Effect of Fluoride Toothpaste Containing Nano-Sized Sodium Hexametaphosphate on Enamel Remineralization: An in situ Study

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Keywords
Dental enamel · Phosphate · Remineralization · Toothpaste · Nano-sized

Abstract

Objective: To evaluate the remineralizing potential of a conventional toothpaste (1,100 ppm F) supplemented with nano-sized sodium hexametaphosphate (HMPnano) in artificial caries lesions in situ. Design: This double-blinded crossed study was performed in 4 phases of 3 days each. Twelve subjects used palatal appliances containing 4 bovine enamel blocks with artificial caries lesions. Volunteers were randomly assigned into the following treatment groups: no F/HMP/HMPnano (Placebo); 1,100 ppm F (1100F); 1100F plus 0.5% micrometric HMP (1100F/HMP) and 1100F plus 0.5% nano-sized HMP (1100F/HMPnano). Volunteers were instructed to brush their natural teeth with the palatal appliances in the mouth for 1 min (3 times/day), so that blocks were treated with natural slurries of toothpastes. After each phase, surface hardness post-remineralization (SH2), integrated recovery of subsurface hardness (ΔIHR), integrated mineral recovery (ΔIMR) and enamel F concentration were determined. Data were submitted to analysis of variance and Student-Newman-Keuls’ test (\(p < 0.001\)). Results: Enamel surface became 42% harder when treated with 1100F/HMPnano in comparison with 1100F (\(p < 0.001\)). Treatment with 1100F/HMP and 1100F/HMPnano promoted an increase of \~23 and \~87\%, respectively, in ΔIHR when compared to 1100F (\(p < 0.001\)). In addition, ΔIMR for the 1100F/HMPnano was \~75 and \~33\% higher when compared to 1100F and 1100F/HMP respectively (\(p < 0.001\)). Enamel F uptake was similar among all groups except for the placebo (\(p < 0.001\)). Conclusion: The addition of 0.5% HMPnano to a conventional fluoride toothpaste was able to promote an additional remineralizing effect of artificial caries lesions.

Introduction

Fluoride toothpastes have made an important contribution on the reduction of dental caries prevalence in many industrialized countries [Rølla et al., 1991] and can be regarded as the best topical method, as it combines the disorganization of dental plaque with the therapeutic ef-
fants of fluoride (F) [Bratthall et al., 1996]. However, owing to the limited effect of these products on caries control, new strategies have been considered to enhance their efficacy in reducing caries in the most affected population groups [Carey, 2014]. Thus, the development of new toothpaste formulations to enhance anticaries effects has been investigated; formulations include the addition of inorganic phosphate salts [Danelon et al., 2015; Takeshita et al., 2015; Danelon et al., 2017]. Among these, micrometric sodium hexametaphosphate (HMP) has a strong affinity to the enamel surface because of multiple binding sites, resulting in a reduced mineral loss when associated with fluoride as demonstrated by da Camara et al. [2014; 2015; 2016].

Nano-sized phosphates (nano) have also emerged as an innovative method with the goal of optimizing the effect of fluoride toothpaste on the demineralization and remineralization processes [Danelon et al., 2015; Dalpasquale et al., 2017]. Dalpasquale et al. [2017] evaluated the effect of conventional toothpaste containing HMPnano at concentrations of 0.25, 0.5, and 1.0% in reducing enamel demineralization, showing that the addition of 0.5% HMPnano significantly enhanced the protective effect of the product. This improved performance is mainly because of physicochemical properties that make them more reactive when compared to micrometric particles [Xu et al., 2010]. This is very important also from a manufacturing perspective; previous studies reported difficulties when adding micrometric HMP at concentrations above 0.5%. The reduction of the particle size facilitates the addition of HMP as well as the manipulation of the formulations.

Given the positive results obtained by the addition of HMPnano with regards to enamel demineralization and considering the absence of studies assessing the effects of remineralization initial caries lesion, the aim of this study was to evaluate the effect of toothpastes containing 1,100 ppm F associated with HMPnano on enamel remineralization in situ. The study’s null hypothesis was that the effect of toothpaste on enamel remineralization would not be influenced by the addition of HMPnano.

Material and Methods

Experimental Design

This study was approved by the Human Ethical Committee of São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil (Protocol: 45716715.0.0000.5420). This was a double-blinded crossed in situ study performed in 4 phases of 3 days each [Danelon et al., 2015]. A sample size of 12 volunteers was based on previous studies [do Amaral et al., 2013; Danelon et al., 2015] considering primary outcomes from surface and cross-sectional hardness analysis, and the mean difference among groups (30 and 1,300, respectively), standard deviation (20 and 900, respectively), an α-error of 5% and a β-error of 20%. Volunteers aged 20–30 years, who were in good general and oral health [Delbem et al., 2005] presented normal salivary flow [Rios et al., 2006] and did not violate the exclusion criteria (use of any form of medication likely to interfere with salivary secretion, use of fixed or removable orthodontic appliances, being an active smoker or having systemic illness) were included in the study. No restrictions were imposed regarding the volunteer’s diet. All participants read and signed informed consent statements prior to study initiation. Enamel blocks were sequentially polished and selected by surface hardness test (SH; Fig. 1b). Blocks were demineralized (Fig. 1c) and submitted to post-demineralization surface hardness (SH1) testing (Fig. 1d). Surface hardness measurements (SH and SH1) were used to eliminate blocks with anomalous properties prior to further testing. Based on the percentage of SH loss (post-demineralization) blocks were divided into 4 treatment groups: no F/HMP/HMPnano (Placebo); 1,100 ppm F (1100F); 1100F plus 0.5% micrometric HMP (1100F/HMP); and 1100F plus 0.5% nano-sized HMP (1100F/HMPnano). After 3 days of the remineralization period (Fig. 1e), surface hardness post-remineralization (SH2; Fig. 1f) was again applied, integrated recovery of surface hardness (ΔSH; Fig. 1g) and integrated mineral recovery (ΔIMR; Fig. 1h), and enamel fluoride (F; Fig. 1k) concentration was also determined.

Processing and Characterization of Nano-Sized HMP

The processing and characterization of nano-sized HMP was based on the study by Dalpasquale et al. [2017]. Initially, 70 g of pure HMP (Na₂P₂O₇H₄, CAS 68915-31-1, average size of 31 ± 33 µm, purity ≥95%, Aldrich Chemistry, CAS 68915-31-1, United Kingdom) was ball milled using 500 g of zirconia spheres (diameter of 2 mm) in 1 L of hexane. After 48 h, the material was filtered and sealed with aluminium foil, and the vials were dried at 75 °C to evaporate the hexane. X-ray diffraction (XRD) was used to identify the crystalline structure and estimate the crystallographic coherence of the HMP, thereafter milled for 48 h (HMPnano). The X-ray diffractograms were obtained from samples in powder form using Shimadzu XRD 6.000 equipment with a CuK radiation source (λ = 1.5406 Å), voltage of 30 kV and current of 30 mA. Measurements were made continuously in the range of 10° ≤2θ ≤80° with a 2° scan speed/min. The structural identification of the samples was carried out by comparing the diffraction patterns obtained with tabulated patterns available in the databases, BJournal Committee on Powder Diffraction Standards – Powder Diffraction File (ICPDS – PDF). The particle morphology of HMP and HMPnano was analyzed by scanning electron microscopy (SEM). The SEM images were collected using a Philips XL-30 FEG.

Toothpaste Formulation and Fluoride and pH Assessment

Toothpastes were produced with the following components: titanium dioxide, carboxymethyl cellulose, methyl p-hydroxybenzoate sodium, saccharin, mint oil, glycerin, abrasive silica, sodium lauryl sulphate and deionized water. Toothpastes containing micrometric or nano-sized HMP were prepared (Aldrich Chemistry, CAS 68915-31-1, United Kingdom) at a concentra-
portion of 0.5% micrometric HMP (HMP) or nano-sized HMP (HMPnano). To these toothpastes, NaF (Vetec, Duque de Caxias, Rio de Janeiro, Brazil) was added to reach a concentration of 1,100 ppm F. In addition, toothpastes without F/HMP/HMPnano (Placebo) as well as with 1,100 ppm F (without HMP/HMPnano) were prepared. Toothpastes used in this study were stored at room temperature and kept properly closed to prevent any change in the samples.

Total fluoride and ionic fluoride concentrations were determined using a fluoride-specific electrode (Orion 9609-BN; Orion Research Inc., Beverly, MA, USA) connected to an ion analyzer (Orion 720 A+; Orion Research Inc.) [Delbem et al., 2009]. Three toothpastes per group were analyzed and the data were presented as micrograms of fluoride per gram of toothpastes. The pH levels of the toothpaste slurries were determined using a pH electrode (2A09E, Analyser, São Paulo, Brazil) calibrated with standard pH levels of 7.0 and 4.0.

Subsurface Enamel Demineralization

Enamel blocks were covered with a protective acid-resistant nail varnish (Risqué®, Brazil), applied on the sides (cut surfaces) and on the bottom of each block, except the enamel surface. Subsurface enamel demineralization was produced (Fig. 1b) by immersing each enamel block in 32 mL of a solution with 1.3 mmol/L Ca, 0.78 mmol/L P in 0.05 mol/l acetate buffer, pH 5.0; 0.03 ppm F; for 16 h at 37 °C [Queiroz et al., 2008].

Palatal Appliance Preparation and Treatments

This was a blind and cross-over in situ study previously approved by the Human Ethical Committee of Araçatuba Dental School, São Paulo State University, Brazil (Protocol: 45716715.0.0000.5420). Palatal appliances were prepared with acrylic resin (Jet-Articles Classic Odontológico, São Paulo, Brazil) as described by Danelon et al. [2015]. Twelve volunteers wore acrylic palatal appliances (Fig. 1e) with 4 demineralized enamel bovine blocks each that were subjected to 4 phases of 3 days each with a 7-day washout period among experimental phases [Danelon et al., 2015]. Treatment with toothpastes was performed 3 times per day with the palatal devices inside the volunteers’ mouths during their habitual oral hygiene routine. They were instructed to initially brush their natural teeth and conduct 3 brushing strokes in each row of enamel blocks on the oral appliance with the natural slurry (saliva/toothpaste) formed. Palatal appliances were employed at all times during each experimental phase (including during sleep) and were to be removed only during the main meals. During the 7-day pre-experimental period and washout periods, volunteers brushed their teeth with an F-free toothpaste. Volunteers received verbal and written instructions prior to the beginning of the study [Danelon et al., 2015].

Hardness Analysis

Enamel SH was determined before (Fig. 1a) and after the induction of subsurface lesions (SH1; Fig. 1d) as well as after each experimental phase (SH2; E) using a Shimadzu HMV-2000 mi-
crohardness tester (Shimadzu Corp., Kyoto, Japan) under a 25-g load for 10 s [Danelon et al., 2015]. Five indentations, spaced 100 µm from each other, were made at the centre of the enamel surface (SH). Indentations for (SH1) and (SH2; Fig. 1f) were spaced 100 µm from each other and from the baseline. For the cross-sectional hardness measurements, enamel blocks were longitudinally sectioned through their center (Fig. 1g) and embedded in acrylic resin (Fig. 1h) with the cut face exposed and gradually polished. One sequence of 14 indentations was created 100 µm apart at different distances (5, 10, 15, 20, 25, 30, 40, 50, 70, 90, 110, 130, 220, and 330 µm) from the outer enamel surface using a Microet 5114 hardness tester (Buehler, Lake Bluff, IL, USA) and the software Buehler OmniMet (Buehler) with a Knoop diamond indenter under a 5-g load for 10 s [Danelon et al., 2013]. The integrated area above the curve (cross-sectional profiles of hardness into the enamel), using the hardness values (KHN), was calculated by trapezoidal rule (GraphPad Prism, version 3.02) in each depth (µm) from the lesion up to sound enamel and subtracted from the integrated area of the post-demineralized enamel, resulting in the integrated recovery of subsurface hardness (ΔIHR; Fig. 1i).

Analysis of Enamel Mineral Concentrations

Enamel blocks (1 mm × 1 mm) of each group were analyzed by micro-computed tomography (MicroCT) operated at 70 kV, 142 mA, aluminium filter of 0.5 mm, with 1.5 mm of spatial resolution, a rotation step at 0.600º and random movement at 15. Projections of the images were rebuilt using NRecon software (version 1.6.10.2, Skyscan1272, Bruker MicroCT, Kontich, Belgium) and smoothing at 5, ring artefact correction at 7 and beam hardening correction at 52%. Following image reconstruction, two-dimensional virtual slices in the sagittal and coronal plane were acquired using Data Viewer software (Skyscan1272). Stacked two-dimensional virtual slices were imported into the ImageJ software to produce an overall mineral concentration (MC; g Hap × cm⁻³) profile as a function of the depth (µm; Fig. 3a). MCs were calculated from the linear attenuation coefficient and expressed
as the mass of pure hydroxyapatite (\( \rho = 3.15 \text{ g cm}^{-3} \)) per unit volume of tissue (\( \beta_{\text{HAp}} \times \text{cm}^{-3} \)) [Dowker et al., 2003; Dowker et al., 2004; Dalpasquale et al., 2017].

To analyze the patterns of remineralization, differential MC profiles were calculated by subtracting the MC values (\( \beta_{\text{HAp}} \times \text{cm}^{-3} \)) of the artificial caries enamel from those of the treated groups (i.e., artificial caries values minus the Placebo, 1100F, 1100F/HMP and 1100F/HMPnano group values) at each depth (Fig. 4b). The integrated area above the curve (differential cross-sectional profiles of MC into the enamel) was calculated by trapezoidal rule (GraphPad Prism, version 3.02) at each depth (\( \mu \text{m} \)) from the mineral recovery area up to sound enamel to yield of integrated mineral recovery values (\( \Delta \text{IMR} \); Fig. 1j).

Analysis of the F Concentration Present in Enamel

Enamel blocks (2 × 2 mm) were obtained from half of the longitudinally sectioned blocks and were fixed to a mandrel. Self-adhesive polishing discs (diameter, 13 mm) and 400-grit silicon carbide (Buehler) were fixed to the bottom of polystyrene crystal tubes (J-10; Injeplast, Sao Paulo, Brazil) and attached to a hand piece (N 270; Dabi Atlante, Ribeirão Preto, Sao Paulo, Brazil) fixed to the top of a modified microscope with a micrometre (Pantec, Sao Paulo, Brazil). One layer of enamel (51.3 ± 2.1 µm) was removed from each block (Fig. 1k) [Weatherell et al., 1985; Takeshita et al., 2009].

The vials, after the addition of 0.5 mL HCl 1.0 mol × L\(^{-1} \), were kept under constant stirring for 1 h [Weatherell et al., 1985; modified by Alves et al., 2007]. For F analysis, specific electrode 9409BN (Thermo Scientific, Beverly, MA, USA) and microelectrode reference (Analyser, Sao Paulo, Brazil) coupled to an ion analyzer (Orion 720A+, Thermo Scientific, Beverly, MA, USA) was used. Electrodes were calibrated with standards containing from 0.25 to 4.00 µg F/mL (100 ppm F, Orion 940907) under the same conditions as the samples. The readings were performed using 0.25 mL of the biopsy solution buffered with the same volume of TISAB II modified by NaOH. The results were expressed in µg/mm\(^3 \) [Akabane et al., 2018].

Results

The XRD pattern of 48-h HMPnano after milling shows broader peaks owing to smaller crystallites (Fig. 2). Figure 3a depicts the SEM images of HMP with large aggregates and particles of smaller sizes (average size of 31 ± 33 µm). Figure 3b shows the SEM images of HMPnano particles with low size distribution and an average size of 91 ± 34 nm.

The mean (SD) total fluoride and ionic fluoride (\( n = 3 \)) were 10.5 (0.1) and 10.0 (1.2) for the Placebo, 1,186.0 (33.2) and 1,102.4 (28.5) for 1100F, 1,168.3 (5.9) and 1,136.5 (42.6) for 1100F/HMP and 1,156.6 (19.7) and 1,100.9 (27.1) for 1100F/HMPnano. The mean (SD) pH value from the groups was 7.2 (0.3), ranging from 6.8 to 7.7.

The mean (SD) initial SH for all blocks was 374.0 (1.0), and the means varied between 371.0 (1.6) up 375.2 (2.0) KHN, with no significant differences among the groups after random allocation (\( p = 0.974 \)). The mean (SD) of SH after demineralization (SH1) was 57.0 KHN (3.7), ranging between 42.5 and 72.4 (\( p = 0.441 \)). The addition of

Statistical Analysis

The analysis was performed using SigmaPlot software (version 12.0, Systat Software Inc., San Jose, CA, USA) at a significance level of 5%. The variables SH2, ΔHHR, ΔIMR and F exhibited normal (Shapiro-Wilk test) and homogeneous (Cochran test) distributions and submitted to one-way, repeated measures analysis of variance, followed by Student-Newman-Keuls’ test. Pearson’s correlation coefficients between ΔKHN and \( \beta_{\text{HAp}} \times \text{cm}^{-3} \) were also calculated.
micrometric HMP to fluoride toothpaste increased the SH2 to approximately 45% when compared with the placebo group ($p < 0.001$) and similar to 1100F ($p > 0.001$). The treatment with 1100F/HMPnano led to SH2 $\sim 42$ and $\sim 41\%$ higher when compared to 1100F/HMP and 1100F toothpastes ($p < 0.001$) respectively. In addition, the capacity to reduce the lesion body ($\Delta$IHR) was $\sim 87\%$ higher with 1100F/HMPnano and $\sim 23\%$ 1100F/HMP ($p < 0.001$) when compared to 1100F (Table 1).

The $\Delta$IMR ($g_{HAp} \times cm^{-3} \times \mu m$) was higher ($\sim 74\%$) for the 1100F/HMPnano when compared to 1100F ($p < 0.001$) and 33% was higher compared to 1100F/HMPnano × 1100F/HMP ($p < 0.001$; Table 1). Figure 4a shows the enamel MC ($g_{HAp} \times cm^{-3}$) profile as a function of depth ($\mu m$), indicating a different profile for all treatments to a depth of 40 $\mu m$. Figure 4b shows the patterns of MC ($g_{HAp} \times cm^{-3}$) according to the treatment groups. The increased mineral content in the subsurface lesion was 1100F/HMPnano 1100F/HMP > 1100F > Placebo groups ($p < 0.001$). Positive and significant correlations were observed between $\Delta$IMR and $\Delta$IHR (Pearson’s $r = 0.720$; $p < 0.001$).

The addition of HMP and HMPnano to the F toothpaste did not influence enamel F concentration, so its effect was similar to 1100F except for the Placebo that featured the lowest concentration ($p < 0.001$; Table 1).

**Discussion**

The addition of nano-sized phosphates to topically applied fluoride products has been shown to significantly enhance their effect on enamel remineralization and demineralization [Danelon et al., 2015; Danelon et al., 2017; Dalpasquale et al., 2017]. Results showed that the addition of 0.5% HMPnano to 1100F led to superior remineralization effects when compared to a conventional toothpaste without HMP or supplemented with HMP micro. Thus, the null hypothesis was rejected. The short-term in situ model used was based on previous studies [Afonso et al., 2013; Danelon et al., 2015] and allows comparing the formulations regarding their potential to boost the remineralization of artificial caries lesions. Lesions induced with this model were shallow ($\sim 50 \mu m$; Fig. 4a), being able to observe enamel remineralization in a shorter time (compared with deeper lesions) as well as a dose-response relationship [Afonso et al., 2013]. It is desirable that a product has the ability to remineralize a mineral loss in a short time, especially when there is high frequency of exposure to sucrose.

The determination of the ideal HMPnano concentration was based on an in vitro study [Dalpasquale et al., 2017] demonstrating that the supplementation of a 1,100 ppm F toothpaste with 0.5% of HMPnano promoted a superior protective effect against enamel demineralization compared to a conventional toothpaste without HMP. As HMP associated to 1,100 ppm F did not enhance enamel F uptake [Dalpasquale et al., 2017; da Camara et al., 2015; da Camara et al., 2016], the superior results with nano-sized HMP is probably due to the higher reactivity of these particles [Dalpasquale et al., 2017]. Given that the anticaries effect of fluoride toothpastes (i.e., without HMP/HMPnano) is usually related to the ability to increase enamel fluoride concentrations, it can be concluded that the mechanism of action of HMP/HMPnano containing fluoride toothpastes is somehow different from products containing fluoride only. Fur-

**Table 1. Mean (SD) values of hardness and fluoride analysis according to the toothpastes**

<table>
<thead>
<tr>
<th>Toothpastes</th>
<th>Variables</th>
<th>SH2 (KHN)$^1$</th>
<th>$\Delta$IHR (vol% $\mu m$)$^2$</th>
<th>$\Delta$IMR ($g_{HAp} \times cm^{-3}$)$^3$</th>
<th>F ($\mu g/mm^3$)$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td>111.2 (7.0)$^a$</td>
<td>1,505.2 (557.5)$^a$</td>
<td>6.3 (3.0)$^a$</td>
<td>0.24 (0.04)$^a$</td>
</tr>
<tr>
<td>100F</td>
<td></td>
<td>160.3 (4.9)$^b$</td>
<td>2,431.9 (227.2)$^b$</td>
<td>11.2 (2.0)$^b$</td>
<td>0.43 (0.09)$^b$</td>
</tr>
<tr>
<td>1100F/HMP</td>
<td></td>
<td>161.7 (5.7)$^b$</td>
<td>2,987.5 (347.9)$^c$</td>
<td>14.7 (3.3)$^c$</td>
<td>0.44 (0.10)$^b$</td>
</tr>
<tr>
<td>1100F/HMPnano</td>
<td></td>
<td>228.0 (0.8)$^c$</td>
<td>4,560.7 (585.0)$^d$</td>
<td>19.6 (5.8)$^d$</td>
<td>0.44 (0.09)$^b$</td>
</tr>
</tbody>
</table>

Distinct superscript lowercase letters a–d indicate statistical significance among groups in each variable (1-way ANOVA, Student-Newman-Keuls test, $p < 0.001$).

1 SH2: surface hardness after each experimental phase – KHN.
2 $\Delta$IHR: integrated loss of subsurface hardness – KHN × $\mu m$.
3 $\Delta$IMR: integrated mineral loss – $g_{HAp} \times cm^{-3}$.
4 F, fluoride concentration in enamel – $\mu g/mm^3$. 

F-Toothpaste with Nano-Sized HMP Enhances Enamel Remineralization
thermore, the procedure used to synthesize HMPnano promoted more reactive particles, with increased adsorption on enamel, as observed by Dalpasquale et al. [2017]. Nano-sized HMP showed to be effective in the remineralization of artificial caries lesions in comparison with 1100F, than micro-sized HMP (Table 1). However, greater reactivity can change its effect on the dynamics of demineralization and remineralization when added at concentrations higher than 0.5% HMPnano [Dalpasquale et al., 2017] or a concentration of HMP over 1% [da Camara et al., 2016]. Recently, a study showed that the adsorption of HMP provides more binding sites to calcium phosphate, and promotes supersaturation of these ions close to the enamel surface [Neves et al., 2018], what explains the capacity of HMP in increasing enamel mineral recovery (Table 1 and Fig. 1). As nano-size is more reactive than micrometric particles, the concentration of HMP in the product can be reduced in half without reducing its remineralizing action. Despite the above-mentioned situation, it may still be argued that HMP nanoparticles might agglomerate within the toothpaste formulation, what could potentially have important implications regarding long-term stability, so that specific analyses of the toothpastes containing HMPnano over time could be instructive. Nonetheless, it should be emphasized that hardness and microCT data obtained provide irrefutable evidence on the enhanced remineralizing effect of HMPnano added to a conventional fluoride toothpaste.

The ability of HMP to induce enamel remineralization in depth had not yet been demonstrated when associated to 1100 ppm F. ΔIHR and ΔIMR values (Table 1) showed the capacity of HMP to enhance a mineral recovery throughout the lesion body (Fig. 4). This effect is greater using the salt in its nano-sized form (~87 and ~78%, respectively, for ΔIHR and ΔIMR) when compared 1100F. To produce this effect, HMP facilitates the diffusion of ions calcium and phosphate into the enamel [da Camara et al., 2015], without any significant effect on enamel fluoride uptake, as previously mentioned. HMP is negatively charged cyclic phosphate that leads to larger amount of electron-donor sites on the enamel surface and, consequently, enhances the adsorption of ionic species as Ca$^{2+}$, H$_2$PO$_4^-$ and CaH$_2$PO$_4^-$ [Neves et al., 2018], which are important in enhancing fluoride, calcium and phosphate diffusion into enamel. This seems to occur more intensively with HMP in its nano-sized form, given that enamel MC increased ~ 33% compared with micrometric particles. A similar finding was arrived at by Danelon et al. [2015], showing that nano-sized TMP increased mineral gain by 44% in relation to its micrometric counterpart, mainly in terms of depth, concluding that the use of nano-sized particles is a strategy that promotes effective remineralization of caries lesions. Although cross-sectional hardness was used to analyze the mineral loss in depth in the above-mentioned study, the present experiment showed a good correlation between cross-sectional hardness (ΔIHR) and MC (ΔIMR) assessed by MicroCT. Both methods were capable of analyzing the mineralization patterns throughout the shallow subsurface lesions induced. ΔIHR and ΔIMR were also shown to be strongly correlated in an in vitro study assessing the effects of HMP on enamel demineralization [Dalpasquale et al., 2017].

When a remineralizing agent is used in the clinical setting, its action is expected to take place within the shortest length of time possible. However, as in vivo demineralization and remineralization processes depend on multiple factors, the accurate determination of the length of time for in situ protocols becomes a difficult task. Therefore, a number of important variables should be considered before determining the experimental period of an in situ study. The type of substrate and depth of the artificial caries lesion seem to be the most important. Bovine enamel has a higher reactivity and porosity, leading to faster remineralization when compared to human enamel [Lynch et al., 2006]. As for the substrate, the depth of enamel demineralization may also interfere with the amount of remineralization time; however, few investigations have considered the depth of the demineralized area in their protocols. It is known that the remineralization process is slower in deep lesions (±100 µm) based on a longer distance for ion diffusion when compared to that seen in the present study [Mellberg, 1991]. Another factor allowing fast remineralization of the 3-day protocol, after using the toothpastes, was the type of lesion. This type of lesion presents faster remineralization rates based on the higher number and diameter of lesion pores, being suitable to compare the efficacy of different remineralizing regimens [Lynch et al., 2006]. Based on previous studies and confirmed by the present results, it is known that the association of HMPnano and F reduces mineral loss, and its effect may be explained by the adsorption of HMPnano on enamel, even with the presence of biofilm on the enamel surface.

To sum up, the addition of HMPnano to a 1,100 ppm F toothpaste promoted a significantly greater mineral recovery compared with a conventional toothpaste supplemented with micrometric HMP or without HMP using a short-term in situ model. Although this approach shows promise, the results should be analyzed with caution, especially considering the need to determine the effects of
this toothpaste in deeper lesions. Therefore, other studies as well as clinical researches should be performed to achieve more conclusive findings.

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References


Disclosure Statement

The authors Marcelle Danelon, Alberto Carlos Botazzo Delbem, Juliano Pelim Pessan and Emerson Rodrigues de Camargo hold a patent request for a product used in the study by the National Institute of Industrial Property – INPI/SP, on 10/17/2014 under number BR 10 2014 025902 3.

Author Contributions