Comparative Effect of Calcium Mesoporous Silica Versus Calcium and/or Fluoride Products on the Reduction of Erosive Tooth Wear and Abrasive Enamel Lesion

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Academic Editor: Alessandro Leite Cavalcanti

Received: 02 March 2020 / Accepted: 20 April 2020 / Published: 25 July 2020

How to cite this article: Justino IBS, Alexandria AK, Canto FMT, Leite KLF, Vieira TI, Cabral LM, et al. Comparative effect of calcium mesoporous silica versus calcium and/or fluoride products on the reduction of erosive tooth wear and abrasive enamel lesion. Pesqui Bras Odontopediatr Clin Integr. 2020; 20:e0025. https://doi.org/10.1590/pboci.2020.146

Abstract

Objective: To evaluate the effect of a calcium nanocompound on the reduction of erosive tooth wear and abrasion. Material and Methods: Bovine enamel specimens (BE), were randomly assigned to the following groups (n = 10): G1 = Calcium mesoporous silica nanoparticles (Ca⁺⁺-MSNs); G2 = casein phosphopeptide-amorphous calcium phosphate (CPP-ACP, 2% CPP-ACP, GC®); G3 = casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP, 2% CPP-ACP + 900 ppm F, GC®); G4 = sodium fluoride NaF (900 ppm F⁻, positive control); and G5 = distilled and deionized water (negative control). Each product was applied to the exposed area for one minute, three times per day for three consecutive days, and followed by the immersion of the specimens in Sprite Zero™ - a low-pH solution (2.58) for five minutes (Coca-Cola™). After the first and last erosive challenges of the day, the specimens were submitted to abrasion in a toothbrush machine for 15 seconds (200 g/BE). The specimens were analysed using 3D non-contact optical profilometry, with tooth structure loss (TSL) measurements and scanning electron microscopy (SEM). TSL values were analysed by Kruskal-Wallis and Mann-Whitney tests (p<0.05). Results: There were no significant differences between G1 (10.95 µm) and G3 (10.80 µm) treatments for TSL values; however both resulted in significantly reduced TSL values compared with the G5 (16.00 µm) (p<0.05). The G4 (12.26 µm) showed no statistically significant difference when compared to the G5 (16.00 µm). The groups G1 and G3 presented higher surface preservation than the G5. Conclusion: Ca⁺⁺-MSNs was effective for reducing tooth surface loss caused by erosive tooth wear and abrasion.

Keywords: Tooth Erosion; Tooth Abrasion; Sodium Fluoride; Calcium.
Introduction

The erosive tooth wear is a process of cumulative loss of tooth structure through a chemical-mechanical process where this dissolution is not caused by bacteria [1,2]. The erosive tooth wear leads to the reduction of microhardness and the softening of the dental surface, which consequently becomes more susceptible to disruption by mechanical impacts [1,3].

Dental abrasion is a clinical condition of pathological tooth structure loss caused by mechanical forces applied to the teeth [3]. More significant levels of tooth wear are caused by the abrascivity of toothpastes than by the mechanical force applied by the toothbrush. Because the tooth structure becomes softened, brittle and more susceptible to wear after the erosive process begins, abrasion becomes more critical, even in cases where toothpaste is not used for brushing [4].

Regarding the preventive effect of different toothpastes abrasivity in an initial erosion-abrasion model, products that containing fluoride exhibited better efficacy for permanent and deciduous teeth [5]. Fluoride compounds have great preventive and therapeutic effects against tooth mineral loss [6,7], due to the formation of fluorapatite, which has significantly lower solubility than hydroxyapatite, and the formation of calcium fluoride (CaF$_2$) [1,8]; CaF$_2$ can act as a physical barrier that prevents the penetration of acids into the underlying enamel layer, and an increasing number of studies have reported that this effect also protects against erosive tooth wear [9-11].

Other compounds have been studied, either alone or in combination with fluoride, in the search for better protection against tooth demineralisation [12,13]. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a nanocomplex that promotes the stabilization of high levels of calcium (Ca) and phosphate (PO$_4$), providing a source of bioavailable ions for use during the remineralisation process [14,15]. In addition, CPP-ACP nanocomplexes can interact with fluoride ions to produce an amorphous calcium fluoride phosphate (ACFP) phase, which can provide an additional benefit due to the presence of fluoride within the nanocomplex [13,16].

Mesoporous silica nanoparticles (MSNs) have also been recently studied in combination with varying dental materials. The structural and chemical properties of MSNs facilitate the aggregation of multiple ions and drugs, which can be selected according to the pore size of the nanoparticles or be immobilized on the nanoparticle surface [17,18]. MSNs have a high pore volume and a large surface area, which enables the association of large amounts of molecules or ions [17]. Thus, as a high-standard carrier, mesoporous silica can increase the bioavailability of aggregated nanoparticles [19], such as calcium, and potentialize the remineralisation process.

Due to the increased incidence of non-carious lesions, resulting from changes in eating habits and the increased consumption of acidic drinks [20,21], there has been a growing demand for studies of substances that are capable of reducing erosive tooth wear. Therefore, this study aimed to evaluate the effect of reducing the enamel dissolution with the use of new mesoporous calcium silica nanoparticles (Ca$_2^+$-MSNs) in vitro after erosion-abrasion challenges, evaluating the loss of tooth structure (TSL). The null hypothesis of the present study is that the application of mesoporous silica nanoparticles to dental enamel does not minimize the loss of tooth structure (TSL) after an erosive and abrasive challenge compared to common products used in dental practice.

Material and Methods

Preparation of the Specimens
Fifty specimens (4×4 mm) were prepared from bovine incisors that were properly stored in a 2% formaldehyde solution. The dental enamel blocks were cut using an Isomet low-speed saw cutting machine (Buehler Ltd., Lake Bluff, Illinois, United States) with two diamond discs (Extec Corp., Enfield, Connecticut, United States), which were separated by a 4-mm-thick spacer. Then, the specimens were polished by using water-cooled silicon carbide paper 600, 800 and 1,200 (Extec Corp., Enfield, Connecticut, United States). The specimens were selected according to superficial microhardness using a microhardness tester (Micromet 5104; Buehler, Mitutoyo Corporation, Tokyo, Japan) containing a Knoop diamond (50 g, 5 s, 3 indentations spaced 100 mm apart). Enamel blocks with the overall average of 321.39 (Kg/mm²) ± 10% were selected for the experiment.

Half of the surface of each specimen was covered with an acid-resistant nail varnish (Colorama, L’Óreal, Clichy, France) to create an unexposed area (control side) that could be compared with the area that was exposed to the erosive/abrasive challenge (experimental side).

Group Allocation
The enamel blocks were randomly allocated among the following groups (n = 10): G1, Ca²⁺MSNs; G2, CPP-ACP slurry (2% CPP-ACP, GC America Inc., Alsip, USA); G3, CPP-ACFP slurry (2% CPP-ACP, 900 ppm F⁻, GC America Inc., Alsip, USA); G4, NaF (900 ppm F⁻, positive control); and G5, water (Milli-Q®, negative control).

A blinded researcher used a pipette to precisely apply 50 µl of each treatment to the corresponding specimens. The treatment remained on the specimens for 1 minute. Then, the samples were rinsed with distilled and deionized water (Milli-Q®). The treatment was performed 3x/day, for three consecutive days, with a two-hour interval between each treatment.

Experimental Protocols
The sample size of 10 specimens was calculated by BioEstat software version 5.3 (Instituto de Desenvolvimento Sustentável Mamirauá, Belém, PA, Brazil), using an α-error level of 5% and a β-error level of 20%, based on previous data, for the detection of a 10% difference in the loss of superficial microhardness [22].

Ca²⁺MSNs suspension was prepared at Laboratório de Tecnologia Industrial Farmacêutica, Faculty of Pharmacy, Federal University of Rio de Janeiro. The CPP-ACP, CPP-ACFP treatments are commercially available as toothpastes were prepared as slurries (toothpaste/deionized water, 1:3 w/w) and NaF.

The specimens were submitted to three days of erosion/abrasion cycles, and the treatments were always performed just before to an erosive/abrasive challenge. Each product was applied to the exposed area for one minute using a pipette (100 µl) three times per day for three consecutive days, with an interval of 2 hours of each treatment on the same day and after treatment, the surfaces were washed with deionized water. The cycle of the erosion challenge was performed in the previously treated blocks using freshly opened bottles of Sprite Zero (pH 2.58, 30 mL/specimen; Coca-Cola Company, Atlanta, Georgia, USA), 4 times daily, for 5 min each [22]. After the erosion protocol, the specimens were rinsed in distilled and deionized water for 5 s.

The specimens were also abraded twice daily, using a mechanical toothbrush machine (Buehler Ltd., Lake Bluff, Illinois, United States) and fresh slurries (0.5 mL/specimen) containing unfluoridated toothpaste (toothpaste/water ratio 1:3; Daut Company, Rio de Janeiro, RJ, Brazil) for 15 s (50 strokes/s), with a weight of 200 g [22], after the first and last erosive challenges each day. After each cycling the specimens remained for
two hours in artificial saliva until the next cycle, and after the last daily cycle, the specimens were stored in artificial saliva (1.5 mmol/L of calcium, 0.9 mmol/L of phosphate, 0.15 M of potassium chloride, Tris buffer, and 0.05 µg of fluoride/mL) overnight at 37°C. (Table 1).

| Products       | Composition                                                                 |
|----------------|-----------------------------------------------------------------------------|
| Ca²⁺-MSNs      | 1g of mesoporous silica doped with calcium powder to 100 mL of Milli-Q® water |
| CPP-ACP        | Pure Water, Glycerol, CPP-ACP (2% CPP-ACP and 900 ppm F⁻), D-Sorbitol, Silicon Dioxide, CMC-Na, Propylene glycol, Titanium dioxide, Xylitol, Phosphoric acid, Guar gum, Zinc Oxide, Sodium Saccharin, Ethyl p-hydroxybenzoate, Propyl p-hydroxybenzoate |
| CPP-ACFP       | Pure Water, Glycerol, CPP-ACP, D-Sorbitol, Silicon Dioxide, CMC-Na, Propylene glycol, Titanium dioxide, Xylitol, Phosphoric acid, Sodium fluoride, Guar gum, Zinc Oxide, Sodium Saccharin, Ethyl p-hydroxybenzoate, Propyl p-hydroxybenzoate |
| NaF            | Calcium Carbonate, Water, ethyl alcohol, Sodium bicarbonate, Sodium Lauryl Sulfate, Sodium Monofluorophosphate (900 ppm F⁻) and Glycerin |
| Unfluoridated Toothpaste | Cellulose Gum, Glycerin, Malva Sylvestris (Mallow) Extract Silica, Sodium Benzoate, Sodium Lauroyl Sarcosinate, Sucrose, Xylitol, Flavor, CI 45430, Aqua |
| Artificial Saliva | 1.5 mmol/L of calcium, 0.9 mmol/L of phosphate, 0.15 M of potassium chloride, Tris buffer, and 0.05 µg of fluoride/mL |

3D Non-Contact Profilometry Analysis

The acid-resistant nail varnish was removed from the surfaces of all samples with acetone P.A. to allow the analysis of experiment results.

The loss of tooth structure (TSL) in µm was analyzed by measuring the height difference between the experimental area exposed to the erosive-abrasive challenge and the unexposed area, using 3D non-contact chromatic confocal chromatic prophylometry (Nanovea PS50 Optical, Nanovea Inc., Irvine, CA, USA). The height differences between the unexposed area and the treated area, after removing the resistant acid varnish, were measured. For correct positioning of the blocks on the profilometer, 1 mm was measured for each side of the center of the block. With this, three measurements were performed with a distance of 100 µm from each. The areas were measured in µm, with the average of the three TSL measurements for each group. Measurements were performed using a chromatic confocal sensor, with an axial source of white light, a scanning speed of 2 m / s and a refractive index of 10,000. 3D profilometry images were also taken to allow the assessment of the topographic characteristics of the superficial enamel at the end of the experiment. In the images, the blue color indicates an increased amount of TSL, while the red color indicates less TSL \[22\].

Scanning Electron Microscopy (SEM) Analysis

Two enamel blocks from each group were randomly selected and prepared for SEM analysis (6460LV; JEOL, Tokyo, Japan) of the qualitative changes to the enamel outer layer and for comparing the two areas of study. The blocks were fixed on stubs with double-faced carbon tape and covered with a 30-µm gold layer. The specimens were examined with the same SEM system operating at 20 kV in a low-vacuum mode (45 Pa). Observations were carefully performed through the enamel and images were acquired in a standard magnification of 2,000×.

The researchers who carried out the analyzes in profilometry (TSL) and in SEM were blind, they did not know which product contained in each group, as well as for the statistical analyzes (Figure 1).
Figure 1. Study flowchart.

Statistical Analysis

The non normal distribution of the data was verified using the Shapiro-Wilk test. The statistical analysis of TSL was performed using the nonparametric Kruskall-Wallis test, followed by the Mann-Whitney U test (p<0.05). SEM and 3D profilometry images were descriptively analyzed.

Results

Only the Ca\textsuperscript{2+} MSNs and CPP-ACF treatments were able to significantly reduce TSL after the erosion/abrasion challenge compared with the negative control (p<0.05). There were no significant differences in TSL among the Ca\textsuperscript{2+} MSN, CPP-ACP, CPP-ACFP and NaF treatments (p>0.05). TSL values for the CPP-ACP and NaF treatments were not significantly different from that for the negative control (p>0.05) (Table 2).

Table 2. Median (maximum and minimum) TSL values for the different treatments.

| Treatments       | Median (Maximum and Minimum) | p-value* |
|------------------|-----------------------------|----------|
| Ca\textsuperscript{2+} MSNs | 10.95 (20.93/9.21) \textsuperscript{a} | 0.018    |
| CPP-ACP          | 12.56 (18.38/10.92) \textsuperscript{ab} | >0.05    |
| CPP-ACFP         | 10.80 (27.06/8.98) \textsuperscript{a} | 0.026    |
| NaF              | 12.26 (12.99/8.19) \textsuperscript{ab} | >0.05    |
| Water (Negative Control) | 16.00 (52.19/11.63) \textsuperscript{b} |          |

Kruskal-Wallis and Mann-Whitney tests; Different letters represent statistical difference (p<0.05); *The p-value represents a statistical difference comparing the products with the negative control.

The 3D images generated by the profilometry analysis are shown in Figure 2. The greatest difference between the unexposed and experimental areas was observed for the G5 group, which was evident based on the colour variability of the image. Colour variability was less evident in images from the other groups compared with the negative control. The same features were observed in the images obtained by SEM analysis (Figure 3); the G5 group showed the greatest differences between the unexposed and experimental areas. Samples treated with CPP-ACP and NaF presented increase in pore depth and areas with more massed surface and
small depressions. The images with fewer signs of porosity and surface alterations correspond with samples from the groups treated with CPP-ACFP and Ca²⁺MSNs.

Figure 2. Image and graphical representation of the surface enamel samples after treatments and erosive/abrasive challenge. (A) Calcium mesoporous silica nanoparticles, (B) CPP-ACP, (C) CPP-ACFP, (D) NaF and (E) Negative control. The number 1 represents the unexposed area, and the number 2 represents the exposed area (treatment and erosive/abrasive challenge).

Figure 3. Surface SEM images of the enamel samples after treatments and erosive/abrasive challenge, at a magnification of 500X. (A) Calcium mesoporous silica nanoparticles, (B) CPP-ACP, (C) CPP-ACFP, (D) NaF and (E) Negative control. The number 1 represents the unexposed area, and the number 2 represents the area exposed to the erosive/abrasive challenge.
Discussion

This study compared the ability of Ca$^{2+}$-MSNs to protect against erosive tooth wear/abrasion with those of other commonly used products with calcium and/or fluoride. The Ca$^{2+}$-MSNs demonstrated a similar degree of effectiveness as CPP-ACFP and performed better than the control group at reducing TSL.

Silica nanoparticles have been demonstrated to have the capability to deliver associated molecules such as EGCG (epigallocatechin-3-gallate-encapsulated) – Nano-hydroxyapatite, Ca and P with good control \([23,24]\). In the present study, the mesoporous silica nanoparticle was used as a novel and high-standard vehicle to increase the bioavailability of calcium molecules and to reduce the erosive tooth wear. This capacity was demonstrated by the positive results obtained from the Ca$^{2+}$-MSNs treated group. The present authors believe that the presence of calcium favoured the reduction of TSL, by the current MSN presenting a high loading capacity of calcium. One may suppose that the calcium available in the MSN could be delivered in the interface among eroded enamel surface and the oral environment created in vitro and therefore being responsible for less erosive loss. Moreover, MSN presents a reasonably low-degradable rate, which means a more stable compound \([25]\).

The CPP-ACFP treatment also demonstrated a protective capability to minimize the effects of erosive tooth wear and abrasion. This protective capability is likely due to the effect facilitated by the addition of fluoride to the nanocomplex, while the ability to reduce tooth TSL against erosive tooth wear combined with abrasion has also been described in the literature for CPP-ACP/NaF varnish (5% NaF, MI Varnish™, GC America, Alsip, IL, USA) \([22]\).

The use of CPP-ACFP resulted in better protection from TSL than the use of nonfluoridated CPP-ACP, which was not able to reduce the abrasion-associated erosive tooth wear, suggesting that the calcium concentration of CPP-ACP was not sufficient enough to act effectively in minimize the effects of erosive tooth wear.

Although both CPP-ACP and CPP-ACFP are stable nanocomplexes that behave as vehicles for calcium and phosphate, only CPP-ACFP has fluoride ions incorporated into its composition \([26]\). Thus, the better performance observed for CPP-ACFP is likely due to the bioavailability of both fluoride and calcium. This beneficial result with the use of CPP-ACP with fluoride is also due to a great affinity for the calcium present in the saliva to form calcium fluoride (CaF$_2$). Products containing calcium and fluoride in their composition, according to recent studies, have formed protective layers to prevent acid contact with the enamel surface and, consequently, the loss of dental tissue \([27]\).

During the de- and remineralization processes, fluoride can be incorporated into hydroxyapatite, making the dental enamel more resistant to the dissolution of minerals \([8]\). In addition, the potential remineralization effects of CPP-ACP on teeth with erosion lesions, either with or without dental abrasions, were observed previously in in situ studies \([18,28]\).

Although the non-fluoridated CPP-ACP does not have the stability provided by fluoride, it has good results in in vitro studies against dental caries because the technology in this nanocomplex adheres to plaques and gradually releases the calcium and phosphate ions onto the tooth surface \([13]\) NaF has been used for the prevention and treatment of mineral loss due to the formation of CaF$_2$, which acts as a physical barrier for the protection of dental structures \([1,8]\). However, in the present study, the observed TSL for the NaF-treated samples was similar to that observed in the control group. This may be due to the 900 ppm fluoride concentration used in the present study, which was below the 1,100 ppm concentration that has been shown to be effective for preventing erosive tooth wear; 900 ppm was selected to allow the concentration of NaF to be similar to the fluoride concentration used in the fluoridated CPP-ACP \([20,29-32]\).
However, studies that used fluoride pastes showed its effectiveness in reducing the loss of tooth structure by erosive tooth wear abrasion, due to its association with the cytosan compound, since the molecule forms stable multilayer at acid pH, resulting in less enamel loss. Furthermore, the presence of this polysaccharide forms multilayers on the enamel surface, since it binds to mucin present in saliva [27].

In this study, Sprite Zero™ was chosen for its ability to provoke erosive processes, reflecting the etiology of noncarious lesions diagnosed in clinical practice and simulating a clinical situation wherein the patient consumes acidic drinks every day. Other in vitro studies have also used the soft drink to promote erosive challenges [33] and in erosive/abrasive challenges [22,27,34]. The use of a control group demonstrated the validity of erosive cycling, with blocks that did not receive treatments having much higher TSL values compared with the other groups.

A toothbrush machine was used to promote the abrasive process after erosion in vitro to simulate tooth brushing after the consumption of an acidic beverage [35]. Although the literature reports that abrasive toothpastes are the primary abrasive agents [4] other agents with less abrasive potential, such as toothbrushes and tongue friction, can promote tooth wear due to the increased brittleness of dental structures after exposure to acids [36] 3D noncontact profilometry was chosen as the method to analyze the results because it is the most commonly used option in studies that perform erosion and abrasion in vitro. In addition, 3D noncontact profilometry does not cause the destruction of the studied blocks and boasts the optimal sensitivity for surfaces that present different levels of height, a characteristic that was utilized in this study to quantify the TSL of the experimental areas [22,36,37]. To complement the 3D noncontact profilometry analysis, which facilitates the measurement of height differences between the control and experimental sides, photomicrographs were obtained by SEM. Thus, it was also possible to qualitatively evaluate the surface alterations on the exposed area of the specimens in response to the erosive/abrasive challenge and to their respective treatments.

Although the in vitro model can be considered a limitation of our study, since it does not fully simulate the oral cavity, artificial saliva and soft drink were chosen to make the experiment as similar as possible with the oral cavity and clinical situation. Another possible limitation would be the comparison of products in the form of solution and toothpaste, as they have different viscosities, however the making of slurries was performed to mimic toothpastes already diluted in the oral cavity.

The null hypothesis of the present study was rejected, since the new product had a positive effect in minimizing the loss of tooth structure when compared to commonly used products, after erosion-tooth abrasion challenges.

Conclusion

This study, which aimed to evaluate the use of Ca²⁺MSNs in reducing the loss of tooth enamel after challenges of erosion and abrasion, comparing with commonly used products, concluded that Ca²⁺MSNs obtained results in minimizing the loss of tooth structure (TSL) similar to a product already marketed as CPP-ACFP, showing less signs of change in topography and changes in surface, showing better results compared to positive and negative control. Future in situ and in vivo studies using Ca²⁺MSNs should be performed to demonstrate its protective effects and ultimately support its use in clinical practice.

Authors' Contributions

IBSJ 0000-0003-1718-7070 Investigation, Formal Analysis, Writing – Original Draft Preparation and Writing – Review and Editing.
Conflicts of Interest

This study was financed in part by the PIBIC, by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Grant No. 303535/2016-4) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ (Process Number E-26/202.924/2017 and E-26/201.865/2018).

Financial Support

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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