The use of a new calcium mesoporous silica nanoparticle versus calcium and/or fluoride products in reducing the progression of dental erosion

Abstract

Objective: There is increasingly common the consumption more times a day of foods and acidic drinks in the diet of the population. The present study aimed to evaluate and compare the effects of a calcium mesoporous silica nanoparticle single application of other calcium and/or fluoride products in reducing the progression of dental erosion. Methodology: Half of the eroded area was covered of 60 blocks of enamel, after which the block was submitted to the following treatments: (Ca\textsuperscript{2+}-MSN), casein phosphopeptide–amorphous calcium phosphate (CPP-ACP); CPP-ACP/F-(900 ppm F\textsuperscript{-}); titanium tetrafluoride (TiF\textsubscript{4} 1%) (positive control); sodium fluoride (NaF 1.36%) (positive control); and Milli-Q® water (negative control) before being submitted to a second erosive challenge. A surface analysis was performed via a three-dimensional (3D) noncontact optical profilometry to assess the volumetric roughness (Sa) and tooth structure loss (TSL) and through scanning electron microscopy (MEV). An analysis of variance (ANOVA) and Tukey’s test were performed. Results: Regarding Sa, all experimental groups exhibited less roughness than the control (p<0.05). The TSL analysis revealed that the Ca\textsuperscript{2+}-MSN and NaF groups were similar (p>0.05) and more effective in minimizing tooth loss compared with the other groups (p<0.05). Conclusions: The Ca\textsuperscript{2+}-MSN and NaF treatments were superior compared with the others and the negative control.

Keywords: Tooth erosion. Mesoporous silica nanoparticles. Fluoride compounds. Tooth remineralization.

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\textsuperscript{1}Submitted: March 2, 2020
\textsuperscript{2}Modification: May 2, 2020
\textsuperscript{3}Accepted: May 19, 2020

J Appl Oral Sci. 2020;28:e20200131
Introduction

Teeth can be exposed to acidic compounds during normal daily activities, especially during the consumption of soft drinks and juices, while taking medications with an acidic composition, or after being subjected to intrinsic acids such as esophageal reflux, these compounds can cause an irreversible and progressive loss of tooth enamel.\(^4\) As a result, many clinical consequences arise, including tooth sensitivity or the loss of dental structure around the restorations, which can lead to a gap that may eventually progress to dentin exposure.\(^3\)

For these reasons, products exist to help minimize the evolution of existing erosion lesions. Fluorides are the adjuncts most commonly used to prevent further enamel structure loss, as they form a protective layer of calcium fluoride (CaF\(_2\)) over the enamel surface.\(^3\) This CaF\(_2\) layer serves as a mineral reservoir on the enamel surface and, in cases of demineralization, it is the first to be dissolved.\(^4\) Other fluoride products such as titanium tetrafluoride (TiF\(_4\)) act by forming a titanium oxide film that prevents erosion.\(^5,6\) In addition, researchers have explored the use of calcium-based products such as CPP-casein phosphopeptide, and CPP-ACP/F\(^{-}\) in the remineralization of dental enamel. CPP provides calcium and phosphate at the enamel surface and which acts in the demineralization-remineralization process.\(^7,8\) Amorphous calcium phosphate, when placed in an acidified pH solution, separates from the CPP, leaving the phosphate and calcium ions to interact with the dental enamel, helping to prevent mineral loss.\(^3\)

Mesoporous silica has great research relevance in the area of health, and with wide application in the field of biomaterials, since it has a great capacity to incorporate molecules within its numerous pores present in its structure and release them in a sustained manner.\(^10\) Positive results have previously been found following use of a novel calcium mesoporous silica nanoparticle (Ca\(^{2+}\)-MSN) to prevent dental caries (unpublished data). In this formulation, silica is present as a mesoporous nanocomposite with high adsorption and is thus able to incorporate gradual-release compounds. Mesoporous silica is a nanocomposite that is capable of incorporating several compounds for its gradual release. In the case of our study, calcium was incorporated as the objective was to release calcium gradually. However, other compounds such as NaF, TiF\(_4\) (unpublished data), substances for medical use are also used.\(^11\)

In addition, the nanocomposite silica has a high surface area since it has a large amount of hexagonal-shaped pores, which increases the loading of substances inside;\(^11\) and adequate thermal stability, that is, mesoporous silica can resist changes in temperature, without however changing its initial composition.\(^12\) Its application has been studied as a means to carry compounds and promote their slow release on an applied surface,\(^13\) which could interfere with mineral loss kinetics.

Therefore, based on the prior established benefits of the novel calcium mesoporous silica material in caries prevention and treatment, since calcium can minimize mineral loss whether in the process of caries or tooth erosion, so, the present study aimed to evaluate and compare the effects of a single application of this compound to those of other calcium and/or fluoride products, specifically in regard to reducing the progression of dental erosion.

Methodology

This in vitro study evaluated the effects of a single application of Ca\(^{2+}\)-MSN in reducing the progression of dental erosion and compared the findings to other calcium and/or fluoride products. The choice of 10 blocks per group was made after performing a sample calculation to detect a significant 50% difference in mean mineral loss in each treatment group as compared with the negative control group while considering a statistical power of 80%, a unilateral test, and a significance level of 5%.\(^14\) The BioEstat 5.3 statistical analysis software program (Institute of Sustainable Development Mamirauá, Tefé, Amazonas, Brazil) was used to successfully detect a significant 50% difference in mean mineral loss in each treatment group in comparison with in the control group, considering a statistical power of 80%, a unilateral test, and a significance level of 5%. The blocks were exposed to an erosive challenge via immersion in a low-pH solution followed by immersion in another solution with neutral pH and a single application of the following products: (1) Ca\(^{2+}\)-MSN solution; (2) CPP-ACP slurry (CG America, Alsip, IL, USA); (3) CPP-ACP/F\(^{-}\)slurry (CPP-ACP + 900-ppm concentration of F\(^{-}\) from
GC Corp., Tokyo, Japan); (4) TiF₄ solution (1%); or (5) sodium fluoride solution (NaF 1.36%); or (6) Milli-Q® water (Millipore Corp., Burlington, MA, USA) as a negative control. Subsequently, the blocks were one again exposed to in vitro erosive challenges and the topography of each block was analyzed via three-dimensional (3D) noncontact optical profilometry and scanning electronic microscopy (SEM).

Sample preparation

Sixty enamel blocks measuring 6×6×2 mm 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) and polished using water-cooled silicon carbide paper 600, 800 and 1,200 (Extec Corp., Enfield, Connecticut, United States) (Figure 1). To select the blocks, microhardness tests were performed following the methodology proposed by 15 (Figures 1A, 1B, and 1C), a using a microhardness machine (Micromet 5104; Buehler, Mitutoyo Corporation, Tokyo, Japan) and the blocks that obtained the same average 342.90 (Kg/mm²) ± 10% were selected for the experiment.

Treatments and erosive challenge

The enamel blocks (n=60) were randomly assigned into six groups (n=10 each) according to the proposed surface treatment. Half of each block was covered with an acid-resistant varnish (untreated area) (Figure 1D) and the other half (i.e., the eroded area) was submitted to the first erosive challenge for three days, three times daily, for five minutes (Figure 1E) in a low-pH solution (2.58; Sprite Zero™, Coca-Cola Co., Atlanta, GA, USA), according to the method proposed by. 16 The blocks were then immersed in artificial saliva (composed of 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)17 for two hours. Upon completion of the first erosive challenge, half of the
eroded area was subsequently covered with the acid-resistant varnish and received a single application of a certain treatment from among the following (n=10 per group): experimental Ca\textsuperscript{2+}-MSN solution; CPP-ACP slurry; CPP-ACP/F\textsuperscript{−} slurry (CPP-ACP + 900-ppm concentration of F\textsuperscript{−}); TiF\textsubscript{4} solution (1%; positive control); and NaF solution (1.36%; positive control). The negative control samples (n=10) received a single application of Milli-Q\textsuperscript{®} water (Figure 1F).

The CPP-ACP (Pure Water, Glycerol, CPP-ACP, 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) and CPP-ACP/F\textsuperscript{−} (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) pastes were established as slurry with a 1:3 composition of one part toothpaste and three parts distilled and deionized water (i.e., Milli-Q\textsuperscript{®}).\textsuperscript{18} The solutions of novel Ca\textsuperscript{2+}-MSN (1g of mesoporous silica doped with calcium powder to 100 ml of MilliQ\textsuperscript{®} water), TiF\textsubscript{4} (1,0% of powder concentration to 100ml of MilliQ\textsuperscript{®} water), and NaF (1.36% of powder concentration to 100ml of MilliQ\textsuperscript{®} water) were developed in the Laboratory of Industrial Pharmaceutical Technology, Federal University of Rio de Janeiro in Rio de Janeiro, Brazil.

The new Mesoporous Silica was developed in the Laboratory of Industrial Pharmaceutical Technology, Federal University of Rio de Janeiro in Rio de Janeiro, Brazil. and after its synthesis, calcium particles were incorporated. It consisted, initially, in the development of an aqueous solution of NaOH, using H2O Milliq\textsuperscript{®}, submitted to magnetic stirring. Then, cetyltrimethylammonium bromide (CTAB) was added, followed by tetraethoxysilane (TEOS), to obtain MNS. Soon after, Ca (NO\textsubscript{3})\textsubscript{2} was added in order to form a precipitate.

A one-minute, single application of each product on the relevant enamel-block surfaces was conducted with the aid of a 100-μL pipette. These surfaces were then washed and dried using absorbent paper (Figure 1G). The enamel blocks of the negative control group received distilled and deionized water (i.e., Milli-Q\textsuperscript{®}) for the same period. After this treatment, all blocks were submitted to a second erosion challenge, as previously described (Figure 1H).

Analysis in noncontact three-dimensional profilometry

After chemically removing the nail varnish protective covering with acetone (Figure 1I), block topography was evaluated (Figure 1). The topographical measurements of the various block areas were recorded using a noncontact 3D profilometer (NanoScope PS50 Optical; NanoScope Inc., Irvine, CA, USA), based on a methodology previously described.\textsuperscript{19} Measurements of volumetric roughness (Sa) were taken for both eroded (Sa\textsubscript{initial}) and treated areas (Sa\textsubscript{final}), with an area of 200×200 μm. The difference between the initial and final roughness findings was determined by the following: R = Sa\textsubscript{final} − Sa\textsubscript{initial}. For tooth structure loss (TSL) analyses, differences between the heights of the eroded and treated areas were analyzed. A qualitative analysis was also performed via 3D image profiling of each surface, considering a color scale varying from red (high peaks with low structure loss) to dark blue (deeper valleys with greater structure loss). All analyses were performed by a blinded examiner who only had access to a random number of the previously demarcated specimens (Figure 1J).

Analysis using scanning electron microscopy

A SEM system was used to qualitatively analyze the enamel surface and the differences between eroded and treated areas as well as the nature of the step formed between them. All areas were covered with a thin layer of gold and analyzed by SEM (Fei Quanta 250; Czech Republic). The SEM operated between 15 kV and 20 kV, with an increased magnification of 500× applied at the interface areas. Photomicrographs were obtained to show the microstructural characteristics of the untreated, eroded, and treated areas (Figure 1K).

Statistical analysis

The Shapiro–Wilk test was applied to evaluate the normality of Sa and TSL data through the Statistical Package for the Social Sciences software version 22.0 (IBM Corp., Armonk, NY, USA). Then, a one-way analysis of variance and Tukey’s test were used to evaluate Sa and TSL measurements between the eroded and treated areas. The level of significance was 95% (p<0.05). The SEM findings were used to explain surface alterations on the areas and differences between untreated, eroded, and treated areas.
Results

The negative control blocks presented higher mean values of Sa in comparison with the difference between the eroded and treated areas of the experimental blocks (p<0.05). Furthermore, the studied products were able to reduce the mean difference of Sa between the eroded and treated areas as compared with the positive control (p<0.05), without statistical differences (p>0.05) (Table 1).

Based on the findings of TSL difference between the eroded and treated areas, the Ca\textsuperscript{2+}-MSN and NaF treatments were found to be similar in terms of effect (p>0.05) and thus more successfully reduced erosion progression than did CPP-ACP, CPP-ACP/F\textsuperscript{-}, or TiF\textsubscript{4}. These latter treatments yielded results similar to those of the negative control (p<0.05) (Table 1).

When the SEM images (Figure 2) were reviewed, the groups that best inhibited the progression of enamel loss (i.e., those that showed the smallest step between the eroded and treated areas) were the novel Ca\textsuperscript{2+}-MSN and NaF groups. The control group presented the largest step and a visible degree of surface loss. The color scale of the 3D profilometry images confirmed that steps had formed between the eroded and treated areas in each product group (Figure 3).

Discussion

The present study aims to compare the effects of a novel Ca\textsuperscript{2+}-MSN and other commonly used products on dental erosion. In present study, the novel Ca\textsuperscript{2+}-MSN exhibited a greater degree of effectiveness in reducing the progression of a previously installed erosion in a

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**Table 1-** Mean ± standard deviation of differences in volumetric roughness (S) and TSL (treated-eroded área) of enamel after application for each product and erosive challenge

|                        | S (Sa treated- Sa eroded) | TSL (treated area-eroded area) |
|------------------------|---------------------------|-------------------------------|
|                        | Mean (SD)                 | Mean (SD)                     |
| Ca\textsuperscript{2+}-MSNs | -0.17(±0.35)\textsuperscript{a} | 11.91(±2.04)\textsuperscript{a} |
| CPP-ACP                | -0.11(±1.55)\textsuperscript{a} | 17.99(±5.04)\textsuperscript{a} |
| CPP-ACP/F\textsuperscript{-} | -0.83(±2.45)\textsuperscript{a} | 19.07(±13.79)\textsuperscript{a} |
| TiF\textsubscript{4}     | -0.21(±1.07)\textsuperscript{a} | 12.79(±5.00)\textsuperscript{a} |
| NaF                    | -0.22(±0.46)\textsuperscript{a} | 9.83(±4.20)\textsuperscript{a}  |
| Water                  | -1.51(±1.90)\textsuperscript{b} | 33.39(±13.35)\textsuperscript{b} |

**Figure 2-** Surface SEM images of enamel of untreated area, eroded area and after treatment in 500X (image of the interface: untreated, eroded and treated areas). (A) Ca\textsuperscript{2+}-MSN, (B) CPP-ACP, (C) CPP-ACP/F\textsuperscript{-}, (D) TiF\textsubscript{4}, (E) NaF, (F) Negative Control (Water). In the images: UA: untreated area; EA: eroded area; TA: treated area. The white arrows represent the border area between EA and TA, where irregular enamel loss and the formation of steps are observed.
manner similar to the NaF treatment, as observed based on the TSL measurements of the eroded versus treated areas. Both the novel Ca\(^{2+}\)-MSN and NaF solutions correlated with a more minor loss of tooth structure than did the other products (i.e., CPP-ACP, CPP-ACP/F\(^-\), and TiF\(_4\)) and water (negative control).

To our knowledge, this is the first study to incorporate calcium into a novel mesoporous silica nanoparticle applied to act as a limiting factor in the progression of dental erosion. The positive results obtained herein in regard to reducing the loss of tooth structure in comparison with other products may possibly be due to the fact that the porous structure of the silica encapsulates the to-be-released compounds, acting as a stabilizer and increasing the reaction by gradually releasing the compounds, thus creating chemical stability.\(^{20,21}\)

Ca\(^{2+}\)-MSN showed results as positive as NaF, which is a product considered the gold standard in the use of remineralization. As a result, Ca\(^{2+}\)-MSN can be considered an important product to be incorporated into future in vivo studies, since it has benefits and positive results as seen in the present study. It is a product with high substantivity, containing many pores that allows a greater incorporation of several substances (in the case of our study calcium was chosen), to be a compound that has a synthesis using a simple process (providing a good cost /benefit), to be a biocompatible product in humans and in addition ,for having a great benefit for its chemical stability capacity,\(^{22,23}\) keeping its properties stable even in the oral cavity that undergoes changes in temperature and pH.

When analyzing the fluoride compounds, positive results were obtained regarding the use of NaF, which can possibly be explained by the fact that fluoride decreases the solubilization of dental enamel (hydroxyapatite) through the formation of CaF\(_2\), leading to establishment of a barrier that is the first to be diluted after an erosive challenge. However, in the present study, TiF\(_4\) was not as effective as NaF in reducing the progression of enamel erosion. Nevertheless, we can speculate that TiF\(_4\) may still be able to prevent dental erosion.\(^{24,25}\) As previously shown by Chevitarese, et al.\(^{26}\) (2004), titanium penetrates more easily into the surface of sound enamel, i.e., into more regular surfaces. Of note, it is possible that the TiF\(_4\) layer may have been thinner or not continuous in our study in areas where a previously eroded surface already existed. A previous study about erosion prevention, found better results for TiF\(_4\) that was indeed able to act positively in preventing erosion\(^{27}\) than in the present study, which evaluated the minimization of the progression of dental erosion. Another reason why the results weren’t positive may have been that TiF\(_4\) has an acidic composition, with low pH values (pH: 1–2), which further damaged the previously eroded surface.\(^{28}\)

In the present study, calcium-based products such as CPP-ACP and CPP-ACP/F\(^-\) were used in comparison with Ca\(^{2+}\)-MSN. Both CPP-ACP products were not as effective as Ca\(^{2+}\)-MSN in protecting already eroded tooth enamel; when related to caries these products usually have a more beneficial effect than when used against dental erosion. Positive results are usually shown in many studies using CPP-ACP and CPP-ACP/F\(^-\) in reducing the progression of caries lesions and increasing resistance to demineralization.\(^{29,30}\)
According to Reynolds31 (2009) and Rose32 (2000), this is due the compound CPP-ACP includes a technology that adheres to the plaque, providing a reserve of calcium and phosphate. However, no benefits were identified as existing for either CPP-ACP and CPP-ACP/F− regarding preventing dental plaque by creating a reservoir of phosphate and calcium ions in the present study, and neither compound presented a similar efficacy against tooth erosion as compared with Ca2+-MSN and NaF. In addition, the flow addition product had a concentration of 900 ppm of F−, which may have been the differential factor, since the CPP-ACP/F− showed more erosion, compared to the positive controls that had 6,153- and 6,135-ppm concentrations of F−, respectively.

In addition, the lesser effectiveness in our study, of CPP-ACP and CPP-ACP/F− products, when compared to the use of more used products such as NaF for example, may have been due to the chosen time of application, studies that chose application times that varied between 3 to 5 minutes, showed better results when compared to our study, for example.33-35

Conclusions

Based on the results of the present study, the discussed novel Ca2+-MSN treatment can prevent the progression of enamel erosion as well as NaF can, likely due to the capacity of the silica to increase the bioavailability and slow the release of the incorporated molecules or ions,27 much like the calcium used in our study, thus acting in the remineralization process. These positive results reveal an array of new possibilities to be tested in future in vitro, in situ and in vivo studies in order confirm the promising viability of Ca2+-MSN.

Acknowledgments

This study was financed in part by the This study was financed in part by the This study was financed in part by the Coordination for the Improvement of Higher Education Personell- (CAPES) –Finance code 001, by CNPq 303535/2016-4 and Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (FAPERJ) Nº E-26/202.924/2017. This study is part of the Master dissertation of the first author.

References

1- Carvalho TS, Colon P, Ganss C, Huysmans MC, Lussi A, Schlüeter N, et al. Consensus report of the European Federation of Conservative Dentistry: erosive tooth wear - diagnosis and management. Clin Oral Invest. 2015;19(7):1557-61. doi: 10.1007/s00578-015-1511-7
2- Lussi A, Schlüter N, Rakhamatullina E, Ganss C. Dental erosion: an overview with emphasis on chemical and histopathological aspects. Caries Res. 2011;45(Suppl. 1):2-12. doi:10.1159/000325915
3- Souza BM, Santi LR, Silva MS, Buzalaf MA, Magalhães AC. Effect of an experimental mouth rinse containing NaF and TiF4 on tooth erosion and abrasion in situ. J Dent. 2018;73:45-9. doi:10.1016/j.jdent.2018.04.001
4- Tenuta L, Cerezetti R, Del Bel Cury A, Tabchoury C, Cury J. Fluoride release from CaF2 and enamel demineralization. J Dent Res. 2008;87(11):1032-6. doi: 10.1177/154405910808701105
5- Wiegand A, Magalhães AC, Sener B, Waldheim E, Altin T. TiF4 and NaF at pH 1.2 but not at pH 3.5 are able to reduce dentin erosion. Arch Oral Biol. 2009;54(8):790-5. doi: 10.1016/j.archoralbio.2009.05.004
6- Wei S, Soboroff D, Wefel J. Effects of titanium tetrafluoride on human enamel. J Dent Res. 1976;55(3):426-31. doi: 10.1177/00220345760550032101
7- Reynolds E, Cai F, Cochrane N, Shen P, Walker G, Morgan M, et al. Fluoride and casein phosphopeptide - amorphous calcium phosphate. J Dent Res. 2008;87(4):344-8. doi: 10.1177/154405910808700420
8- Cross K, Huq N, Reynolds E. Casein phosphopeptides in oral health-chemistry and clinical applications. Curr Pharm Des. 2007;13(8):793800. doi: 10.2174/138161207780363086
9- Alencar CR, Oliveira GC, Magalhaes AC, Buzalaf MA, Machado MA, Honorio HM, et al. In situ effect of CPP-ACP chewing gum upon erosive enamel loss. J of Appl Oral Sci. 2017;25(3):258-64. doi: 10.1590/1678-7757-2016-030
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10- Gisbert-Garzarán M, Manzano M, Vallet-Regi M. Mesoporous silica nanoparticles for the treatment of complex bone diseases: bone cancer, bone infection and osteoporosis. Pharmaceutics. 2020;12(1):83. doi:10.3390/pharmaceutics12010083

11- Wang Y, Zhao Q, Han N, Bai L, Li J, Liu J, et al. Mesoporous silica nanoparticles in drug delivery and biomedical applications. Nanomedicine. 2015;11(2):313-27. doi:10.1016/j.nano.2014.09.0143

12- Mercuri LP, Carvalho LV, Lima FA, Quayle C, Fantini MC, Tanaka GS, et al. Ordered Mesoporous silica SBA-15: a new effective adjuvant to induce antibody response. Small. 2006;2(2):254-6. doi:10.1002/sml.200500274

13- Selvam P, Dapurkar S. Catalytic activity of highly ordered mesoporous VMCM-48. Appl Catal A Gen. 2004;276(1-2):257-65. doi:10.1016/j.apcata.2004.08.012

14- Alexandria AK, Valença AM, Cabral LM, Maia LC. Fluoride varnishes against dental erosion caused by soft drink combined with pediatric liquid medicine. Braz Dent J. 2017;28:482-8. doi:10.1590/0103-6440201701567

15- Vieira TI, Câmara JV, Cardoso JG, Pintor AV, Villaça JC, Cabral LM, et al. Cytotoxicity of novel fluoride solutions and their influence on enamel mineral loss from enamel exposed to a Streptococcus mutans biofilm. Arch Oral Biol. 2018;91:57-62. doi:10.1016/j.archoralbio.2018.04.008

16- Magalhães AC, Levy FM, Rios D, Buzalaf MA. Effect of a single application of TiF4 and NaF varnishes and solutions on dentin erosion in vitro. J Dent. 2010;38(2):153-7. doi:10.1016/j.jdent.2009.09.015

17- Nassur C, Pomerico L, Sousa VP, Cabral LM, Maia LM. Characterization of a new TiF4 and β-cyclodextrin inclusion complex and its in vitro evaluation on inhibiting enamel demineralization. Arch Oral Biol. 2013;58(3):239-47. doi:10.1016/j.archoralbio.2012.11.001

18- Alexandria AK, Vieira TI, Cardoso JG, Pintor AV, Villaça JC, Cabral LM, et al. In vitro enamel erosion and abrasion-inhibiting effect of different fluoride varnishes. Arch Oral Biol. 2017;77:39-43. doi:10.1016/j.archoralbio.2017.01.010

19- Alexandria AK, Meckelburg ND, Puettet UT, Salles JT, Souza IP, Maia LC. Do pediatric medicines induce topographic changes in dental enamel? Braz Oral Res. 2016;30(1):S1806-83242016000100211. doi:10.1590/1807-3107BOR-2016.vol30.0011

20- Cauda V, Schlossbauer A, Bein T. Bio-degradation study of colloidal mesoporous silica nanoparticles: effect of surface functionalization with organo-silanes and poly(ethylene glycol). Microporous and Mesoporous Mater. 2010;132(1-2):60-71. doi:10.1016/j.micromeso.2009.11.015

21- Wang S. Ordered mesoporous materials for drug delivery. Microporous and Mesoporous Mater. 2009;117(1-2):1-9. doi:10.1016/j.micromeso.2008.07.002

22- Slowing II, Trewny BG, Lin VS. Mesoporous silica nanoparticles for intracellular delivery of membrane-impermeable proteins. J Am Chem Soc. 2007;129(28):8845-9. doi:10.1021/ja0719780

23- Narayan R, Nayuk U, Raichur A, Garg S. Mesoporous silica nanoparticles: a comprehensive review on synthesis and recent advances. Pharmaceutics. 2018;10(3):118. doi:10.3390/pharmaceutics10030118

24- Castilho AR, Salomão PM, Buzalaf MA, Magalhaes AC. Protective effect of experimental mouthrinses containing NaF and TiF4 on dentin erosive loss in vitro. J Appl Oral Sci. 2015;23(5):486-90. doi:10.1590/1678-775720150127

25- Magalhães AC, Santos MG, Comar LP, Buzalaf MA, Ganss C, Schlueter N. Effect of a single application of TiF4 varnish versus daily use of a low-concentrated TiF4/NaF solution on tooth erosion prevention in vitro. Caries Res. 2016;50(5):462-70. doi:10.1159/000448146

26- Chevitarese AB, Chevitarese O, Chevitarese LM, Dutra PB. Titanium penetration in human enamel after TiF4 application. J Clin Pediatr Dent. 2004;28(3):253-6. doi:10.17796/jcpd.28.3.jn8625287675053

27- Canto FM, Alexandria AK, Vieira TI, Justino IB, Cabral LM, Silva RF, Maia LC. Comparative effect of calcium mesoporous silica versus calcium and/or fluoride products against dental erosion. Braz Dent J. 2020;31(2):164-70. doi:10.1590/1678-7757202002557

28- Nobrega CB, Fujibara FY, Curaj YA, Rosalen PL. TiF4, Varnish – A,N,F-NMR stability study and enamel reactivity evaluation. Chem Pharm Bull (Tokyo). 2008;56(1):139-41. doi:10.1248/cpb.56.139

29- Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds E. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res. 2004;38(6):551-6. doi:10.1159/000080585

30- Reynolds E, Cai F, Shen P, Walker G. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res. 2003;82(3):206-11. doi:10.17771/154405910308200311

31- Reynolds E. Casein phosphopeptide-amorphous calcium phosphate: the scientific evidence. Adv Dent Res. 2009;21(1):25-9. doi:10.1177/0895937409335619

32- Rose R. Binding characteristics of Streptococcus mutans for calcium and casein phosphopeptide. Caries Res. 2000;34(5):427-431. https://doi.org/10.1159/000016618

33- Somani R, Jaidka S, Singh DJ, Arora V. Remineralizing potential of various agents on dental erosion. J Oral Biol Craniofac Res. 2014;4(2):104-8. doi:10.1016/j.jobcr.2014.05.001

34- Carvalho FG, Brasil VL, Silva TJ Filho, Carlo HL, Santos RL, Lima BA. Protective effect of calcium nanophosphate and CPP-ACP agents on enamel erosion. Braz Oral Res. 2013;27(6):462-70. doi:10.1590/1807-3107BOR-20130127

35- Pirca K, Balbín-Sedano G, Romero-Tapia P, Alviltez-Temoche D, Robles G, Mayta-Tovalino F. Remineralizing. Effect of casein phosphopeptide-amorphous calcium phosphate and sodium fluoride on artificial tooth enamel erosion: an in vitro study. J Contemp Dent Pract. 2019;20(11):1254-9