ALKALI-SOLUBLE FLUORIDE DEPOSITION ON ENAMEL AFTER PROFESSIONAL APPLICATION OF TOPICAL FLUORIDE *IN VITRO*

FORMAÇÃO DE FLÚOR FRACAMENTE LIGADO AO ESMALTE APÓS A APLICAÇÃO TÓPICA PROFISSIONAL DE FLÚOR IN VITRO

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Since the efficacy of topical fluoride products is related to the fluoride (F) availability and its reactivity with enamel, this study was conducted. The F concentration of the following materials was verified: I- acidulated phosphate fluoride (APF) gel (1.23% F), II- APF foam (1.23% F) and III- Varnish (2.26% F). Forty blocks of bovine enamel were divided into 4 groups and treated according to the materials described, being one of them used as control. Loosely bound fluoride ("CaF_2") was determined on enamel after extraction with 1.0M KOH and analyzed by ion-selective electrode. Total F concentration found in gel was 12,642, in foam 12,755 and in varnish 23,183 mg F/g. All products formed statistically higher amounts of "CaF_2" on enamel compared to the control group ($p < 0.05$), but the difference between them was not significant ($p > 0.05$). Thus, "CaF_2" formation was not proportional to the total F content in the products, suggesting that the pH and the vehicle used are more important.

UNITERMS: Fluorine; Topical fluorides; Dental enamel; Professional topical fluoride.

INTRODUCTION

Fluoride (F) has been proven to be an effective anti-caries agent when delivered in many vehicles and concentrations, including a variety of professionally applied F[17]. The efficacy may depend on the reaction products formed after F topical application, which can be loosely (calcium fluoride-like – "CaF_2") and firmly bound F[16]. "CaF_2" is the major reaction product formed during F topical treatment of dental hard tissues. The formation of "CaF_2" is important because it interferes with the de- and remineralizing phases of the caries process[14], acting as a pH-controlled reservoir of F ions on enamel or in dental plaque to be released during cariogenic challenges[9,10,11]. Besides, the "CaF_2" formed on the enamel surface could be rapidly lost, but it might result in further fluorapatite formation by supplying F ion to the enamel mineral[5].

The formation of products when F reacts with the enamel depends on the F concentration, duration, pH, frequency and the treatment method[9]. Although the general efficacy of F under these varied conditions is intriguing, it is clear that the more products are formed after application, the greater will be the inhibition of the dynamic process of caries development[12]. Thus, it is likely that the higher the F concentration in a product, the larger the F uptake would be expected.

There are several commercially available products for professional topical application presented as gels, foams and varnishes. However, their efficacy in the formation of alkali-soluble fluoride ("CaF_2") as to the differences found in vehicles, F concentrations, pH and procedures of application is still unknown.

Therefore, the aim of this study was to determine the *in vitro* formation of "CaF_2" on bovine enamel surface after professional topical application of F gel, foam and varnish.

MATERIAL AND METHODS

Evaluation of F concentration

The products presented in Table 1 were analyzed according to the total F content. Three samples of foam and gel from different batches were weighed (± 0.01mg) and vigorously homogenized in distilled deionized water. As regards the varnish, two samples from different batches were analyzed. A beaker containing 90mL of distilled deionized water was placed in a scale (± 0.01mg) and an amount of varnish was added to the water and weighed. The water was boiled for 5min for extraction of F from the lacquer matrix and the volume was completed to 100mL. Each sample was buffered with an equal volume of TISAB II for all analyses (1.0M acetate buffer pH 5.0, 1.0M NaCl, 0.4% CDTA). Fluoride was analyzed using an ion-selective electrode Orion 96-09 and an ion analyzer Orion EA-940, previously calibrated with F standard solutions containing 1.25 to 15.0 µg F/mL. Analyses were made in triplicate.

Fluoride Application

Forty enamel blocks (5 x 5 x 2 mm) were obtained from bovine incisor teeth stored in 2% formaldehyde solution, pH 7.0, for at least one month [6]. The blocks were mounted in dental wax, leaving only the enamel surface exposed (5 x 5mm). The blocks were randomly divided into 4 groups, which received the following treatments: I- acidulated phosphate fluoride (APF) gel; II- APF foam; III- varnish and IV- control (without treatment). The enamel surface, which was submitted to gel and foam, was treated for 4min using a cotton swab and after application the blocks were washed with distilled deionized water for 1min. Varnish was spread over the enamel surface with a plastic scalpel and the blocks were stored at 100% humidity and 37°C for 24 h. After this period, the varnish lacquer was removed using another plastic scalpel and the small remnants of resin were cleaned with acetone using a cotton swab until every signs of remnants disappeared[13].

Determination of "CaF_2"

The enamel blocks, treated or not, were individually immersed in plastic tubes containing 0.5mL 1.0M KOH solution and gently agitated at room temperature for 24h, according to Caslavska, et al[4]. After this period, the extracts were neutralized with 0.5mL TISAB II containing 1.0M HCl and analyzed as previously described, but standard solutions containing from 0.0312 to 4.00 µg F/mL were used. The amount of "CaF_2" formed on each enamel block surface was calculated and expressed as µg F/cm^2.

Statistical Analysis

The data were processed using one-way ANOVA followed by the Tukey test to determine the significance of the treatments ($p < 0.05$).

RESULTS

Table 2 shows the concentration of F (mg F/g) in the products used for topical F treatments. The F concentration found in the gel and foam was 12,642 and 12,755 mg F/g, respectively, whereas in the varnish it was 23,183 mg F/g.

The mean amount of "CaF_2" formed on the enamel after treatment is expressed as micrograms of F per square centimeter (Figure 1). The quantities of "CaF_2" produced by the gel, foam, varnish and control were 31.72 ± 22.18, 44.57 ± 22.06, 19.39 ± 16.53 and 0.17 ± 0.04 mg F/cm^2, respectively. Statistical analysis showed that all products formed higher amounts of "CaF_2" on enamel in comparison with the control group ($p < 0.05$). There was no statistical difference ($p>0.05$) between gel and foam and also between gel and varnish, however the varnish statistically differed from foam ($p<0.05$).

DISCUSSION

The present study showed that the F concentration found in gel, foam and varnish are in accordance with the

TABLE 1- Products according to the manufacturers

Type	Manufacturer	Composition	pH
Gel (APF)	Nupro (Dentsply, Petrópolis, Brazil)	1.23% F (NaF and HF) in 0.1 M phosphoric acid	3.6 to 3.9
Foam (APF)	Topical fluoride foam (Laclede, USA)	1.23% F (NaF and HF) in 0.1 M phosphoric acid foam	3.5
Varnish	Duraphat™ (A. Nattermann & Cie. GmbH, Cologne, Germany)	5% NaF in an alcoholic solution of natural resins (colophonium base)	Neutral (Dijkman *et al.*[7])

manufacturers' specifications (12,300 mg F/g for APF products and 22,600 mg F/g for varnish), as shown in Table 2. The mean variation above the estimated values was no greater than 3.7% for all products. These analyses confirmed the high F content in APF products and almost twice their concentration in the varnish.

All products investigated caused deposition of "CaF_2" on enamel (Figure 1) and they showed statistically similar results. It is clear that gel and foam did not differ because both products present similar F concentrations (Table 2) and had the same reaction time. However, the formation of "CaF_2" in the foam group was numerically higher than after gel and varnish applications. One possible difference between both products that could explain this fact is the slightly lower pH of the foam when compared to gel.

Although varnish shows the highest F concentration among all topical products and remained in contact with enamel for 24 h, it did not significantly differ from the gel and foam groups and showed a lower mean "CaF_2" formation. This result is in accordance with Dijkman, et al.[8], who also found comparable results for the APF gel and varnish treatments, reporting that Duraphat™ formed less "CaF_2" than expected. Some characteristics of the varnish could account for this result. Sodium fluoride (NaF) is present in Duraphat™ as a suspension in an alcoholic base and not as a solution. Thus, only a negligible percentage of F can be expected to be available in the varnish to react with enamel. According to Brunn, Givskov [3], only 17% of the NaF in Duraphat® was found to be soluble in water after 18h. In addition, the varnish presents a neutral pH, while gel and foam are acidic. Considering that only in the varnish group the enamel was cleaned with acetone to remove the remaining resin, this procedure could raise doubts in causing damage to the product formed. Brunn, Givskov [3] tested this hypothesis and showed that acetone was not able to extract any "CaF_2" from the enamel treated with varnish. Also, "CaF_2" is not soluble in acetone.

Regarding the limitations of an *in vitro* model and considering its advantages, the results found in this study could help the understanding of some basic aspects of these products. In this study, it was possible to evaluate the formation of reaction products from these materials and to relate it to the F concentration of the product. Considering the experimental design made, the findings should be experimentally confirmed using different *in vitro*, *in situ* and *in vivo* models.

Although F varnishes have been the standard of care in practice for the professional application of topical F in Western Europe, Scandinavia and Canada for 25 years, FDA has not approved F varnish as a caries-preventing agent yet[1]. The reason for that is the lack of clear evidences, mainly on clinical trials. Also, it has been reported that the percentage of caries reduction by F varnish and gels has decreased during the last decade[2]. At the same time, concerns regarding fluorosis, ingestion and toxicity have spurred recent researchers to reevaluate the clinical efficacy of topical F agents. The potential for ingestion and toxicity from the use of F varnishes does exist. Most of the varnish applied to the tooth surface is ingested and not expectorated, since the patients are instructed not to brush their teeth for 24 h[15]. Considering the results found in this study, we suggest further evaluations on the effectiveness of topical F application products, mainly on varnish, due to its high F concentration and limited efficacy.

Finally, the data showed that all professional topical products formed a significant amount of "CaF_2" on the enamel surface when compared to the control group. However, "CaF_2" formation was not proportional to the total F content in the products. We concluded that the efficacy of products for professional topical application in the formation of "CaF_2" is more related to the pH and the vehicle used than to the F concentration in the product.

TABLE 2- Means + standard deviations of fluoride concentrations (µg F/g) in fluoridated gel, foam and varnish

Products	µg F/g	
	Estimated	Observed
Gel	12,300	12,642.5 + 428.3 (n=3)
Foam	12,300	12,756.0 + 623.6 (n=3)
Varnish	22,600	23,183.2 + 719.5 (n=2)

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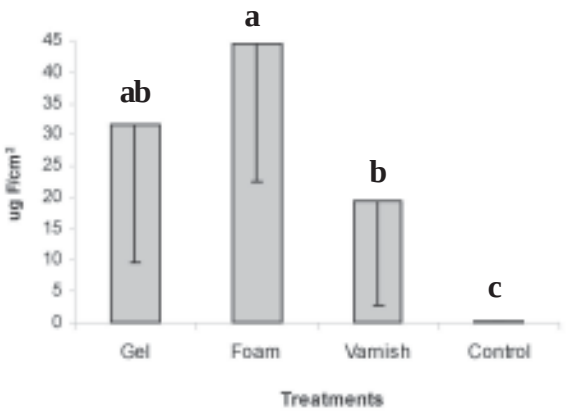

FIGURE 1- "CaF_2" (µg F/cm²) formed on enamel after the treatments (mean, sd; n=10)

Treatments with means followed by distinct letters are statistically different ($p < 0.05$)

RESUMO

Considerando que a eficácia dos produtos para aplicação tópica profissional de flúor (géis, espumas e vernizes) está relacionada com a reatividade do flúor (F) com o esmalte e sendo esta dependente da disponibilidade do F em cada produto, este estudo foi conduzido. A concentração de F nos seguintes produtos foi estudada: I - Flúor Fosfato Acidulado (FFA) gel (1,23% F), II - FFA espuma (1,23% F) e III- Verniz fluoretado (2,26% F). Foram confeccionados 40 blocos de esmalte bovino, tratados de acordo com os grupos descritos, sendo um deles utilizado como controle. O F fracamente ligado ("CaF_2") ao esmalte foi determinado após a extração com 1.0 M KOH e analisado em eletrodo específico. A concentração de F encontrada no gel foi de 12.642, na espuma 12.755 e no verniz 23.183 mg F/g. Todos os produtos formaram uma quantidade significantemente maior de "CaF_2" na superfície do esmalte, comparado ao grupo controle ($p < 0,05$), mas entre eles, esta diferença não foi significante ($p > 0,05$). Assim, a formação de "CaF_2" na superfície do esmalte não foi proporcional ao conteúdo de F nos produtos, sugerindo que o pH e o veículo utilizado são mais importantes.

UNITERMOS : Flúor; Flúor tópico; Esmalte; Flúor tópico profissional.

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