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# Fluoride gel supplemented with sodium hexametaphosphate reduces enamel erosive wear *in situ*

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ABSTRACT

*Objective:* This study evaluated the effect of fluoride gels, supplemented or not with sodium hexametaphosphate (HMP), on enamel erosive wear *in situ*.
*Methods:* Twelve healthy volunteers wore palatal appliances containing four bovine enamel discs. Subjects were randomly allocated into four experimental phases (double-blind, crossover protocol) according to the gels: Placebo (no fluoride or HMP), 1% NaF, 2% NaF, and 1% NaF + 9% HMP. Enamel discs were selected after polishing and surface hardness analysis, and treated only once with the respective gels prior to each experimental phase. Erosion (ERO) was performed by extra-oral immersion of the appliance in 0.05 M citric acid, pH 3.2 (four times/day, five minutes each, 5 days). Additional abrasion (ERO + ABR) was produced on only two discs by toothbrushing with fluoridated dentifrice after ERO (four times/day, 30 s, 5 days). The specimens were submitted to profilometry and hardness analysis. The results were analyzed by two-way ANOVA and the Student–Newman–Keuls test ($p < 0.05$).
*Results:* The 1% NaF + 9% HMP gel promoted significantly lower enamel wear for ERO compared to the other groups, being statistically lower than 1% NaF and Placebo for ERO + ABR. Similarly, the lowest values of integrated lesion area were found for 1% NaF + 9% HMP and 2% NaF, respectively, for ERO and ERO + ABR.
*Conclusion:* The addition of HMP to the 1% NaF gel promoted greater protective effect against ERO and ERO + ABR compared to the 1% NaF gel, achieving similar protective levels to those seen for the 2% NaF gel.
*Clinical significance:* Gel containing 1% NaF + 9% HMP showed a high anti-erosive potential, being a safer alternative when compared to a conventional 2% NaF gel.



## 1. Introduction

Topical fluoride application has an inhibitory effect on dental erosion [1,2], which depends on the concentration of fluoride and the frequency of application. The addition of inorganic polyphosphate salts to fluoridated products has been shown to be effective in increasing their effectiveness against caries [3,4]. One of the most promising candidate is sodium hexametaphosphate (HMP), which is able to inhibit the formation of dental calculus [5], has antimicrobial action [6], and also prevents the formation of extrinsic stains [7]. HMP consists of a cyclic polyphosphate, presenting strong attraction to calcium in the hydroxyapatite in relation to other phosphates [8], being mixable in water and insoluble in organic solvents [9,10]. It is a variant of the longer pyrophosphate chain and presents greater possibility of binding to the enamel surface, protecting it from acid dissolution [11].

*In vitro* and *in situ* studies demonstrated that the association of fluoride and HMP in conventional dentifrices (1100 μg F/g) promoted greater protection against enamel demineralization compared to their counterparts without HMP [12–14]. A synergistic effect was also demonstrated when HMP was added to a dentifrice with reduced fluoride concentration (250 μg/g), achieving mineral loss values similar to those observed for the positive control (1100 μg F/g) [15]. Moreover, the addition of HMP to a dentifrice containing stannous fluoride promoted lower erosive wear in enamel and dentin compared to the positive control [16].

Concerning the addition of HMP to fluoridated gels, only one study has evaluated the effects of this association against dental caries *in vitro*, demonstrating that the 1% NaF gel supplemented with 9% HMP presented greater ability to inhibit enamel demineralization compared to a 1% NaF gel without HMP, as well

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as similar results to the 2% NaF gel [17]. However, the effect of gels containing fluoride and HMP on erosion has not yet been investigated. Considering that gels are widely used in dental practice and for normal oral hygiene in some countries, it would be interesting to analyze their effectiveness against erosion as an adjunct to normal oral hygiene with a fluoridated dentifrice.

Therefore, this *in situ*/*ex vivo* study analyzed the effects of fluoridated gels supplemented or not with HMP on enamel erosion, followed or not by abrasion. The null hypothesis was that the use of a fluoridated gel supplemented with HMP is not different from a gel with the same fluoride concentration without HMP in preventing erosive wear and mineral loss of enamel.

## 2. Material and methods

### 2.1. Volunteers' selection and ethical aspects

This study was approved by the Institutional Review Board of Araçatuba Dental School, UNESP (CAAE n. 5421712.2.0000.5420, report n. 154.616). Twelve healthy adult volunteers were selected [18], aged 20 to 33 years old (4 male, 8 female) living in areas with fluoridated water supply and presenting good general and oral health. Sample size was determined based on a previous study with similar methodology [19] considering the mean difference among the groups of 0.30 and standard deviation of 0.20 from enamel wear data, and an $\alpha$-error of 5% and a $\beta$-error of 20%. The inclusion criteria comprised normal salivary flow (1–3 mL/min, stimulated saliva, determined as described by Pessan et al. [20]) and no use of drugs that might interfere with salivary composition and flow [21]. The exclusion criteria were smokers, individuals presenting active caries lesions, receiving fluoride applications up to two weeks before the study, using antidepressants, narcotics, diuretics and antihistamine drugs in the last two months, submitted to radiation therapy, practicing water activities, working in environments polluted by compounds with low pH, and presenting systemic diseases (xerostomia, type I diabetes, autoimmune diseases, malnutrition, gastroesophageal problems, regurgitation disorders and vomiting). Exclusion criteria during the study comprised volunteer dropout, health changes with consequent alteration in salivary flow or need of antibiotics, utilization of mouthrinses or dentifrices different than those supplied by the investigators, or failure to follow the experimental design. If any volunteer was excluded, a new volunteer would be selected to complete all study phases. Before the experimental phase, the volunteers received written and verbal instructions on the study protocol, and were supervised to follow the study conditions. All volunteers used the same standard 1100 μg F/g dentifrice (NaF-1100 μg F/g-Sorriso Fresh Plus Gel, Colgate) throughout the study.

### 2.2. Experimental protocol

This *in situ*/*ex vivo* study was conducted in 4 experimental phases of 5 days each, with a lead in and wash out periods of 7 days. Volunteers wore palatal appliances containing 4 enamel discs, which were treated with Placebo, 1% NaF, 2% NaF and 1% NaF + 9% HMP gels, following a double-blind, crossover protocol. Enamel discs were subjected to *ex vivo* erosive challenges (immersion in 0.05 M citric acid), followed or not by abrasion by toothbrushing with a silica-based NaF toothpaste (1100 ppm F). Subjects were instructed to keep the palatal appliances in the mouth during all times throughout the experimental phases, except for eating and drinking and for performing oral hygiene procedures.

In each phase, all volunteers received a kit containing one tube of 1100 μg F/g dentifrice, one soft bristle toothbrush (Sorriso Kolynos Original Macia—Colgate), 5 disposable cups with visible indication of 50 mL, one orthodontic appliance case, gauze, one dropper bottle containing the dentifrice suspension (ratio 1 g of dentifrice: 3 mL of deionized water) and one liter of citric acid pH 3.2.

### 2.3. Preparation of enamel discs

Permanent mandibular central incisors were used, obtained from bovines aged 2 to 3 years and maintained in plastic flasks with 2% formalin solution pH 7.0 for 30 days [4]. Enamel discs ($n = 192$, approximate 4.5 mm diameter) were obtained from the flattest portion on the buccal surface of crowns, using a bur (DS 07-882457) connected to a bench drill (MOD. FGC-16, reamer 5/8). The discs were then marked with a round diamond bur to identify the cervico-occlusal direction. The dentin was adjusted (thickness ±2 mm) for achievement of parallel surfaces between enamel and dentin. Then, enamel surfaces were turned upwards, and polished with sandpaper grits 400 (20 s), 600 (30 s), 800 (30 s) and 1200 (30 s), under water cooling. The surface was then polished with polishing cloth (Polishing Cloth BUEHLER 40-7618) and diamond suspension (METADI Diamond Suspension 1 micron Blue Color Polish Spray, Water Base 40-653). Finally, the discs were rinsed with deionized water for 20 s and stored in a humid environment with gauze soaked in deionized water, for the initial enamel surface microhardness analysis [22–24].

### 2.4. Determination of initial enamel hardness (SHi) and sample selection

The surface microhardness of enamel discs was evaluated using the microhardness meter Shimadzu Micro Hardness Tester HMV-2.000 (Shimadzu Corporation—Kyoto—Japan) using Knoop indenters, static load of 25 grams and time of 10 s, connected to the image analysis software CAMS-WIN (NewAge Industries, USA).

For the initial surface microhardness (initial SH), five indents were made on the central region of the enamel disc, equidistant at 100 μm. Discs presenting mean hardness values within the confidence interval 353.7 (2.7) to 354.2 (1.4) were selected. The discs were identified according to their initial microhardness value.

### 2.5. Formulation and assessment of fluoride and pH in the experimental gels

The gels were prepared at the laboratory of Pediatric Dentistry of Araçatuba Dental School, São Paulo State University (Brazil), containing the following ingredients: carboxymethyl cellulose, saccharin, glycerin, mint oil and deionized water. The sodium fluoride concentrations were 1% and 2% (NaF, Merck, Germany). Ssodium hexametaphosphate (HMP, Sigma, USA) was added to the 1% NaF gel at a concentration of 9%, based on a previous study assessing the effects of gels on enamel demineralization in vitro [17]. Also, a gel without NaF or HMP was prepared and used as negative control (Placebo).

For fluoride analysis, approximately 100 mg of each gel was weighed and sequentially diluted in distilled/deionized water. Following, the flask contents were transferred to volumetric flasks and the volume was completed up to 100 mL with distilled/deionized water. Three dilutions were made for each product. Thereafter, two samples of 1 mL were obtained and buffered with TISAB II [25]. The solutions were then analyzed using a fluoride ion-specific electrode (9609 BN—Orion) connected to an ion analyzer (Orion 720 $A^+$), previously calibrated with the five standards (2.0; 4.0; 8.0; 16.0 and 32 μg F/mL). The total fluoride (TF) and ionic fluoride (IF) were analyzed. Data obtained in mV were converted into μg F/mL. The pH of the gels was assessed in