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ORIGINAL ARTICLE

In Vitro Evaluation of Fluoride in Saliva After Topical Application of Professional Use Products

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Abstract

Objective: To evaluate in vitro the presence of fluoride in saliva after applying professional use products on the surface of dental enamel. **Material and Methods:** Experimental groups were composed by: Cariostatic 12% (CA), Fluoridated Varnish 5% (FV), Fluorine Acidulate Gel 1.23% (AG) and Fluorine Neutral Gel 2% (NG). Fluoridated dentifrice (FD) and Artificial Saliva (AS) were used as controls. Products (10 µL) were applied to the surface of bovine enamel blocks (4×4×1 mm, n = 18) and immersed in 10 mL of artificial saliva at room temperature. Aliquots of artificial saliva (750 µL) of each sample were collected 1, 2, 4, 8, 24 and 48 hours after application of the products. Analyses were performed in triplicate, using a fluoride ion-specific electrode coupled to a potentiometer. The equipment was calibrated using a standard curve for fluoride analysis from 0.125 to 64 µgF-/mL. **Results:** Greater fluoride concentration (µgF-/mL) was observed after 1h application, as follows: 197.40 (NG), 172.21 (AG), 20.25 (CA), 14.49 (FV) e 11.81 (FD). Fluoride concentration increased overtime for all groups. After 48h, the following fluoride concentrations were assessed: 428.12 (AG), 267.25 (NG), 65.36 (FV), and 62.52 (CA). **Conclusion:** Greater fluoride release was observed for AG and NG groups, mostly after 1h application.

Keywords: Fluoride; Cariostatic Agents; Artificial Saliva; In Vitro Techniques.

Introduction

Dental caries is a disease caused by the exposure of the cariogenic biofilm to fermentable dietary carbohydrates, which when converted to acids by bacterial metabolism causes a decrease in the pH of the area and, consequently, demineralization of dental enamel [1,2]. The presence of fluoride during the cariogenic challenge, however, negatively regulates the progression of dental caries lesions, through the reduction of demineralization [1-4].

The bioavailability of fluoride during the cariogenic challenge is directly proportional to the anti-caries effect [1-4]. Thus, products of professional use allow high concentration of fluoride to contact calcium ions present in the oral cavity, forming CaF2 reservoirs in the dental enamel, which results in the gradual release of fluoride over time [4-7]. Therefore, the topical application of fluoride products for professional use is an alternative for the prevention and control of dental caries [2,4,8].

These products are indicated according to the individual's experience of dental caries [9,10]. Oral hygiene performed regularly with conventional fluoride dentifrice (1000/1450/1500 ppm F-) and appropriate brushing technique is sufficient to maintain good oral conditions [11-13]. However, individuals with a high risk of caries (active caries, high sugar consumption and increased retention of biofilm by the use of orthodontic appliance) require additional methods to increase the intra-oral fluoride concentration in order to reduce their risk. Alternatives can be found in the form of solution, gel or varnish [2,12-14].

Products that promote greater fluoride release overtime are effective in preventing and controlling dental caries [2,14]. Therefore, determining the fluoride concentration present in saliva after topical application of professional use products in necessary to assess their efficacy [6,14-16]. Then, the aim of this study was to evaluate the presence of fluoride in saliva, after the topical application of professional use fluoridated products.

Material and Methods

Study Design

An in vitro experimental study with inductive approach, descriptive-comparative procedure and direct laboratory documentation technique was carried out [17].

To select the bovine incisors, the absence of caries, stains, cracks or other enamel defects were considered. After the bovine incisors selection, two parallel mesial-distal and cervical-incisal cuts were obtained from the middle third of the buccal face of the bovine's incisors crown. These cuts were performed using an automatic cutting machine (ISOMET Low Speed Saw cutting machine - model n 11-1280-170, Lake Bluff, IL, USA). Each tooth originated two blocks of enamel (4 × 4 × 1 mm), which were submitted to planning and polishing in Metalographic polisher (APL4, Arotec, Cotia, SP), with the use of silicon oxide sandpapers at granulations #400, #600, #800 and #1200. Prior to experiments, the enamel blocks were stored at 100% humidity (in contact with a moist paper). The enamel blocks were randomly selected to inclusion in the study using a random mathematic function available in Microsoft Excel software.

Topical Application of the Fluoride Product on Dental Enamel

Bovine dental enamel blocks were submitted to one of the following experimental groups: Cariostatic 12% (CA), Fluoridated Varnish 5% (FV), Fluorine Acidulate Gel 1,23% (AG) and Fluorine Neutral Gel 2% (NG). The groups consisting of Fluoridated Dentifrice (FD 0.14%) and no-treatment (artificial saliva - AS) were used as controls (Table 1).

Table 1. Experimental groups according to the evaluated professional use products.

Groups	Product / Manufacturer	Presentation Form	Fluoride Concentration
CA	Cariestop®/ Biodinâmica, Ibiporã, Brazil	Solution	12% Silver Diamine Fluoride
FV	Fluorniz® / SS White, Rio de Janeiro, RJ, Brazil	Varnish	5% Sodium Fluoride
AG	Flúor Gel® / Nova DFL, Rio de Janeiro, RJ Brazil	Gel	1.23% Acidulated Phosphate Fluoride
NG	Flugel® / Nova DFL, Rio de Janeiro, Brazil	Gel	2% Sodium Fluoride
FD (Control)	Tripla Ação – Menta Original® Colgate-Palmolive, São Bernardo do Campo, SP, Brazil	Dentifrice	0.14% Sodium Fluoride
AS (Control)	Artificial Saliva	Solution	Without fluoride

The professional-use fluoridated products (10 µL) were applied to the surface of bovine dental enamel blocks (4 × 4 × 1 mm, N = 18) and, after 5 minutes, immersed in 10 mL of artificial saliva at room temperature. Saliva aliquots (750 µL) of each sample were collected 1, 2, 4, 8, 24 and 48 hours after the application of the tested product on the buccal face of the bovine dental enamel samples [7].

Analysis of Fluoride Concentration in Saliva

Analyses were performed using a fluoride ion specific electrode (Orion Star Series, Thermo Scientific, Singapura) coupled to a potentiometer (Orion Star Series, Thermo Scientific, Singapura) [7]. The calibration of the equipment was performed in duplicate, using a calibration curve of 0.125 to 64 µgF-/mL (r2 = 0.997; Slope = -53.5) (Figure 1). For this purpose, the calibration curve was obtained by means of serial dilutions from a standard solution of 100 µgF-/mL.

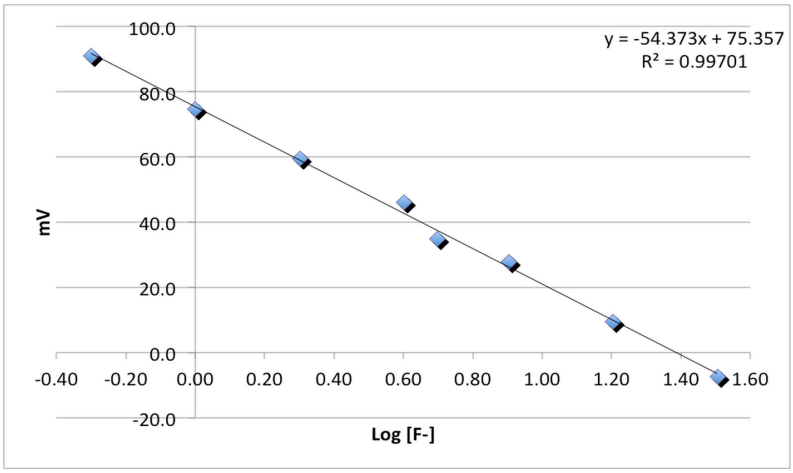


Figure 1. Calibration curve of the fluoride-specific ion electrode coupled to a potentiometer (millivolts (mV) per Log [F-]).

The standard curve values were drawn from 750 µL of each of these standards plus 750 µL of Total Ionic Strength Adjustor Buffer (TISAB II). The mean values of the electrical conductivity (mV) were transferred to a spreadsheet and the fluoride concentration was calculated using a linear regression equation based on the relationship between the electrical conductivity (mV) read and the [log F-] (r2 = 0.997; Slope = -53.5). There was no difference greater than 5% between the expected and the calculated concentrations, within each point of the curve.

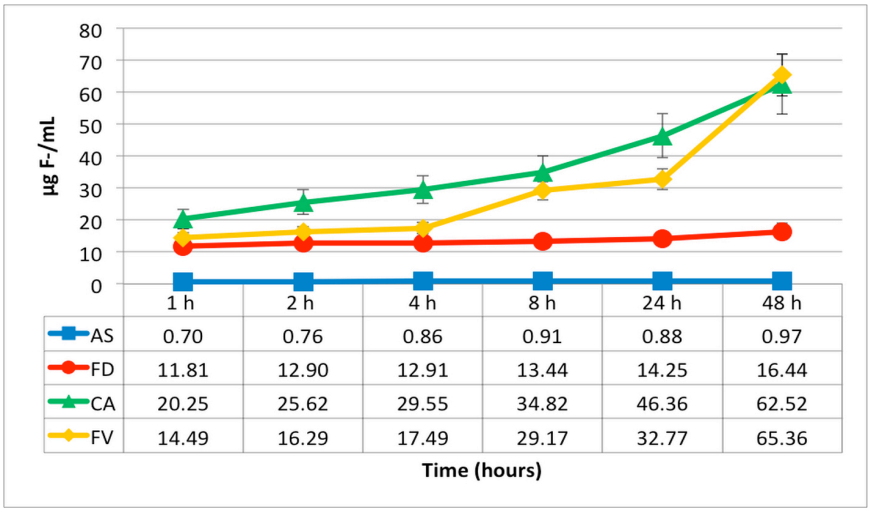
Wihtin each time point (1, 2, 4, 8, 24 and 48 hours), aliquots (750 µL) of artificial saliva from each experimental group was added to 750 µL of TISAB II and fluoride concentrations were determined using the calibration curve, by transforming the mV readings on µgF-/mL concentration. Proper dilutions were made in the case mV readings were not within calibration curve range.

Data Analysis

The data were analyzed descriptively through the use of means and standard deviation, as measures of central tendency and dispersion, respectively. Data were presented in time-lapsing charts.

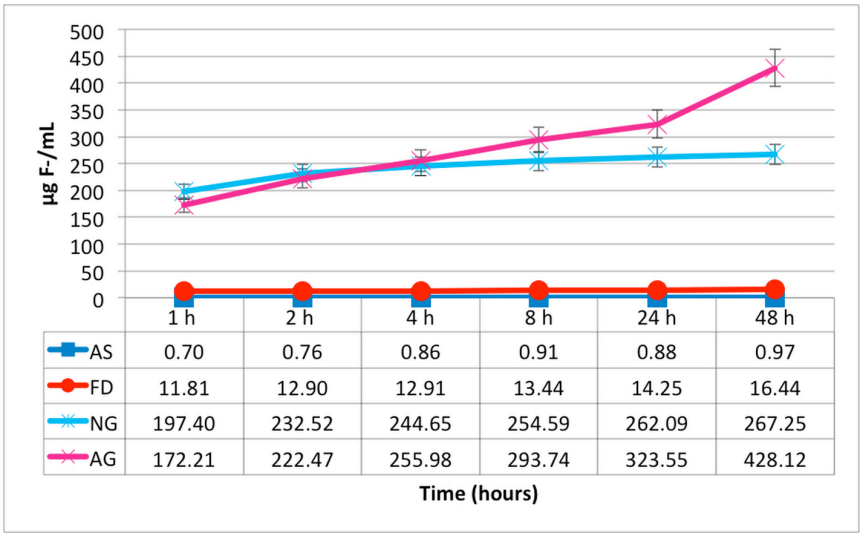
Results

Greater fluoride concentration (µgF-/mL) was observed 1 h after application of enamel surface, as follows: 197.40 (NG), 172.21 (AG), 20.25 (CA), 14.49 (FV) e 11.81 (FD) (Figures 2 to 4). CA and FV groups presented progressive fluoride concentrations between 2 and 48 h, being observed at 48 h the following fluoride concentrations: 62.52 µgF-/mL (CA) and 65.36 µgF-/mL (FV). Greater fluoride concentrations irrespective of the time was observed for AG and for NG, which varied between 172,21 µgF-/mL (1h) to 428.12 µgF-/mL (48 h) (Figure 3).



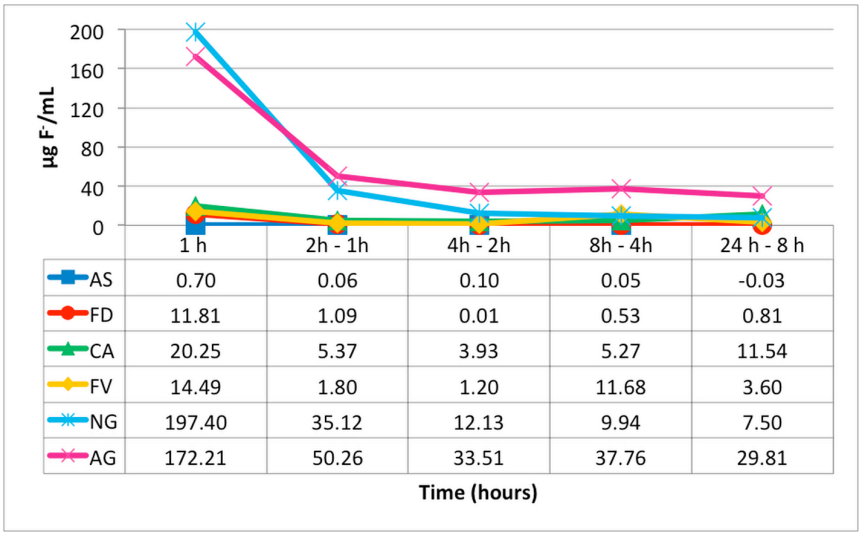
	1 h	2 h	4 h	8 h	24 h	48 h
AS	0.70	0.76	0.86	0.91	0.88	0.97
FD	11.81	12.90	12.91	13.44	14.25	16.44
CA	20.25	25.62	29.55	34.82	46.36	62.52
FV	14.49	16.29	17.49	29.17	32.77	65.36

Figure 2. Fluoride concentration (µg F-/mL) in saliva within groups: no application (Artificial Saliva - AS Control), Fluoridated Dentifrice (FD), Cariostatic (CA) and Fluoridated Varnish (FV) at 1, 2, 4, 8, 24 and 48 hours (Maximum y-axis: 80 µg F-/mL).



	1 h	2 h	4 h	8 h	24 h	48 h
AS	0.70	0.76	0.86	0.91	0.88	0.97
FD	11.81	12.90	12.91	13.44	14.25	16.44
NG	197.40	232.52	244.65	254.59	262.09	267.25
AG	172.21	222.47	255.98	293.74	323.55	428.12

Figure 3. Fluoride concentration (µg F-/mL) in saliva within groups: no application (Artificial Saliva - AS Control), Fluoridated Dentifrice (FD), Neutral Fluoride Gel (NG) and Acidulated Fluoride Gel (AG) at 1, 2, 4, 8, 24 and 48 hours (Maximum y-axis: 500 µg F-/mL).



	1 h	2h - 1h	4h - 2h	8h - 4h	24 h - 8 h
AS	0.70	0.06	0.10	0.05	-0.03
FD	11.81	1.09	0.01	0.53	0.81
CA	20.25	5.37	3.93	5.27	11.54
FV	14.49	1.80	1.20	11.68	3.60
NG	197.40	35.12	12.13	9.94	7.50
AG	172.21	50.26	33.51	37.76	29.81

Figure 4. Fluoride release (µg F-/mL) in saliva within groups: no application (Artificial Saliva - AS Control), Fluoridated Dentifrice (FD), Cariostatic (CA), Fluoridated Varnish (FV), Neutral Fluoride Gel (NG) and Acidulated Fluoride Gel (AG) at 1, 2, 4, 8 and 24 hours (Maximum y-axis: 200 µg F-/mL)

Discussion

A higher concentration of fluoride in saliva was observed one hour after application of the fluoridated products. Studies have observed higher concentration of fluoride one hour after the application of dentifrices [18] and fluoridated varnishes [15], as observed in the present investigation. In addition, another study observed greater fluoride release one hour after the application of Duofluorid® varnishes and experimental varnish containing 5% of CaGP (calcium glycerophosphate). However, the experimental varnish containing 1% CaGP presented higher fluoride release 8 hours after application [19]. In the present study, the fluoride concentration in saliva increased for all professional use products, but greater concentrations were also seen for

fluoridated gels. This result can be explained by the greater fluidity and solubility of the gels, being released in the saliva in small intervals of time; whilst other products, such as fluoride varnish, release small concentrations of fluoride at longer time intervals, which exceed the 48 hours evaluated in the present study [19].

The concentration of fluoride increased overtime (1-48 hours), whilst the fluoride release tax decreased. Even though, the concentrations of fluoride in saliva observed in subsequent analyses (2, 4, 8, 24 and 48 hours) can also prevent the development of dental caries [20]. Studies corroborate that low fluoride concentrations, i.e. 0.1-1 ppm [20], present continuously in buccal fluids (saliva and biofilm liquid) interfere negatively with the cariogenic process, being able to reduce demineralization and increase remineralization of dental enamel [2,3,13,20]. One study observed that above 135 ppm of fluoride there is no additional significant decrease in tooth enamel demineralization. Therefore, the optimum range for the reduction of enamel demineralization is between 0.1 and 135 ppm [21]. Therefore, it is ideal that fluoridated products have relatively low concentrations and slow release over time [21].

The CA group, silver diamine fluoride - Cariestop 12%®, presented enough fluoride release (>0.1 ppm) able to paralyze or reverse the dental enamel demineralization [21,20]. However, there are few studies that have evaluated the effect of silver diamine fluoride at 12%, considering the fluoride bioavailability. Studies have observed that this product at 38% positively interferes with the cariogenic process [11,22-25]. In addition to its anti-caries effect, silver diamine fluoride has also been demonstrated as antimicrobial agent, which would aid dental biofilm control [26].

In the FV group, fluoridated varnish was used based on 5% NaF, which among the products tested, presented lower fluoride release during the period evaluated. However, the fluoride concentration evaluated within 48 h can reduce demineralization and increase remineralization (>0.1 ppm) [20,21]. One study observed different concentrations of fluoride released by varnishes containing 5% NaF [15]. The efficacy of fluoride varnishes for prevention and control of dental caries has been proven in the literature [11,27,28]. This behavior is due to a fluoridated controlled release system, which demonstrates activity for several days [29].

The groups that had the highest concentration of fluoride released were AG and NG, respectively. The higher fluoride release observed in the AG group can be explained by the increase in the reactivity of calcium and fluoride to form CaF2 reservoirs due to the acid present [5,30]. One study observed higher fluoride concentration after application in biofilms treated with acid dentifrices than with their neutral counterparts [18]. This aspect, however, has not been proven in this investigation. In a clinical condition, the topical application of fluoridated gel confers greater comfort to the patient and the professional. Thus, the results of this study indicate that the fluoride release after professional use of fluoridated gel may represent a relevant measure for the control of dental caries [7].

The highest concentrations of fluoride released by fluoridated products may be due to the composition and viscosity of the product [19]. This can be explained because the more fluid

products can be spread over a larger area of the block, which increases the contact surface with the surrounding saliva and provides greater release of fluoride [19]. In addition, the longer contact time of the product with the enamel provides greater efficacy against dental caries [1,3,7].

The fluoridated products evaluated released fluoride concentrations able to interfere with the cariogenic process (>0.1 ppm) [20,21]. Thus, dental surgeons can use these products in individuals with high dental caries risk. As the products are available in varied forms, the clinician should consider the better convenience. This is especially important to populations with high risk of dental caries, such as cerebral palsy children, in which preventive measured should be associated with instructions directed to caregivers [31].

Because it is an in vitro study, the dynamics of human saliva, which may interfere with the release of fluoride [15,28], as well as other physiological conditions inherent to humans, such as swallowing and chewing and body temperature [16]. However, despite the limitations, the present study provides application in the clinical scenario, since it guides the choice of fluoridated products by dental professionals according to the patients' needs. Besides that, this study may guide the convergence of resources and practices for the improvement of the oral health of populations [9,10,15].

Conclusion

Greater fluoride release is observed within 1 h after professional-use fluoridated products. Greater fluoride concentration is observed for acidulated and neutral fluorine gels, compared to other presentation forms.

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Conflict of Interest: The authors declare no conflicts of interest.

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