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In situ evaluation of fluoride-, stannous- and polyphosphate-containing solutions against enamel erosion

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ABSTRACT

Objective: To evaluate the anti-erosive effect of solutions containing sodium fluoride (F: 225 ppm of fluoride), sodium fluoride + stannous chloride (F + Sn: 225 ppm of fluoride + 800 ppm of stannous), sodium fluoride + stannous chloride + sodium linear polyphosphate (F + Sn + LPP: 225 ppm of fluoride + 800 ppm of stannous + 2% of sodium linear polyphosphate), and deionized water (C: control), using a four-phase, single-blind, crossover in situ clinical trial.

Methods: In each phase, 12 volunteers wore appliances containing 4 enamel specimens, which were submitted to a 5-day erosion-remineralization phase that consisted of 2 h of salivary pellicle formation with the appliance in situ, followed by 2 min extra-oral immersion in 1% citric acid (pH 2.4), 6x/day, with 90 min of exposure to saliva in situ between the challenges. Treatment with the test solutions was performed extra-orally for 2 min, 2x/day. At the end of the experiment, surface loss (SL, in μm) was evaluated by optical profilometry. Data were analyzed using ANOVA and Tukey tests ($\alpha = 0.05$). The surface of additional specimens was evaluated by x-ray diffraction after treatments ($n = 3$).

Results: C (mean SL ± standard-deviation: 5.97 ± 1.70) and F (5.36 ± 1.59) showed the highest SL, with no significant difference between them ($p > 0.05$). F + Sn (2.68 ± 1.62) and F + Sn + LPP (2.10 ± 0.95) did not differ from each other ($p > 0.05$), but presented lower SL than the other groups ($P < 0.05$). Apatite and stannous deposits on specimen surfaces were identified in the x-ray analysis for F + Sn and F + Sn + LPP.

Conclusions: Sodium fluoride solution exhibited no significant anti-erosive effect. The combination between sodium fluoride and stannous chloride reduced enamel erosion, irrespective of the presence of linear sodium polyphosphate.

Clinical significance: Under highly erosive conditions, sodium fluoride rinse may not be a suitable alternative to prevent enamel erosion. A rinse containing sodium fluoride and stannous chloride was shown to be a better treatment option, which was not further improved by addition of the sodium linear polyphosphate.

1. Introduction

Dental erosion is a complex condition that affects different age groups in populations worldwide [1]. The overall increase in its presence could be related to changes in lifestyles and nutritional habits, with a higher consumption of acidic foods and beverages [1,2]. In addition to avoiding exposure to erosive sources, the use of fluoridated products is highly recommended for patients with erosion [3]. However, their effectiveness against erosion seems to be dependent on the type of fluoridated compound, F concentration and pH. Many studies have tested the anti-erosive ability of F solutions containing metal cations, such as stannous (Sn), with promising outcomes [4–7]. Sn can incorporate into enamel through a complex process of demineralization and reprecipitation; it can also induce the surface deposition of acid-resistant precipitates [8]. In situ investigations have shown that a solution containing 500 ppm F and 800 ppm Sn was able to reduce

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enamel and dentin loss in the range of 45–67% and 47–68%, respectively [4,5].

Despite these positive results, studies have demonstrated that the protection offered by F and Sn can be increased by combining them with some polymers. A dentifrice containing F, Sn and the biopolymer chitosan showed improved enamel erosion protection compared with a dentifrice containing F + Sn alone [9]. A previous in vitro investigation by our group demonstrated that the addition of a phosphate polymer (sodium linear polyphosphate − LPP) could increase the protection of a solution containing 225 ppm F and 800 ppm Sn by 11% under highly erosive conditions [6], irrespective of the presence of simulated salivary pellicle [7].

The salivary pellicle is important when evaluating film-forming agents such as LPP, due to the possibility of competition for binding sites on the enamel surface [10]. The salivary pellicle formed in vitro is known to differ from the in situ because, among other changes, the proteins of the saliva collected can undergo alteration or degradation [11,12]. Considering this fact, this study sought to evaluate the protective effect of the combination of F + Sn + LPP against erosion under more clinically relevant conditions, such as those achieved in in situ models. Our hypothesis was that LPP would improve the protective effects of F + Sn against enamel erosion.

2. Materials and methods

2.1. Experimental design

This study consisted of a four-phase, single-blind crossover *in situ* clinical trial, involving 12 volunteers who met the inclusion/exclusion criteria described in detail elsewhere [13]. Briefly, the volunteers were at least 18 years old, with good general and oral health, without any allergy or any other condition that could compromise their safety. Their unstimulated and stimulated salivary flow rate had to be ≥ 0.5 ml/min and ≥ 1 ml/min, respectively. The exclusion criteria were: pregnancy (or intention to become pregnant) during the study period, nursing, concomitant participation in another research study, and inability to comply with study procedures. In each study phase, the volunteers used removable mandibular devices containing 4 specimens of bovine enamel. The study followed a completely randomized experimental design, with test solution as the single experimental factor, at 4 levels: C: Control (deionized water); F: Sodium fluoride solution (11.83 mM of NaF; 225 ppm of fluoride; pH 4.5); F + Sn: Sodium fluoride plus stannous chloride solution (11.83 mM of NaF + 10.75 mM of $SnCl_2$; 225 ppm F, 800 ppm Sn; pH 4.5); F + Sn + LPP: Sodium fluoride, stannous chloride and sodium linear polyphosphate solutions (11.83 mM of NaF + 10.75 mM of $SnCl_2$ + 2% of LPP; 225 ppm F, 800 ppm Sn; pH 4.5). The response variable was tooth surface loss, in μm, determined by optical profilometry at the end of the clinical phase. As an additional test, the surface of extra enamel specimens was evaluated by x-ray diffraction after treatments (n = 3).

2.2. Ethical aspects

This study was conducted in the Restorative Dentistry Department of School of Dentistry, University of São Paulo, São Paulo, SP, Brazil. The study protocol was reviewed and approved by the local Ethics Committee on Research with Humans (CAAE: 27621214.9.0000.0075). To participate in the study, all subjects had to sign a term of free and informed consent.

2.3. Sample size

For this *in situ* study, 12 subjects were recruited. This sample size was chosen based on a previous study [14] with a similar design, which showed significant difference between experimental groups using a sample size of 10 individuals.

2.4. Study population

The recruitment of the subjects was carried out in the Restorative Dentistry Department of School of Dentistry, University of São Paulo. First, the subjects were informed about the nature of the study, its possible risks and data confidentiality. After agreeing to participate, their medical and dental history was evaluated. Unstimulated and stimulated salivary flow rates were measured using established procedures, as previously described [13].

The subjects who met the inclusion/exclusion criteria received an oral hygiene kit containing a toothpaste (Colgate Cavity Protection, 1500 ppm F, Colgate-Palmolive, Osasco, SP, Brazil), a regular toothbrush (Colgate Twister Fresh, Colgate-Palmolive, Osasco, SP, Brazil) and dental floss, to be used on the 7 days before the study began (lead-in phase) and throughout the entire study period. They were not allowed to use any other oral hygiene products. Subjects were instructed to perform oral hygiene twice a day, with the oral appliance removed from the mouth. They were also advised not to brush their teeth with toothpaste in the 2 h prior the beginning of the experimental procedures, and also in the 30 min after eating.

All the eligible subjects were identified by a unique study number. In each week, they were randomly assigned to the treatment solutions according to a standard randomization table. Before the study began, the subjects were thoroughly trained in all experimental procedures, and they received a written protocol containing all the instructions. In each study phase, they also received a schedule and a digital timer to guide their conduct and recording of the experimental procedures.

2.5. Intraoral device

An impression of each subject's mandibular arch was taken with heavy consistency condensation silicone (Clonage®, DFL, Jacarepagua, RJ, Brazil). From the impressions, bi-lateral mandibular intraoral appliances were prepared with acrylic resin [15]. In these devices, four niches of approximately 4 mm x 4 mm x 2 mm were made on the buccal surfaces of the premolars and molars.

The intraoral devices were disinfected with 2% chlorhexidine solution for 10 min before and after each study phase, and rinsed with tap water. Before mounting the specimens in the appliances, they were sterilized with gamma radiation (Experimental irradiator Cobalt-60, Gamacell 220, IPEN, São Paulo, SP, Brazil). The day before each phase began, the sterilized specimens received adhesive unplasticized polyvinyl chloride (UPVC) tapes on their polished surface, leaving a central area of 3 mm x 1 mm exposed. The specimens were fixed in the 4 niches with sticky wax, so that their surfaces remained 1 mm below the appliance surface, to avoid abrasion of the buccal soft tissues.

2.6. Specimen preparation

Enamel specimens were prepared from bovine incisors that were firstly cleaned with periodontal curettes (Hu-Friedy, Chicago, USA), and subjected to prophylaxis with a mixture of pumice and water applied with rubber cup at low speed. The teeth were kept in 0.1% thymol solution (Sigma-Aldrich Co.), at 4 °C, until the experiment began. The crowns were separated from the roots. Then enamel specimens measuring 3 mm x 3 mm x 1.5 mm were sectioned from the buccal sides of the crowns, by using a precision cutting machine (Isomet 1000, Buehler Ltd, Lake Buff, Illinois, USA). The pulp surfaces of the specimens were flattened with a polishing machine (Buehler Ltd, Lake Buff, Illinois, USA), fitted with a #600 grit abrasive disc (Buehler Ltd), under constant water cooling. Subsequently, the buccal surfaces were ground flat and polished using a sequence of abrasive discs with decreasing granulations: #600, #1200, #2400 and #4000 (Buehler Ltd), and polishing cloth sprayed with diamond suspension (1 μm, Buehler Ltd) for 3 min. At the end of the polishing procedures, the specimens were sonicated with distilled water for 3 min. Specimens without any cracks or