varnishes could be either a result of their higher F concentration (60% greater than that of Duraphat®) or of their composition and viscosity. Duraphat® was found to be more viscous than Duofluorid® and the two experimental varnishes, so the latter varnishes could be spread over a larger area of the acrylic blocks. This increased their surface area in contact with the surrounding water and possibly led to greater release of F. The experimental varnishes had the same F content as Duofluorid®, so these varnishes might have been expected to release similar amounts of F whereas, in fact, the experimental varnishes released significantly greater amounts of F than Duofluorid®. This suggests that the mechanism involved in F release is probably related to the presence of CaGP. Furthermore, it might be that there is a saturation effect of the CaGP, since the varnish containing 5% CaGP released less F than the one containing 1% CaGP.

Even though the experimental varnishes released significantly greater amounts of F than the other varnishes, this did not increase their preventive effect in the present experimental model. Therefore, we can hypothesize that a high F release does not indicate high cariostatic effect of these varnishes. The primary action of F in caries prevention, however, is more closely related to its presence in the fluid phases of the oral cavity, where F must be constantly present at low concentrations⁽⁴⁾. So, although greater amounts of F are released by the experimental varnishes, this

does not necessarily imply a greater formation of CaF₂-like structures on enamel. Moreover, the increased amount of F released from the experimental varnishes will probably have no significant preventive impact on tooth demineralization. A previous study showed that the preventive action of F varnish is limited to the area to which varnish has been applied.²⁷ So the dynamic of ionic changes between varnish/water (or varnish/saliva) is probably different to that between varnish/enamel. In any case, further experiments are necessary to actually identify the mechanism of the experimental varnishes and to explore why the varnishes with lower F concentrations and later F release apparently promoted greater enamel protection. Although these questions need to be further investigated, it may be speculated that F can bind either loosely or firmly to the enamel surface,^{22,27} and the CaF₂ layer will probably be more stable *in situ*.²⁸

Conclusion

The present study shows that (1) the addition of CaGP to F varnishes significantly increased F release, but (2) these varnishes did not have a greater preventive effect against *in vitro* enamel demineralization using the present experimental model.

Acknowledgements

The authors thank the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES; www.capes.gov.br) and the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq; www.cnpq.br) for financial support. The authors would also like to express their gratitude to the post-graduates and professors at the Pediatric Dentistry Department (*Faculdade de Odontologia – Universidade de São Paulo - FOU SP*) for their valuable comments and discussions.

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