

Novel fluoride and stannous -functionalized β -tricalcium phosphate nanoparticles for the management of dental erosion

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ARTICLE INFO

Keywords:

Tooth erosion
Enamel
Dentin
Fluoride
Stannous
 β -TCP nanoparticles

ABSTRACT

Objective: To evaluate the anti-erosive effect of solutions containing β -tricalcium phosphate (β -TCP) nanoparticles functionalized with fluoride or with fluoride plus stannous on enamel and dentin.

Methods: β -TCP nanoparticles were synthesized and characterized by X-ray diffraction (XRD) and transmission electron microscopy (TEM). Sixty enamel and dentin specimens were randomly allocated into the groups ($n = 10$): Control (water); F (NaF, 225 ppm F⁻); F + Sn (NaF + SnCl₂, 800 ppm Sn²⁺); F + β -TCP (F + 40 ppm β -TCP); F + Sn + β -TCP (F + Sn + 40 ppm β -TCP); F + Sn + 100 β -TCP (F + Sn + 100 ppm β -TCP). Specimens underwent erosion-remineralization cycling (5 min immersion into 1 % citric acid solution and 60 min exposure to artificial saliva, 4 \times /day, 5 days). Immersion in the test solutions was performed for 2 min, 2 \times /day. Surface loss (SL, in μ m) was determined by optical profilometry at the end of cycling. Data were analyzed using one way-ANOVA and Tukey's tests ($\alpha = 0.05$).

Results: XRD confirmed the β -TCP phase. TEM micrographs showed differences between the bare nanoparticle and the β -TCP functionalized with F and Sn. All enamel groups presented lower SL than the control, with F + Sn, F + Sn + β -TCP, and F + Sn + 100 β -TCP showing the lowest values. For dentin, all the groups had lower SL than the control. F + β -TCP presented the lowest SL, significantly differing from all the other groups.

Conclusion: β -TCP nanoparticles functionalized with fluoride showed improved anti-erosive effect compared to the fluoride solution on dentin. There was no significant effect of the β -TCP nanoparticles functionalized with fluoride plus stannous in both substrates.

Clinical Relevance: β -TCP nanoparticles are a promising agent to be added to oral health products to improve the protective effect of fluoride against dentin erosion.

1. Introduction

Erosive tooth wear can influence the long-term health of the dentition [1]. This condition is associated with loss of the hard dental tissues by the chemical action of dietary or stomach acids. The enamel demineralizes, resulting in a softened superficial layer that can be removed due to prolonged demineralization or by the impact of cyclical mechanical forces [2]. Studies have shown that as demineralization progresses, minerals from dentin are removed, exposing the organic components [3]. This process slows erosion since the acids will have to

diffuse through the organic layer to reach the minerals located underneath [4].

Patient education and preventive measures are recommended to control the progression of erosive tooth wear [5]. Frequent application of fluoride-containing products is recommended to manage the progression of this condition [6]. The mechanism proposed to explain the protective effect of fluoride in erosion is the formation of a globular CaF₂-like layer, which, by its dissolution under erosive conditions, temporarily protects the underlying dental tissue [7]. When using polyvalent metal fluorides, i.e. stannous fluoride, either a coating of

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metal-rich surface precipitates or a metal-rich surface layer (Sn incorporate into the dental surface) is formed, which is more acid-resistant [8]. Promising results were obtained when stannous fluoride or the combination of fluoride and stannous (as different salts) was tested, although they are not able to completely inhibit tissue loss caused by the erosive process [9–12].

Although surface protection provided by the CaF₂-like deposits has been proposed as the primary anti-erosive mechanism of conventional fluoridated compounds, the amount of bioavailable calcium in oral fluids may limit the deposition of these compounds [13]. Thus, to potentially enhance the fluoride’s protection against dental erosion, the use of a calcium pre-rinse before the fluoride rinse would increase the calcium availability in the oral environment, allowing for deposition of higher amounts of CaF₂-like compounds [13,14]. Besides calcium, phosphates would also play an essential role in the stability of CaF₂-like materials, reducing its solubility [15]. In the presence of low pH values, the phosphate groups would bind to the protons thus releasing fluoride to the environment [16]. Therefore, it would be interesting to have fluoride, calcium and phosphate ions in the same material.

Studies suggest that high concentrations of calcium and fluoride in the same formulation decreases the bioavailability of fluoride because these ions would have a premature reaction [17,18]. It was demonstrated that the addition of calcium plus fluoride in dental materials could lead to the opposite outcome- by the formation of insoluble compounds such as calcium fluoride [19]. For that, novel compounds have been tested to deliver available calcium, phosphates, and fluoride ions, in order to improve the formulation of oral care products. Among the new compounds, β-tricalcium phosphate (β-TCP) particles can act as a source of mineralizing components. It is a calcium phosphate system with reduced solubility in comparison to other calcium salts and minerals, improving the fluoride compatibility in water-based preparations [20]. Although the use of β-TCP seems to be promising, only a few studies have provided data regarding the interaction between β-TCP and fluoride, and fluoride + stannous for the treatment of erosive tooth wear. Two previous in situ studies dealing with the remineralization of eroded enamel observed that the use of a rinse containing 225 ppm F and 40 ppm F of functionalized β-TCP induced more remineralization than a solution with a similar amount of fluoride, but without the functionalized β-TCP [21,22]. Another study employing an erosion cycling model observed that, after 20 days of cycling, a solution containing 225 ppm fluoride plus 20 ppm of a functionalized β-TCP agent promoted more protection than a solution containing fluoride only [22].

Considering the promising effects of β-TCP on dental erosion, the present study aimed to evaluate the anti-erosive effect of novel nanoparticles of beta-tricalcium phosphate (β-TCP) functionalized with fluoride or with fluoride plus stannous on human enamel and dentin. The tested hypotheses were: (1) the β-TCP nanoparticles functionalized with fluoride and with fluoride + stannous would show improved protection on enamel erosion than its respective solutions without the β-TCP nanoparticles, and (2) the β-TCP nanoparticles functionalized with fluoride and with fluoride + stannous would show improved protection on dentin erosion than its respective solutions in absence β-TCP nanoparticles.

2. Material and methods

2.1. Synthesis of experimental beta-tricalcium phosphate powder

β-TCP powder was synthesized by the co-precipitation method, wherein the phosphoric acid (H₃PO₄, Synth – Brazil) diluted in MilliQ water (0.3 M) was slowly dropped (8 mL./min rate) into a suspension of calcium hydroxide (Ca(OH)₂, Synth- Brazil) and magnesium hydroxide (Mg(OH)₂, Synth Brazil) (0.5 M). The initial (Ca + Mg)/P ratio was fixed at 1:5. After the precipitation and aging processes, the solution was filtered and washed several times with MilliQ water to remove free

ions and then dried at 60 °C overnight. Finally, the dried powder was heat-treated at 1000 °C for 30 s, using an homemade adapted microwave oven [17].

2.2. β-TCP functionalization

The concentration of the β-TCP nanoparticles was determined in preliminary tests (not shown). The F⁻ and Sn²⁺ concentrations used were chosen based on previous studies [18,19]: 225 ppm F⁻, as NaF, and 800 ppm Sn²⁺, as SnCl₂.

For the β-TCP functionalization with fluoride and/or stannous ions, 40 ppm or 100 ppm of β-TCP were dispersed in 100 mL of MilliQ water. The suspension was sonicated in a water bath for 5 min. A second solution was prepared dissolving 2.3 g/L of gluconic acid sodium salt (Acros Organic; Geel, Belgium) in 400 mL of MilliQ water for stability purposes. The F⁻ solutions were prepared by adding a concentration of 0.497 g/L of sodium fluoride into the second solution (NaF; Sigma-Aldrich, St. Louis, MO, USA). For the Sn²⁺-containing solutions, stannous chloride (SnCl₂; Sigma-Aldrich, St. Louis, MO, USA) was used at a concentration of 1.277 g/L. After the complete salts’ dissolution, the last solution was added to the β-TCP suspension and the mixture was sonicated for an extra 5 min. The pH of all the solutions, except the water (negative control), was adjusted to 4.5, with HNO₃ solution, to eliminate pH as bias [18] (Table 1).

2.3. X-ray diffraction

Powder X-ray diffraction (XRD) patterns for β-TCP were collected by a Multiflex Rigaku diffractometer using the Cu Kα radiation.

2.4. TEM micrographs (TEM) and energy dispersive spectroscopy (EDS)

The bare β-TCP nanoparticles were dispersed in absolute isopropanol and dropped on a copper grid to be observed by transmission electronic microscopy (JEM 2100 - JEOL), and to be qualitatively analyzed by energy dispersive spectroscopy (EDS).

2.5. Specimen preparation

The study protocol was approved by the local Ethics Committee in Research of the University of São Paulo, School of Dentistry (process #2.585.009). The teeth were stored in 0.1 % thymol solution at 4 °C until the beginning of the experimental procedure. Sixty dentin and enamel slabs (3 mm × 3 mm × 1.5 mm) were cut from roots and crowns of human third molars (lingual and buccal surfaces) using a cutting saw machine under constant irrigation (Isomet 1000 Buehler Ltd, Lake Buff, Illinois, EUA). The test surfaces of the slabs were ground flat and polished using aluminum oxide papers (grits #600, #1200 and #4000) under water-cooling. Between each paper and at the end of the polishing procedures, the specimens were sonicated in distilled water for 3 min, in order to remove debris.

The specimens were then submitted to profilometric analysis to

Table 1
Study design and experimental groups.

Group	Experimental Solution
W	Control (MilliQ water)
F	Sodium fluoride solution (NaF; 225 ppm F ⁻ ; pH = 4.5)
F + Sn	Sodium fluoride (NaF; 225 ppm F ⁻) + Stannous chloride (SnCl ₂ ; 800 ppm Sn ²⁺) solution; pH = 4.5
F + β-TCP	40 ppm β-TCP plus F solution
F + Sn + β-TCP	40 ppm β-TCP plus F + Sn solution
F + Sn + 100β-TCP	100 ppm β-TCP plus F + Sn solution

Experimental unit: enamel and dentin specimens (n = 10 for each substrate).
Response variable: surface loss (in μm) evaluated post cycling.

Table 2
Daily cycling sequence used in the erosion-remineralization model.

	Sequence	Procedures
1	Erosive Challenge	5 min (1 % citric acid)
	Re-mineralization	30 min (artificial saliva)
	Treatment	2 min (experimental solution)
2	Re-mineralization	30 min (artificial saliva)
	Erosive Challenge	5 min (1 % citric acid)
	Re-mineralization	60 min artificial saliva
3	Erosive Challenge	5 min (1 % citric acid)
	Re-mineralization	60 min (artificial saliva)
4	Erosive Challenge	5 min (1 % citric acid)
	Re-mineralization	30 min (artificial saliva)
	Treatment	2 min (experimental solution)
	Re-mineralization	30 min (artificial saliva)

select those with a surface curvature < 0.3 μm. This analysis was performed with an optical profilometer (Proscan 2100; Scantron, Venture Way, Tauton, UK). 120 specimens with no fractures or other visual imperfections, and with initial curvature lower than 0.3 μm were selected. Unplasticized polyvinyl chloride (UPVC) tapes were then placed on the polished surfaces of selected specimens, leaving a central window of 3 × 1 mm exposed for subsequent testing. The specimens were randomly allocated into 6 experimental groups according to the test solutions.

2.6. Erosive cycling

Enamel and dentin slabs (n = 10) were fixed to the lids of 12-well cell culture plates using sticky wax. Table 2 illustrates the daily sequence of the 5-day erosion-remineralization model used. Briefly, the specimens were immersed for 5 min in 1 % citric acid solution (pH ~2.4), followed by a 60 min immersion in artificial saliva (0.213 g/L CaCl₂·2H₂O; 0.738 g/L KH₂PO₄; 1.114 g/L KCl; 0.381 g/L NaCl; 12 g/L Tris Buffer, pH adjusted to 7.0 with concentrated HCl solution). Thirty min after the first and fourth erosive challenges, the specimens were immersed into the experimental solutions for 2 min. After erosion and before saliva exposure, the specimens were rinsed for approximately 20 s with distilled water and gently dried with absorbent paper. The citric acid and the test solutions were renewed after each exposure. Artificial saliva was renewed once a day at the beginning of the cycling (see Table 2). All the procedures were performed at room temperature (~ 24 °C) and without agitation. The specimens were kept in 100 % relative humidity condition at 4 °C until the beginning of the cycling, between the days of cycling, and until the profilometric test was performed.

2.7. Enamel and dentin surface loss assessment

Surface loss (SL) was measured by an optical profilometer (Proscan 2100) at the end of the 5 days of erosive cycling. For the readings, the UPVC tapes were removed from the specimens' surfaces, and a central area of 2 mm long (X) × 1 mm wide (Y) was scanned. The length covered both the treated area and reference surfaces. The step size was set at 0.01 mm and the number of steps at 200 in the (X) axis; and at 0.1 mm and 10, respectively, in the (Y) axis. The depth of the treated area was calculated based on the subtraction of the average height of the test area from the average height of the two reference surfaces by using the dedicated software (Proscan Application software v. 2.0.17). For this measure, a 3-point height tool was applied. In order to avoid collagen shrinkage, dentin specimens were analyzed in a moistened condition [21].

2.8. Statistical analysis

Surface loss data from enamel and dentin were analyzed independently. For both substrates, data were checked for normal distribution and homoscedasticity with Shapiro-Wilks and Brown-Forsythe tests, respectively. Since both statements were satisfied, data were analyzed with one way-ANOVA and Tukey tests. The significance level was set at 5 %. The software SigmaPlot 13.0 was used for the calculations.

3. Results

Fig. 1(A) illustrates the results of the EDS analysis, confirming the presence of Sn and F ions, besides Ca and P; and (B) Spherical aggregations of β-TCP functionalized with F + Sn sized between 150 and 300 nm. Fig. 2 (A) TEM micrograph, in which an aggregation of small nanometric particles in the range of 10–20 nm can be observed; and (B) shows the X-ray diffraction of the bare β-TCP shows the single beta-tricalcium phosphate phase of the nano powder

The means and standard deviations (SD) of enamel surface loss (in μm) for the experimental groups are shown in Fig. 3. Group F + Sn had the lowest SL, not significantly differing from the F + Sn + β-TCP and F + Sn + 100β-TCP groups. All groups, in turn, differed from the negative control. The means and standard deviations (SD) of dentin surface loss (in μm) for the experimental groups are shown in Fig. 4. Group F + β-TCP had the lowest SL, being significantly different from all the other groups. All groups presented lower SL than the negative control.

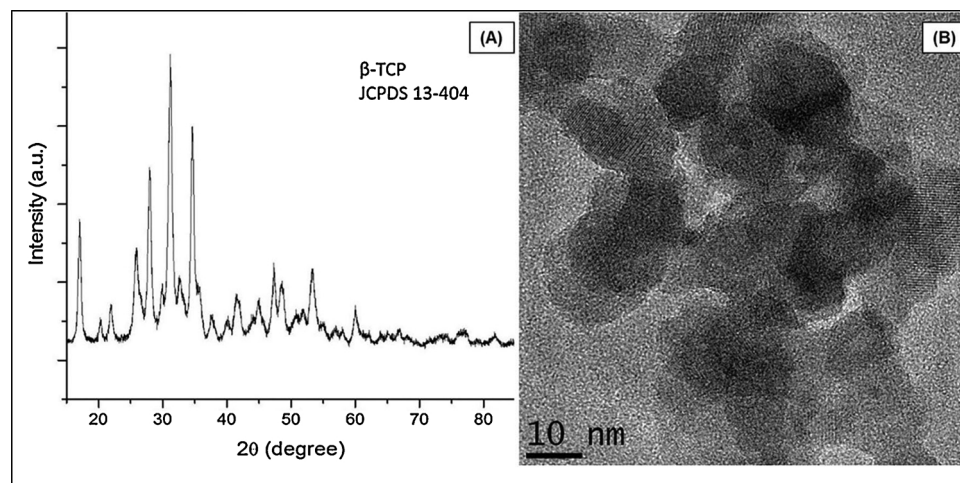


Fig. 1. (A) XRD confirming the presence of Sn and F ions; and (B) Spherical aggregations sized between 150 and 400 nm of β-TCP functionalized with F + Sn.

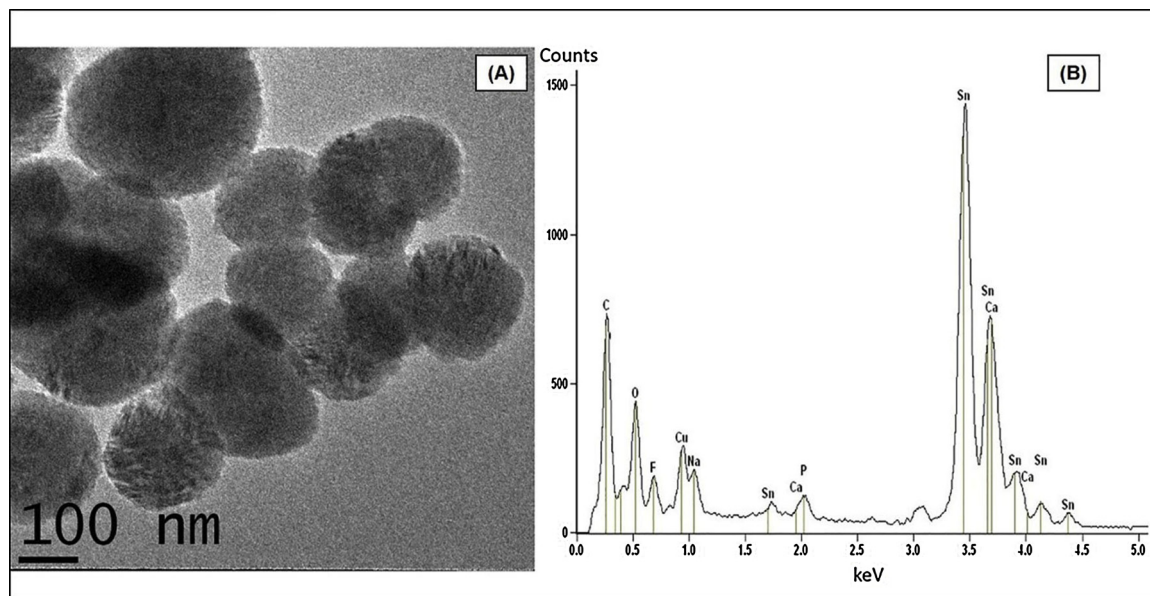


Fig. 2. (A) Transmission electron microscope (TEM) analyses of the bare β -TCP nanoparticle showing an aggregation of small nanometric particles in the range of 10–20 nm; and (B) EDS analysis of the bare β -TCP showing the single beta-tricalcium phosphate phase of the nano powder.

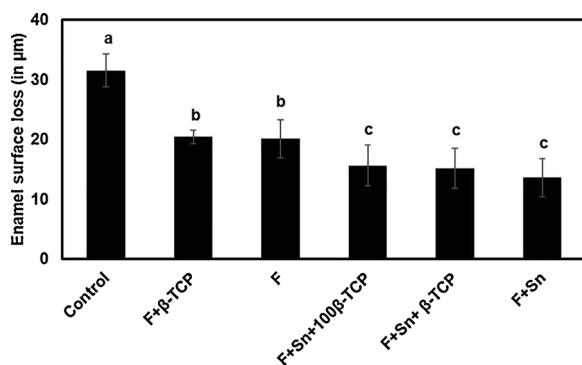


Fig. 3. Mean (SD) of enamel surface loss (in μm) for the experimental groups. Different letters denote statistical difference between groups ($p < 0.05$).

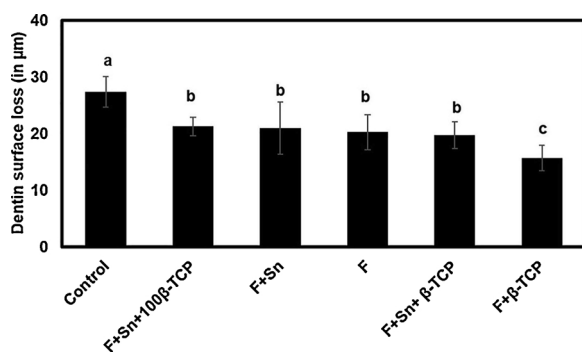


Fig. 4. Mean (SD) of dentin surface loss (in μm) for the experimental groups. Different letters denote statistical difference between groups ($p < 0.05$).

4. Discussion

The characterization of the bare β -TCP powder by XRD (Fig. 1) confirms the presence of pure β -TCP phase, which is comprised of nanometric spherical particles (~ 10–20 nm) that forms agglomerates in aqueous solution (TEM image). After its complexation with fluoride and stannous ions, the functionalized β -TCP showed a new conformational structure stabilized as spherical nano-sized particles in the range of

150–300 nm. The elemental qualification by energy dispersive spectroscopy (EDS) corroborates the composition of the functionalized β -TCP sample, composed by Sn, F, in addition to Ca and P ions. The experimental solutions were stable during the entire experiment assay (5 days), with no particle’s precipitation visible to the naked eye.

On enamel, the β -TCP particles functionalized with fluoride or fluoride and stannous did not show improved erosion protection when compared to the F and the F + Sn solutions without β -TCP. Thus, our first hypothesis was rejected. This result is in disagreement with a previous investigation, where a synergistic anti-erosion effect on enamel was observed between fluoride and β -TCP particles functionalized with urea and silica [23]. According to the authors, β -TCP particles functionalized with silica and urea can promote improved erosion protection [24], because the former agent would bind to the tooth defects in acidic environments, and the latter can penetrate within the enamel encouraging increased calcium, phosphate, and fluoride uptake by this substrate [25]. Despite the different particles tested on these studies, the use of distinct study models can also explain the contrary results. Whereas in this previous investigation, the specimens were treated with the solutions three times daily for twenty days, in the present study, the treatment was performed twice daily for five days. This prolonged and higher exposure to the treatments could have enhanced its benefits, by a cumulative action of the agents. Moreover, this study evaluated surface loss at the end of the cycling, as opposed to surface and longitudinal hardness measurements of the previous study, which could also implicate different outcomes depending on the magnitude of the lesions created by the challenges. It is also worth mentioning that the erosion model employed in the present study was more prone to demineralization, and that many investigations are supporting the synergistic effect between fluoride and β -TCP in the context of remineralization of the eroded substrate [26–28]. The supplementation of fluoride solutions with functionalized β -TCP is thought to increase fluoride-based nucleation activity, which would be then followed by remineralization [24]. Although we intended to test whether the presence of a calcium source would promote increased CaF_2 -like material deposition on the tooth surfaces, thereby resulting in additional surface protection, it could be suggested that the aggressiveness of erosive challenges performed left only small room for mineral deposition, reducing one of the effects of β -TCP on this substrate.

The F + Sn solution presented the best results for enamel, showing the lowest SL, not significantly differing from F + Sn+ β -TCP and

F + Sn + 100 β -TCP. The protective effect of fluoride plus stannous-containing solutions on the enamel erosion has been demonstrated in many *in vitro* and *in situ* studies [9–12]. This can be attributed to a complex reaction that forms less soluble precipitates, such as Sn₂OHPO₄, Sn₃F₃PO₄, and Ca(SnF₃)₂ [29]. Also, under cyclic conditions of erosion and remineralization, stannous was shown to incorporate within a few micrometers into the enamel surface (10–20 μ m), increasing its resistance. Likewise, some studies have also shown a protective effect with the F solution against erosion, although it is usually limited and less pronounced than F + Sn [9,11].

For dentin, β -TCP nanoparticles functionalized with fluoride ions (40 ppm of β -TCP) were able to promote the highest anti-erosion protection—more so than the F solution. However, the addition of the β -TCP nanoparticles functionalized with fluoride + stannous, at either 40 or 100 ppm, was not able to show superior protection compared to the F + Sn solution. Thus, our second hypothesis was partially accepted. Considering that surface protection is the primary mechanism of action of fluorides against erosion [6], it can be suggested that the β -TCP nanoparticles functionalized with fluoride allowed an increased deposition of CaF₂-like material on the dentin surfaces (due to higher calcium availability), therefore resulting in higher protection against the acid challenges. Such effect was predominantly observed for dentin, possibly because dentin contains a higher organic phase with the capacity to retain large amounts of CaF₂-like material [30,31]. β -TCP can increase fluoride's ability to promote surface protection and mineral deposition at the eroded substrate [26,27]. In demineralized dentin, mineralization mostly takes place by the growth of residual crystals [32]. In the present study, the organic matrix of dentin was not enzymatically removed. This organic portion of dentin was also found to be relevant to the anti-erosive effect of fluoride [32]. Thus, it could be assumed that the functionalized β -TCP nanoparticles could diffuse within this organic layer, acting on the mineral portion of dentin by supplying fluoride, calcium, and phosphate as needed at a deeper level.

There is an indication that, in dentin, the presence of stannous might play a reduced role in erosion protection than in enamel, which resulted in the similar behavior presented by F and F + Sn solutions on this substrate. A similar result was also observed previously [18,33]. Perhaps for dentin, the increased deposition of CaF₂-like material promoted by functionalized β -TCP nanoparticles was more relevant than the presence of stannous for a significant anti-erosive effect. A similar explanation previously given for enamel could also help to explain the lack of difference between the F + Sn and F + Sn + β -TCP solutions.

One limitation of the present study was the lack of reproduction of some important clinical aspects, such as the role of the plaque and the soft oral tissues as additional sources for fluoride retention. These tissues and components act as a reservoir, slowly releasing fluoride for the improvement of the treatment [34,35]. Another point that needs to be addressed is the use of profilometry to measure surface loss in demineralized dentin where the organic matrix is present. To avoid the shrinkage of this matrix, caution was taken to scan the specimens in standardized moist conditions. Nevertheless, when evaluating our results, it should be taken into consideration that clinically, this organic matrix is likely to be degraded by enzymatic action. The model used by the present study followed the guidelines designed for dental erosion studies [36]. Prior to conducting the study, pilot tests (not shown) were performed in order to find the best concentration of the functionalized β -TCP to be added without affecting the stability of the solution. Based on these results and previous reports [26,27], it was opted to use two distinct concentrations of β -TCP (40 ppm and 100 ppm functionalized with F + Sn). The concentration of 40 ppm has shown higher effectiveness in preventing erosion.

The new, functionalized β -TCP used in the present work was synthesized as nanoparticles (Fig. 1). The thermal treatment made the particles more spherical, homogeneous, and stable. The ability to devise a calcium and phosphate material in the nanoscale that can coexist with fluoride, and that has the potential to provide greater efficacy in

comparison to fluoride ions alone, presents an opportunity to improve the therapeutic effects of fluoride, enhancing its dental health benefits. The β -TCP nanoparticles functionalized with fluoride could present numerous advantages for dental care. The material is biocompatible and bioactive [37]; an available source of calcium and phosphate related to its higher solubility when compared to other calcium salts and minerals; and can coexist and act synergistically with fluoride ion in aqueous solutions, enhancing the protective benefits in comparison with fluoride alone [24,26]. Considering the promising results observed with the β -TCP nanoparticle functionalized with fluoride on dentin, further studies are still necessary to make this agent suitable for enamel erosion protection. Modifications to be tested include the evaluation of different concentrations of fluoride and β -TCP particles, different concentrations of stannous, as well as different β -TCP particle sizes. Lastly, this innovative product would be suitable for patients at risk of erosion in the form of mouthwash, a self-administrated product that can be used daily and at a high frequency.

5. Conclusion

The novel β -TCP nanoparticles functionalized with fluoride was the most effective agent for the control of dentin erosion. For enamel, the β -TCP nanoparticles functionalized with fluoride did not show improved erosion protection when compared to the fluoride solution. There was also no significant superior effect of the β -TCP nanoparticles functionalized with fluoride + stannous in both substrates.

Declaration of Competing Interest

The authors declare that they have no compete of interests.

CRediT authorship contribution statement

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Acknowledgements

This work was supported by the National Council for Scientific and Technological Development [CNPq - process#141068/2018-3]; and the Sao Paulo Research Foundation [FAPESP - process#2015/15629-7].

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