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Fluoride concentration and amount of dentifrice influence enamel demineralization *in situ*

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ABSTRACT

Objectives: This study evaluated the effect of conventional (CD, 1100 ppm F) and low-fluoride (LFD, 550 ppm F) dentifrices, applied in different quantities, on enamel demineralization, and on fluoride (F) concentrations in the dental biofilm formed *in situ*.

Methods: Five combinations of dentifrices and quantities were tested: placebo (P–F-free) applied on all brush bristles; LFD applied by the transversal technique (0.3 g–T1) or on all bristles (0.6 g–T2); and CD applied in a pea-sized amount (0.15 g–T3) or by the transversal technique (0.3 g–T4), in order to produce comparable intensities (F concentration in the dentifrice × amount applied to the brush). Volunteers (n = 13, 20–36 years old) wore palatal devices containing 4 bovine enamel blocks, and performed cariogenic challenges (30% sucrose solution) 6×/day, and brushing 3×/day, following a double-blind, cross-over and randomized protocol. On the 8th day, biofilm was collected 5 and 60 min after brushing. The percentage of surface hardness loss (%SH), integrated loss of subsurface hardness (ΔKHN) and biofilm F concentrations (solid and fluid phases) were determined. Data were analyzed by repeated-measures ANOVA, Student-Newman-Keuls test, and Pearson's correlation coefficient ($p < 0.05$).

Results: Significantly lower ΔKHN was observed for treatments with higher intensity (T2 and T4) in comparison with the lower intensity (T1 and T3). A strong correlation was observed between ΔKHN and F concentrations in total biofilm ($r = -0.71$) and biofilm fluid ($r = -0.72$) 5 min after brushing.

Conclusions: The treatment intensity has a significant influence on the development of caries lesions *in situ*. Clinical significance: The intensity of treatment (amount of dentifrice × concentration) during brushing seems to be a more relevant parameter of clinical efficacy than simply observing the F concentration of the product. The use of a small amount of CD significantly reduced the protective effects against enamel demineralization.

1. Introduction

A comprehensive Cochrane review assessing the relative caries-protective effectiveness of dentifrices with different fluoride (F) concentrations concluded that toothbrushing with dentifrices with concentrations of 1000 ppm F or above is an effective measure in preventing caries in children and adolescents [1]. Given that the early use of fluoridated dentifrices has been shown to be a potential risk factor for the development of dental fluorosis [2], professionals and health authorities have advocated the use of dentifrices with F concentrations above 1000 ppm F, but applied in very small quantities for young children [3,4], under the assumption that this measure would minimize systemic F exposure from this source without compromising the clinical efficacy of the product.

Considering that the clinical effects of F are known to be dose-dependent, this strategy would be valid except for the fact that the dentifrice is diluted in saliva during toothbrushing. It could be anticipated, therefore, that reducing the amount of dentifrice on the brush would reduce the F concentration in the natural dentifrice-saliva slurry formed during toothbrushing, which, in turn, could affect the clinical efficacy of the dentifrice [5]. While previous studies have addressed the impact of the amount of dentifrice used during toothbrushing on salivary F concentrations, enamel remineralization and enamel F uptake [6,7], the effect of the amount of dentifrice as a function of F concentration in the product has only recently been assessed. It was demonstrated that brushing with a low-F dentifrice (LFD, 550 ppm F) applied using the

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transversal technique (*i.e.*, perpendicular to the long axis of the brush) led to significantly higher salivary F concentrations than a conventional dentifrice (CD, 1100 ppm F) using a pea-sized amount [8], confirming the hypothesis that the dilution of the dentifrice in saliva during toothbrushing has a direct impact on intraoral F concentrations.

Based on dose-response considerations, the above-mentioned results could have important clinical implications, raising questions on the appropriateness of the current recommendation of dentifrices to children. Nonetheless, as only one intraoral variable was assessed, and given the short-term nature of that study [8], these results cannot be fully extrapolated to a clinical situation. Considering that dental caries is the net result of the slow mineral loss occurring in dental enamel covered by a cariogenic biofilm [9], the aim of this study was to assess the effects of treatment intensity (*i.e.*, F concentration in the dentifrice versus the amount used during toothbrushing) on enamel mineral loss, as well as on biofilm F concentrations. The null hypothesis was that the treatment intensity would not influence the variables assessed.

2. Materials and methods

2.1. Ethical aspects and inclusion criteria

This study was approved by the IRB of School of Dentistry, Araçatuba–UNESP (CAAE 44712715.5.0000.5420). Participants signed an informed consent form and received written and verbal instructions on the research protocol prior to the beginning of the study. Thirteen individuals (20–36 years old), living in the city of Araçatuba-SP, Brazil (0.6–0.8 mg F/L in the public water supply) [10], were enrolled. The inclusion criteria involved participants in good general and oral health. Individuals who used drugs that could interfere with the formation of the dental biofilm or salivary flow, smokers, those who received fluoride applications 2 weeks before the experiment, were using orthodontic appliances or had systemic diseases could not participate in the study [11].

2.2. Experimental design

Sample size was based on a previous study assessing the effects of dentifrices containing 500 and 1100 ppm F on enamel demineralization and biofilm F concentrations [12]. A sample of 14 volunteers was calculated considering α-error of 5%, β-error of 20% [13], and dropout rate of 20%. The protocol used was *in situ*, double-blind and crossover. The volunteers were randomly divided into 5 groups, totaling 4 combinations of dentifrice and quantity, namely: 550 ppm F applied by the transversal technique (0.3 g) or on all bristles of the brush (0.6 g), and 1100 ppm F applied in a pea-size amount (0.15 g) or by the transversal technique (0.3 g). A placebo dentifrice (F-free) applied on all bristles of the brush (0.6 g) was also included. The dentifrice tubes were coded and the quantities used during brushing were determined by an examiner not involved in sample collection and analysis.

2.3. Formulation and determination of F in the experimental dentifrices

The experimental dentifrices were produced in the laboratory of Pediatric Dentistry from School of Dentistry, Araçatuba, using the same basic formulation with the following components: titanium dioxide, carboxymethylcellulose, methyl *p*-hydroxybenzoate, sodium saccharine, oil peppermint, glycerin, silica abrasive, sodium lauryl sulfate, water and sodium fluoride (NaF, Merck®, Germany 550 e 1100 μg F/g). A F-free formulation without F (placebo) was also used. The determination of the ionic and total F concentrations [14] and pH [15] of the dentifrices was performed prior to the beginning of the study.

2.4. Enamel blocks and appliance preparation

Enamel blocks (n = 260) measuring 4 × 4 x 2 mm were obtained from bovine incisors previously stored in 2% neutral formaldehyde solution (pH 7.0) for 1 month. They were sequentially polished (600, 800, 1200 grit), followed by polishing with felt paper and 1 μ diamond suspension. Four enamel blocks were fixed in 4-mm deep spaces of custom-made acrylic palatal devices, approximately 1 mm below the level of the acrylic surface, in order to allow biofilm accumulation on the enamel specimens. A plastic mesh covered the enamel blocks to prevent biofilm disturbance by mechanical forces [12].

2.5. Intraoral procedures

Two drops of a 30% sucrose solution were dispensed on each enamel block *ex vivo*, 6 times/day, at previously established times (8:00, 11:00, 14:00, 17:00, 19:00, 21:00 h). The appliances were left to rest during 5 min before being returned to the oral cavity [12]. The treatment of the blocks with the dentifrices was performed 3 times/day (7:30, 13:00 and 22:00 h), during 7 days. The amount of dentifrice to be used was demonstrated individually to all volunteers, in each experimental phase. They also received photographs via cell phone showing the quantity to be applied on the brush. Volunteers were instructed to brush their natural teeth with the appliance in the oral cavity for 1 min, and to swish the natural suspension of dentifrice/saliva for 30 s. Volunteers then completed the cleaning of their natural teeth without the appliance in their mouths, and rinsed the appliances (20 mL) and their mouths (10 mL) with tap water. They were instructed to remove the appliances only during meals and ingestion of liquids, with no restrictions on their diet. Prior to each experimental phase (lead in/wash out), the volunteers used a F-free dentifrice for 7 days.

2.6. Sample collection

On the morning of the 8th day, the volunteers attended the laboratory of Pediatric Dentistry, School of Dentistry, Araçatuba, while fasting, having been instructed not to brush their teeth and not to perform the cariogenic challenge on the specimens. Volunteers then performed the brushing procedures as previously described, but with the dentifrices weighed by the researchers (Shimadzu Balance AUY220, Kyoto–Japan). Biofilm from 2 blocks was randomly collected at 5 and 60 min after brushing, being immediately transferred to a microcentrifuge tube filled with mineral oil. The tube was weighed (before and after collection) and centrifuged (21,000*g*, 5 min, 4 °C) to separate the solid and fluid phases of the biofilm. Following, a small fraction of the fluid phase was collected, using a micropipette.

2.7. Fluoride analysis

Biofilm fluid samples were transferred to the surface of an inverted ion-specific electrode (Orion 9409), immersed in mineral oil, and placed on drops of TISAB III previously placed on the electrode membrane, at a 10:1 ratio (sample:TISAB III), as described by Vogel et al. [16]. F was determined in the total biofilm after HCl extraction and buffering with TISAB III, using a F ion-specific electrode (Orion 9409), and a reference electrode (Orion 900200), both coupled to an ion analyzer (Orion 720A$^+$) [17].

2.8. Hardness analysis

Surface hardness was determined prior to the experiments (SH_i) using the Micromet 5114 hardness tester (Buehler, Lake Bluff, USA and Mitutoyo Corporation, Kanagawa, Japan), under 25 g for 10 s (five indentations, spaced 100 μm from each other, made at the center of the block) [18]. Blocks included in the study had SH_i between 330–370 KHN, and were randomly distributed among the study groups. After the experimental phases, final surface hardness (SH_f) was determined as described for SH_i, 100 μm from the initial indentations. The percentage of SH loss (%SH) was calculated using the formula: %SH = 100 x (SH_f –