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Insoluble NaF in Duraphat® May Prolong Fluoride Reactivity of Varnish Retained on Dental Surfaces

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There is no consensus about the clinical recommendation of the time that Duraphat® varnish should be maintained on enamel surfaces without suffering mechanical disturbance by the patient. Considering the importance of calcium fluoride (CaF_2)-like reservoirs on the anticaries effect of professional fluoride application, an *in vitro* study was designed to test the reactivity of Duraphat® varnish with enamel forming these reservoirs as a function of time. Since most fluoride in Duraphat® varnish is insoluble to react and form products on enamel, the relative contribution of the varnish soluble and insoluble fluoride fractions to the reactivity was also evaluated. For this, whole-varnish, containing soluble and insoluble fluoride (total fluoride concentration of 23699±384 µg F/g), or centrifuged varnish, containing only soluble fluoride (fluoride concentration of 258±97 µg F/g), were applied in a standardized manner on enamel slabs (n=8/varnish group/time), which were immersed in continuously renewed artificial saliva for up to 36 h. CaF_2-like reservoirs formed on enamel by varnish application were extracted using 1 M KOH and fluoride concentration was measured with ion specific electrode. The results were expressed as µg F/cm² of enamel area. Whole varnish formed significantly higher fluoride concentration on enamel than centrifuged varnish, reaching maximum concentration at 24 h (22.0±4.5 µg F/cm²). Centrifuged varnish reached maximum concentration at 6 h (3.20±0.81 µg F/cm²). In conclusion, a longer varnish retention time than the usually recommended could improve the anticaries effect of Duraphat® varnish, allowing that NaF particles, initially insoluble in the varnish matrix, prolong the reactivity with enamel.

Key Words: fluorides, topical fluorides, calcium fluoride.

Introduction

Among commercial formulations for professional fluoride application, the anticaries effect of fluoride gel and varnish (Duraphat®) is based on evidence (1,2). Their mechanism of action is attributed to the reactivity of the fluoride present in these formulations with enamel/dentine, forming loosely-bound, calcium fluoride (CaF_2)-like reservoirs (3), whose effect on enamel demineralization is concentration-dependent (4). However, while in gels all fluoride is available for an immediate (1-4 min) reaction with enamel/dentine because the NaF salt used is dissolved in water (5, 6), in the varnish the colophony hydrophobic matrix is dissolved in ethanol, in which only a small percentage of NaF is soluble.

Based on studies suggesting that the reaction of Duraphat® with enamel is time-dependent (7,8), a prolonged retention of NaF varnishes has been clinically recommended to allow for a more thorough fluoride reaction with enamel. However, there is no consensus among the authors about the time recommended to maintain the varnish, ranging from 4 h (9) to the next morning after application (10-12). This may have arisen from the limitations of the studies to establish a precise time for varnish maintenance on dental surfaces. Thus, Retief et al. (7) showed that fluoride uptake by enamel was increased when the contact with Duraphat® varnish was raised from 1 to 24 h, but only total fluoride was measured, and not the loosely-bound fluoride reservoirs. On the other hand, Bruun and Givskov (8) could not observe differences in the reactivity of Duraphat® varnish with enamel after 6 or 18 h, but a simulation of the intraoral salivary clearance was not performed. Therefore, a detailed kinetics study on the reactivity of fluoride varnish with enamel is still lacking. Also, in both cited studies the relative importance of the fraction of fluoride that is soluble in varnish for an immediate reaction with dental hard tissues forming CaF_2-like products versus the insoluble NaF particles present in the varnish matrix that could prolong the reaction when dissolved by saliva was not tested.

Thus, it was hypothesized that the reaction of the varnish with enamel would be dependent, on the short-term, on its soluble fluoride concentration, and on the long-term, on dissolution of insoluble NaF in the matrix. Therefore, it was used whole varnish and the supernatant of the centrifuged varnish, in which all fluoride is soluble, to isolate the effect of total and soluble fluoride on the kinetics of CaF_2-like formation on enamel.

Material and Methods

Experimental Design

This was an *in vitro*, randomized, blind study, in which 112 bovine enamel slabs (3x3 mm) prepared from sound bovine incisors and with their surfaces polished flat, were randomly allocated (n=8 in each varnish group and time), into the study factors: 1. *composition of the varnish*, i.e., whole varnish (containing soluble and insoluble fluoride (Duraphat®, A. Natherman & Cie, Cologne, Germany. Lot N°. 011001)) and centrifuged varnish (the supernatant of centrifuged Duraphat® (5 min, 11.000 *g*), containing only soluble fluoride) (Fig. 1); and 2. *reaction time*, from 5 min to 36 h. Additionally, 16 untreated slabs were used for baseline loosely-bound (KOH-soluble) fluoride concentration determination. The dimension of each slab surface was measured with a digital caliper (± 0.01 mm). Only the outer enamel surface was exposed to the treatments, and the other surfaces were isolated with wax.

The reactivity test was performed by applying whole or centrifuged varnish (5.0±0.5 mg) on the surface of each enamel slab, which was immersed in constantly renewing artificial saliva (13) for 5 min, 30 min, 2 h, 6 h, 12 h, 24 h or 36 h. In each test, 4 slabs of the same varnish group and time were immersed together in 40 mL of artificial saliva (10 mL/slab). Saliva was continuously renewed (0.5 mL/min/slab), simulating salivary flow rate, using two peristaltic pumps, which kept saliva volume within a narrow oscillation range (±1 mL) during the immersion periods. Each test was repeated twice for each of the experimental times, totalizing n=8 slabs/time/group. At the end of each test period, the system was disassembled, the slabs were collected, the varnish removed with acetone-imbibed cotton swab and each slab thoroughly rinsed with acetone for 30 s to ensure complete removal of the varnish but not of the CaF_2-like reservoirs formed (8,14), followed by a 30 s rinse with purified water. CaF_2-like concentration formed on enamel was determined.

Determination of CaF_2-like Concentration on Enamel

Slabs were individually immersed in 0.4 mL of 1 M KOH (4.44 mL/cm²) for 24 h under agitation at room temperature (15). Fluoride in the alkali extract was determined using a specific ion electrode (Orion model 96-09, Orion Research, Cambridge, MA, USA) coupled to an ion analyzer (Orion EA-740) after buffering with TISAB II containing 1 M HCl, and expressed as µg F/cm² of enamel area.

Determination of Fluoride Concentration in Varnish

Fluoride concentration in whole and centrifuged Duraphat® varnish (Fig. 1) was determined after a standardized extraction using acetone. Varnish was weighed (10±0.1 mg) in a centrifuge microtube to which 100 µL of acetone was added. After vigorous homogenization in vortex for 15 s to ensure complete varnish dissolution, the extracts of whole or centrifuged varnishes were transferred to 100 or 10-mL volumetric flasks, respectively, and the microtube was washed 3x with 1 mL of water and once again with 100 µL of acetone for total varnish removal. The extract was diluted to 100 or 10 mL using purified water, for whole or centrifuged varnish, and fluoride concentration was measured in this solution using a specific ion electrode after buffering with TISAB II.

Determination of Fluoride Concentration In Artificial Saliva

Aliquots (n=2 to 11) of artificial saliva exiting the flow system were collected at different time-points for determination of fluoride concentration using the fluoride electrode, after buffering with TISAB III.

Statistical Analysis

CaF_2-like fluoride data were analyzed by two-way ANOVA, considering as factors type of varnish (two levels) and application time (seven levels). The assumption of normality of errors and homogeneity of variances were satisfied by transforming the data to the potency of 0.3 for analysis. The SAS system (SAS Institute Inc., version 9.2, Cary, NC, USA) was used in the analyses, with significance level set at 5%.

Results

Fluoride concentrations measured in whole and centrifuged varnish were 23699.1±384.3 ppm F (n=4) and 258.8±97.1 ppm F (n=8), respectively.

Baseline CaF_2-like concentration in the untreated enamel blocks was 0.11±0.02 µg F/cm². Exposure to varnishes significantly increased CaF_2-like concentration

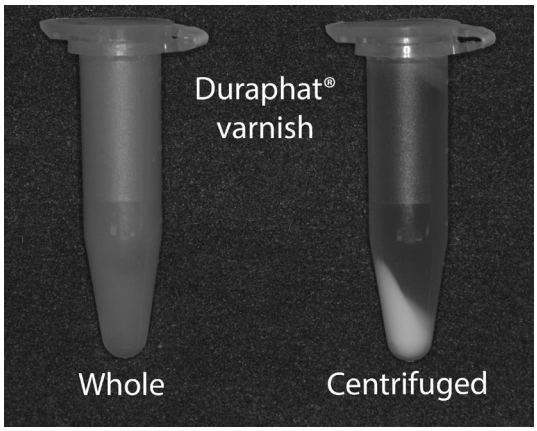

Figure 1. Whole and centrifuged Duraphat® varnish used in the experiment. Note the precipitation of NaF particles by centrifugation, while in the cloudy whole varnish, they are dispersed in the matrix.

on enamel (Fig. 2). This increase was significantly higher when the whole varnish was applied when compared to the centrifuged varnish ($p<0.05$). Moreover, although the reactivity of the whole varnish with enamel was stabilized only after 24 h (Fig. 3), for the centrifuged varnish the maximum CaF_2-like concentration was reached after 6 h.

Fluoride concentration in the continuously flowing artificial saliva was below the detection limit of the fluoride analysis (0.03 µg F/mL) for the collections made after 6 h for the whole varnish and for all times for the centrifuged. In the whole varnish group, the maximum fluoride concentration in artificial saliva (Fig. 3) was observed at 30 min (0.07±0.01 µg F/mL).

Discussion

Since most fluoride in Duraphat® varnish is insoluble and the CaF_2-like fluoride reservoirs chemically formed on enamel from soluble fluoride reactivity are considered responsible for the anticaries mechanism of action of professional fluoride application (4), it is in principle challenging to explain how this product is effective to control caries (1).

The current study presents an explanation to the reaction with enamel surface of the soluble and insoluble fluoride fractions in the varnish, and also extends the findings of Retief et al. (7) and Bruun and Givskov (8), which support the importance of retaining the varnish applied on dental surfaces for longer time. Furthermore, our results provide a clue on how the anticaries potential of different varnishes formulation currently found in the market should be evaluated (16).

The findings (Fig. 2) showed that Duraphat® reaction with enamel is time-dependent and that the insoluble fluoride in the formulation plays a significant role in the process. The greater fluoride concentration formed on enamel by the whole varnish than by the centrifuged one may be attributed to the dissolution of insoluble NaF particles present in the varnish matrix (8) and the increase

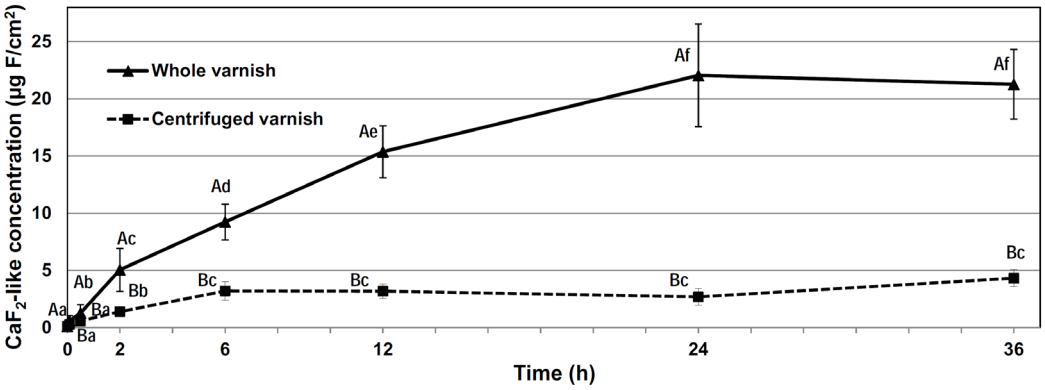

Figure 2. CaF_2-like (KOH-soluble fluoride) concentration (µg F/cm²) formed on enamel by the whole or centrifuged varnish according to the time under continuous artificial saliva flow (avg±sd;n=8). Capital letters represent the statistical differences between the varnishes conditions, and lower case among the application times for each condition ($p<0.05$).

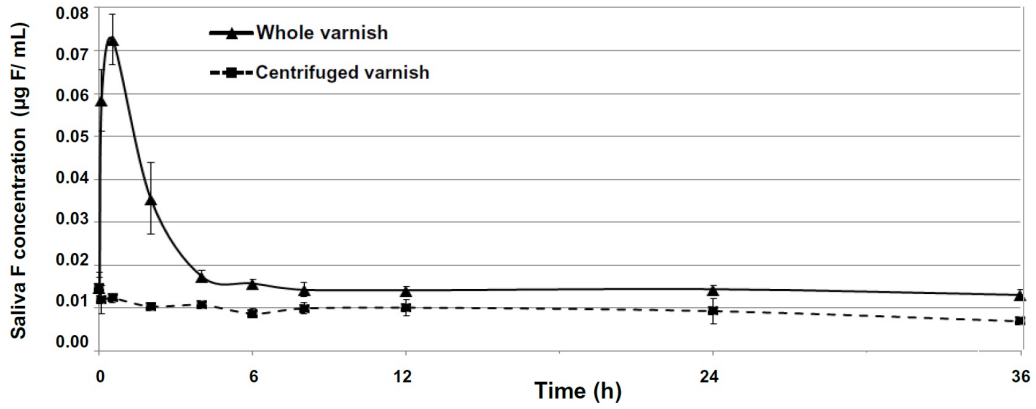

Figure 3. Fluoride concentration (avg±se; n=2 to 11) in artificial saliva according to the time (h) that the varnishes were kept on enamel surfaces.

of free fluoride concentration at the varnish-enamel surface interface, but not to the fluoride released to saliva. Indeed, the concentration of fluoride found in saliva was very low (Fig. 3) to explain the increase of CaF_2-like fluoride formed on enamel overtime (17). Therefore, fluoride release to saliva from fluoride varnishes may not be considered the best predictor of their anticaries potential because it is short-lived and the anticaries effect of these products is maintained for longer periods, giving support to their use 2 to 4 times/year (1). Also, fluoride varnish should not be considered a slow-releasing material able to protect all dentition against caries because its site-specific action is based on the formation of CaF_2-like reservoirs on the dental surface where it was applied, with limited reactivity with the surrounding dentition (18). These loosely-bound fluoride reservoirs would be released to a biofilm newly formed on the treated surface (4,12). Likewise, microscopic remnants of the varnish retained on retentive surfaces for long periods of time might function as slow-release fluoride reservoirs, but with limited local effect.

Any model has limitations to adequately simulate the *in vivo* conditions (19). The use of bovine enamel as a surrogate of human enamel can be considered acceptable given that similar reactivity with both of them has been observed after the use of professional fluoride products (4,6). Also, in the present study, although artificial saliva was used as the immersion media to simulate the kinetics of varnish imbibition and insoluble NaF dissolution, the specimens were subjected to continuous flow simulating what happens in the oral cavity. Furthermore, the relationship between CaF_2-like concentration and time that varnish is retained on dental surfaces should be tested regarding its effect on enamel-dentine demineralization. Finally, the kinetics of CaF_2-like formation as a function of varnish retention time should also be tested *in situ* or *in vivo* giving support to a later removal (20).

In conclusion, the findings of the present study show that insoluble fluoride in whole-varnish is an important fluoride source that would be dissolved during intraoral exposure, increasing fluoride reactivity with enamel overtime. Considering the anticaries importance of loosely-bound fluoride reservoirs formed by professional fluoride application, the results suggest that the recommendation that patients avoid removing the varnish applied on dental surfaces could actually improve the anticaries effect of Duraphat®.

Resumo

Não há consenso sobre a recomendação clínica do tempo que o verniz Duraphat® deve ser mantido nas superfícies de esmalte sem ter perturbação mecânica pelo paciente. Considerando a importância dos reservatórios tipo fluoreto de cálcio ("CaF_2") no efeito anticárie da aplicação profissional de fluoreto, um estudo *in vitro* foi delineado para testar a reatividade do verniz Duraphat® com o esmalte na formação desses reservatórios em função do tempo. Como a maioria do fluoreto no verniz Duraphat® é insolúvel para reagir e formar produtos no esmalte, também se avaliou a contribuição relativa das frações solúvel e insolúvel do verniz na reatividade. Assim, verniz total, contendo fluoreto solúvel e insolúvel (concentração total de 23699±384 µg F/g), ou verniz centrifugado, contendo somente o fluoreto solúvel (concentração de 258±97 µg F/g), foram aplicados de maneira padronizada sobre blocos de esmalte (n =8/grupo de verniz/tempo), os quais foram imersos em saliva artificial com renovação continua por até 36 h. Os reservatórios tipo "CaF_2" formados no esmalte pela aplicação do verniz foram extraídos com KOH 1 M e a concentração de F foi medida utilizando um eletrodo específico. Os resultados foram expressos como µg F/cm². O verniz total formou significativamente maior concentração de fluoreto no esmalte que o verniz centrifugado, atingindo concentrações máximas após 24 h (22,0±4,5 µg F/cm² de área de esmalte). O verniz centrifugado apresentou a máxima formação após 6 h (3,20 ± 0,81 µg F/cm²). Em conclusão, um tempo maior de retenção do que habitualmente recomendado poderia melhorar o efeito anticárie do verniz Duraphat®, pois partículas de NaF, inicialmente insolúveis na matriz do mesmo, possibilitam uma reatividade prolongada do verniz com o esmalte em função do tempo.

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References

1. Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2002:CD002279.
2. Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride gels for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2002:CD002280.
3. ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. Eur J Oral Sci 1997;105:461-465.
4. Tenuta LM, Cerezetti RV, Del Bel Cury AA, Tabchoury CP, Cury JA. Fluoride release from CaF_2 and enamel demineralization. J Dent Res 2008;87:1032-1036.
5. Villena RS, Tenuta LM, Cury JA. Effect of APF gel application time on enamel demineralization and fluoride uptake *in situ*. Braz Dent J 2009;20:37-41.
6. Calvo AF, Tabchoury CP, Del Bel Cury AA, Tenuta LM, da Silva WJ, Cury JA. Effect of acidulated phosphate fluoride gel application time on enamel demineralization of deciduous and permanent teeth. Caries Res 2012;46:31-37.
7. Retief DH, Sorvas PG, Bradley EL, Taylor RE, Walker AR. *In vitro* fluoride uptake, distribution and retention by human enamel after 1- and 24-hour application of various topical fluoride agents. J Dent Res 1980;59:573-582.
8. Bruun C, Givskov H. Formation of CaF_2 on sound enamel and in caries-like enamel lesions after different forms of fluoride applications *in vitro*. Caries Res 1991;25:96-100.
9. Du M, Cheng N, Tai B, Jiang H, Li J, Bian Z. Randomized controlled trial on fluoride varnish application for treatment of white spot lesion after fixed orthodontic treatment. Clin Oral Investig 2012;16:463-468.
10. Weintraub JA, Ramos-Gomez F, Jue B, Shain S, Hoover CI, Featherstone JD, et al.. Fluoride varnish efficacy in preventing early childhood caries. J Dent Res 2006;85:172-176.
11. Arruda AO, Senthamarai Kannan R, Inglehart MR, Rezende CT, Sohn

W. Effect of 5% fluoride varnish application on caries among school children in rural Brazil: a randomized controlled trial. Community Dent Oral Epidemiol 2012;40:267-276.
12. Skold-Larsson K, Modeer T, Twetman S. Fluoride concentration in plaque in adolescents after topical application of different fluoride varnishes. Clin Oral Investig 2000;4:31-34.
13. ten Cate JM, Duijsters PP. Alternating demineralization and remineralization of artificial enamel lesions. Caries Res 1982;16:201-210.
14. Retief DH, Bradley EL, Holbrook M, Switzer P. Enamel fluoride uptake, distribution and retention from topical fluoride agents. Caries Res 1983;17:44-51.
15. Caslavska V, Moreno EC, Brudevold F. Determination of the calcium fluoride formed from *in vitro* exposure of human enamel to fluoride solutions. Arch Oral Biol 1975;20:333-339.
16. 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.
17. Arends J, Nelson DG, Dijkman A, Jongebloed WL. Effect of various fluorides on enamel structure and chemistry. In: Cariology Today. Guggenheim (Editor). Zürich: Karger; 1984. p. 245-258.
18. Attin T, Lennon AM, Yakin M, Becker K, Buchalla W, Attin R, et al.. Deposition of fluoride on enamel surfaces released from varnishes is limited to vicinity of fluoridation site. Clin Oral Investig 2007;11:83-88.
19. Tenuta LM, Cury JA. Laboratory and human studies to estimate anticaries efficacy of fluoride toothpastes. Monogr Oral Sci 2013;23:108-124.
20. Miller EK, Vann WF, Jr. The use of fluoride varnish in children: A critical review with treatment recommendations. J Clin Pediatr Dent 2008;32:259-264.

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