EXPERIMENTAL MOUTH RINSE WITH NANO-HAP TO BE USED AT HOME FOR DENTAL EROSION TREATMENT

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ABSTRACT:

Objective: The aim of this in vitro study was to evaluate — by X-ray fluorescence technique (XRF), surface Vickers microhardness (VM) and SEM — the effect of the following products in bovine enamel submitted to acidic challenges: nanoparticles of calcium hydroxyapatite (nano-HAp), associated or not with fluoride, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) associated or not with fluoride, sodium fluoride, and saliva. **Methods:** 58 bovine incisors were sectioned into fragments and randomly divided into 8 groups and 2 specimens for initial SEM images. All teeth were initially evaluated and over a 10-day experimental period, the enamel samples were subjected to erosive demineralization for 6 X 2 min per day. The samples received the corresponding treatments: professional pastes (nano-HAp; nano-HAp and fluoride; CPP-ACP; CPP-ACP and fluoride) and aqueous solutions (NaF; nano-HAp and nano-HAp + NaF). The samples were rinsed again with distilled water for 1 min and stored in artificial saliva. The time between cycles was 1.5 h. All specimens were evaluated again. **Results/Conclusions:** All treatments carried out in groups of professionals pastes were considered effective on mineral deposition of tooth enamel surface subjected to erosive challenge cycle. The groups that received treatment with solutions were effective, matching the final results to the control group, considering VM and SEM, except for the sodium fluoride solution (0.05%). The nano-HAp solution associated with fluoride promoted full recovery in P and Ca count during cycling and had increased VM similar to all groups of professional pastes.

KEYWORDS:

Dental erosion. Nanohydroxyapatite. CPP-ACP. Fluoride.

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INTRODUCTION

Dental erosion is defined as the irreversible loss of tooth structure due to chemical dissolution by acids not of bacterial origin, triggered by intrinsic source acids such as the acid from the gastric fluid or extrinsic acids.[1-3]

The etiology of dental erosion is multifactorial and not fully understood. There are predisposing factors and different etiologies of the disease. The interaction of chemical, biological and behavioral factors is essential and helps to explain why some individuals exhibit more dental erosion than others, even if they are exposed to erosive challenges with the same acids in their diets.[4,5] There are three stages to the dental erosion development process: the first is the loss of salivary organic substances covering the tooth surface, followed by the loss of mineral from the tooth surface because of the presence of a decalcifying agent, and finally, the destruction of the tooth surface decalcified by a biochemical, biophysical and/or mechanical action.[6]

With regard to prevention, one of the most important points is the identification of patients at risk of erosion to begin primary prevention measures. In this case, it is important to evaluate the different etiological factors that can lead to tooth erosion.[7] Thus, we may take actions to reduce exposure to acids and mechanical impact, increase the quality and quantity of saliva and acquired film, and reduce the consumption of drinks and acidic solutions. Another preventive strategy is the application of products rich in calcium and/or fluoride.[8] Patient education is crucial to the process, since most of them do not know how much their habits contribute to the destruction of their teeth.[9] However, these preventive strategies may have a limited effect, since they largely depend on patient cooperation. In this way, it would be interesting to develop preventive strategies that rely less on patient cooperation.

Saliva has the capacity to act directly on the erosive acids, causing its dilution, cleaning, neutralization, and buffering. It also forms the acquired pellicle, a bacteria-free organic film formed *in vivo* as a consequence of selective adsorption of salivary proteins and glycoproteins in tooth surface. It also contains, in lower concentrations, other macromolecules such as lipids. The organic components of this pellicle play important roles, such as lubrication and protection of the under-

lying tooth surface, reduce the demineralization rate, and increase remineralization, providing calcium, phosphate, and fluoride both for enamel and dentin lesions.[10,11] Some studies have indicated that acid-resistant proteins present in the acquired pellicle, such as mucin, can be used in dental products to prevent erosion.[11,12]

Several substances have been suggested in the literature with the aim of reducing and controlling erosion lesions, such as the compounds of fluoride, casein phosphopeptide (CPP), and nano-hydroxyapatite.

Conventional toothpastes that have fluoride in its composition do not seem to be able to efficiently protect the growing process of erosive challenge.[13] Several types of fluoride compounds have been tested for this purpose. Among the most-used fluorides are sodium fluoride (NaF), stannous fluoride (SnF_2), and titanium tetra fluoride (TiF_4), which show different levels of protection. Promising results were obtained with the TiF and SnF [14-16] and also with the combination of $SnCl_2$ and NaF[1].

Casein, a milk-derived protein, and some of its derivatives, such as casein phosphopeptide (CPP), are widely studied in relation to its protective action around the dental hard tissues, protecting them against demineralization.[17,18]

Hydroxyapatite is one of the most biocompatible bioactive materials and is widely used to coat areas of radicular dentine exposure. Nanoparticles of hydroxyapatite have a similarity to the hydroxyapatite crystals of enamel in morphological structure and crystalline structure.[19] When used for mineral deposition and treatment of dentinal hypersensitivity, associated with fluoride, seem to more easily penetrate inside the microcracks in enamel, providing a high-quality sealing and restoring the microstructure and composition of the tooth surface.[19]

Most of the bioactive materials have been tested before and seem to be effective in dental erosion treatment.[14-20] These products are commercially available for dentists and patients, but they are very expensive for most of population or need to be used in office by a dentist in periodic appointments. On the other hand, although the results using fluoride[14-20] in dental erosion treatments are not the best, this product is still the most used, usually in mouth rinses used at home, exactly because of its cost and because it is easy to be used by the patient, without professional supervision. So, one ideal option would be a domestic treatment modality with one of these bioactive materials.

Thus, the aim of this *in vitro* study was to evaluate the effect of an experimental mouth rinse with nano-HAp, associated or not with fluoride, to be used at home for dental erosion treatment, comparing with other methods existing on the market.

MATERIALS AND METHODS

Fifty-eight bovine incisors were used in this study. The samples were stored in thymol (0.05%) for up to a week before conducting the tests. The teeth were cleaned with periodontal curettes and prophylaxis with pumice and water.

The teeth were sectioned in fragments of approximately 4 x 4 mm, obtained from the flattest area of the labial portion, and were stored in ultrapure water at 4°C. Then, the fragments were embedded in epoxy resin with the aid of wax, with the labial surface parallel to the horizontal plane of the bench, and they were contained in standard teflon devices for X-Ray Fluorescence (XRF) testing and Vickers Microhardness (VM) testing, and in acrylic pipes for the SEM evaluation, for 24 hours, to wait for the polymerization of the resin. The tooth/base set was flattened and polished in a metallographic polishing machine (APL-4, Arotec Ind e Com, Cotia, SP, Brazil), with #400, 600, 800, and 1200 silicon carbide sandpaper and abundant water. Then, the specimens were immersed for 5 minutes in an ultrasonic bath and then identified.

Study design

The 58 specimens were randomly divided into 8 groups (n=7) and 2 specimens for initial SEM images. Materials used in each group are described in Table 1.

Table 1:

Materials used in the study.

GROUP	MATERIALS	MANUFACTURER
1 (control)	Artificial saliva	University Pharmacy, Rio de Janeiro Federal University, Rio de Janeiro, RJ, Brazil
2	Experimental Desensibilize Nano P - Calcium hydroxyapatite nanoparticles	FGM Prod Odontológicos, Joinville, SC, Brazil
3	Desensibilize Nano P - Calcium hydroxyapatite nanoparticles and sodium fluoride (9000 ppm)	FGM Prod Odontológicos, Joinville, SC, Brazil
4	GC Tooth Mousse - Casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) — Recaldent™	GC Europe, Leuven, Belgium
5	GC Tooth Mousse - Casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) — Recaldent™ + sodium fluoride (900 ppm)	GC Europe, Leuven, Belgium
6	Sodium fluoride aqueous solution (0.05%)	Nova Era Manipulation Pharmacy, Rio de Janeiro, RJ, Brazil
7	Calcium hydroxyapatite nanoparticles aqueous solution (0.375%)*	
8	Calcium hydroxyapatite nanoparticles aqueous solution (0.375%) + sodium fluoride (0.05%)*	-

* Aqueous solutions of calcium hydroxyapatite nanoparticles (nano-HAp) were manipulated by diluting 6.25 grams of NanoXIM Care Paste (Fluidinova S.A., Porto, Portugal) in 250 ml of distilled water in pure solution at G7 and in 250 ml of sodium fluoride aqueous solution (0.05%) at G8. Process conducted at room temperature with the aid of a 250 ml flask.

X-ray fluorescence (XRF) by energy dispersion multi-elemental analysis

In each of the 8 groups, 5 specimens were initially evaluated with XRF (Artax™200, Bruker Corporation, Massachusetts, USA), in order to obtain an initial count of P and Ca elements. In each specimen, one measurement with XRF equipment was conducted before and one after treatment, at 5 predefined points, and an X-ray beam was positioned in the central region of the points. These points were recorded by calculating the distance between two starting points registered in the Teflon device, which made possible the final measurement to be performed at the same point of the initial measurement. This also allowed the maintenance of the distance between the X-ray beam and the detector in the final measurement, in case of loss or gain of structure in the tooth surface after treatment. The XRF spectra were analyzed using the ARTAX equipment software.

Vickers Microhardness testing (VM)

Initially, with the help of a universal vise and a parallelometer, the specimens were fixed and positioned in a plane parallel to the table of the microhardness testing equipment (Micromet 5114, Buehler, Illinois, USA) and perpendicular to the indentation tip. The microhardness testing was conducted using a 50x magnification lens. In each specimen, 5 indentations were performed (50 gf, 15 sec), with a distance of 100 µm between them, before and after surface treatments. Vickers hardness was calculated by the division between the applied force (Kgf) and the indentation area (mm^2).

SEM evaluation

Two treated specimens in each group and two fragments of untreated teeth were selected for SEM qualitative evaluation of treated and untreated surfaces. SEM images of gold-sputtered specimens were obtained at 500x and 1000x magnification (secondary electron, 12.50 and 20.00 kV) in a QUANTA 250 SEM equipment (FEI Company, Oregon, USA).

Erosive challenge cycle

During a 10-day trial period, the specimens were subjected to a cyclic process of several daily acid attacks, as well as applications of test solutions.

The simulation of a high erosive challenge was performed by immersing the specimens in 40ml of 0.05 M citric acid (pH 2.3), six times (2 min) daily, with a 1.5-hour interval between cycles, at room temperature and with gentle agitation

on a magnetic stirrer (Scilogex, CT, USA). Subsequently, specimens were thoroughly rinsed for 1 minute with ultra-pure water and then immersed in artificial saliva[21].

Manipulation of the experimental mouth rinse

Aqueous solutions of calcium hydroxyapatite nanoparticles (nano-HAp) were manipulated by diluting 6.25 g of NanoXIM Care Paste (Fluidinova S.A., Porto, Portugal) in 250 ml of distilled water in pure solution at G7 (0.375% calcium hydroxyapatite nanoparticles aqueous solution) and in 250 ml of 0.05% sodium fluoride aqueous solution at the G8 (0.375% calcium hydroxyapatite nanoparticles aqueous solution + 0.05% sodium fluoride) — process conducted at room temperature with the aid of a 250 ml flask.

Treatment with the products

Specimens in G1 were stored in artificial saliva for 10 days, and specimens in G2, G3, G4, and G5 were treated with their respective products with a disposable applicator, actively, for 10 seconds in each of the seven specimens in each group. The product was kept for 5 minutes, and then the excess was removed with gauze. Samples were stored in artificial saliva. This process was carried out in the 4th, 7th, and 10th day.

In G6 and in the groups with experimental mouth rinse with 0,375% Nano-HAp, without fluoride and with fluoride, G7 e G8 respectively, specimens were immersed in an aqueous solution of the respective products, two times per day, for ten days, and gently stirred on a magnetic stirrer for 1 minute, simulating the use of a daily mouth rinse at home.

Specimens were kept in an incubator (37° C) between cycles for 10 days. New XRF, VM, and SEM measurements were performed after 10 days of cycling.

Statistical analysis

Student's t-test was used to compare the initial and final averages within each group and to compare groups of different treatments with a 5% significance level, using SPSS for Windows 10.0.1 software.

RESULTS

Initially, the authors obtained the amount of P and Ca elements with XRF, and they also obtained initial values of Vickers microhardness. Table 2 shows the average amounts of P and Ca and the microhardness means in each group, with respective standard deviations (SD), before any treatment.

Table 2:

Mean amount of P and Ca, microhardness (**HV**), Standard deviation (**SD**), and Coefficient of variation (**CV**) in each group, before any treatment.

GROUP	P			CA			MICROHARDNESS		
	MEAN	SD	CV (%)	MEAN	SD	CV (%)	MEAN	SD	CV (%)
G1	1046	23	2.2	51080	229	0.4	361	10	2.7
G2	1078	17	1.6	52118	321	0.6	357	10	1.9
G3	1116	18	1.6	52077	456	0.9	349	21	6.1
G4	1113	41	3.7	51544	818	1.6	351	19	5.3
G5	1109	24	2.1	52119	551	1.1	360	9	2.4
G6	1095	22	2.0	51077	421	0.8	360	14	3.8
G7	1094	21	1.9	51542	1392	2.7	357	18	5.0
G8	1098	23	2.1	51513	554	1.1	329	16	4.9

After treatments in each group, the mean amount of P and Ca was obtained by means of XRF testing, and final Vickers Microhardness was evaluated.

Table 3 shows the average amounts of P and Ca and also the microhardness means, in each group, with respective standard deviations (SD), after 10 days of treatment.

Students' t test was initially used to compare the initial and final means within groups with distinct treatments, with 5% significance level.

Statistically significant differences were found between the P counts before and after treatment in G1 (control group), demonstrating reduction in counts ($p < 0.05$). There was a statistically significant increase in P count in G5 after treatment ($p < 0.05$). In all other groups, the authors did not find statistically significant difference in P count before and after treatments ($p > 0.05$).

Table 3:
Mean amount of P and Ca, microhardness (**HV**), Standard deviation (**SD**), and Coefficient of variation (**CV**) in each group, after 10 days of treatment.

GROUP	P			CA			MICROHARDNESS		
	MEAN	SD	CV (%)	MEAN	SD	CV (%)	MEAN	SD	CV (%)
G1	957	23	2.4	44659	1434	3.2	122	6	4.6
G2	1103	37	3.3	51102	917	1.8	159	22	14.1
G3	1105	28	2.5	51044	927	1.8	159	17	10.4
G4	1136	19	1.7	52759	463	0.9	157	9	5.5
G5	1136	19	1.7	52632	628	1.2	154	7	4.7
G6	1130	22	2.0	51973	1869	3.6	136	16	11.5
G7	1083	67	6.2	50410	3137	6.2	139	8	5.4
G8	1121	42	3.7	51838	868	1.7	135	8	6.2

Statistically significant differences were found between the Ca counts before and after treatment in G1 (control group), demonstrating reduction in counts ($p<0.05$). There was statistically significant increase in P count in G4 after treatment ($p<0.05$). In all other groups, the authors did not find statistically significant difference in Ca count before and after treatments ($p>0.05$).

Statistically significant differences were found between the initial and final microhardness means for all groups, demonstrating loss of hardness in all groups ($p<0.05$).

The student's t test was also used to compare the differences of final/initial means of each group, with 5% significance level. Figures 1 and 2 show the relative intensity difference between the initial and final means (P and Ca and microhardness) in each group.

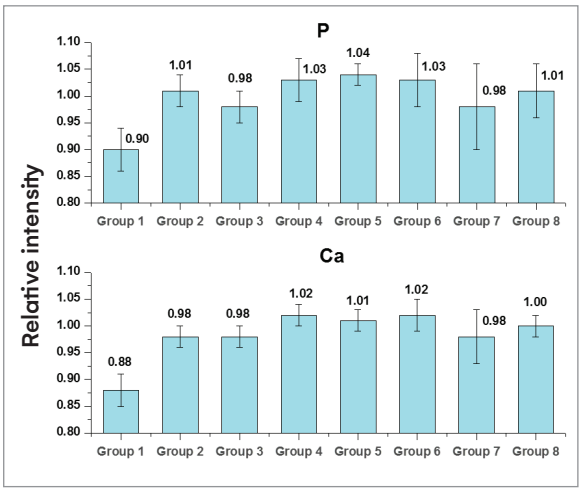

Figure 1:
Relative intensity of final/initial means of all groups regarding P and Ca amount.

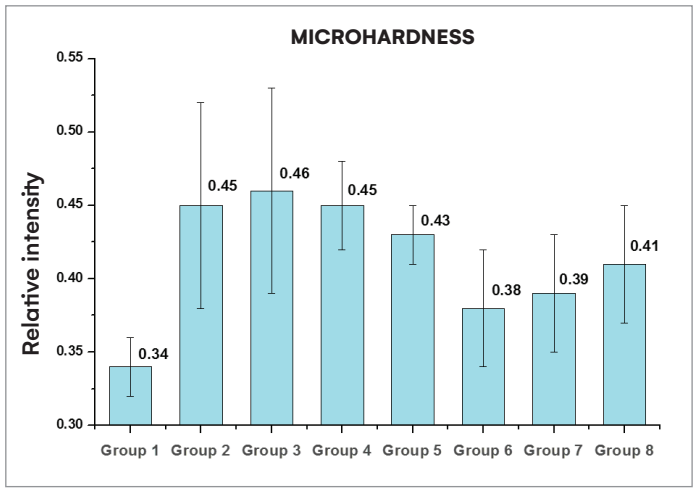

Figure 2:
Relative intensity of final/initial means of all groups regarding microhardness.

Regarding P count, statistically significant differences were found between G1 and all other groups ($p < 0.05$). There was no statistically significant difference between G1 and G7 ($p > 0.05$). G3 had a P count loss statistically higher to G5 ($p < 0.05$).

Regarding Ca count, statistically significant differences were found between G1 and all other groups ($p < 0.05$). The authors also found statistically significant differences between G2 and G4; G2 and G5; G3 and G5 ($p < 0.05$).

Regarding Vickers Microhardness, statistically significant differences were found between G1 and all other groups ($p < 0.05$), and there was no statistically significant difference between G1 and G6 ($p > 0.05$). Statistically significant differences were found between G7 and G4 ($p < 0.05$).

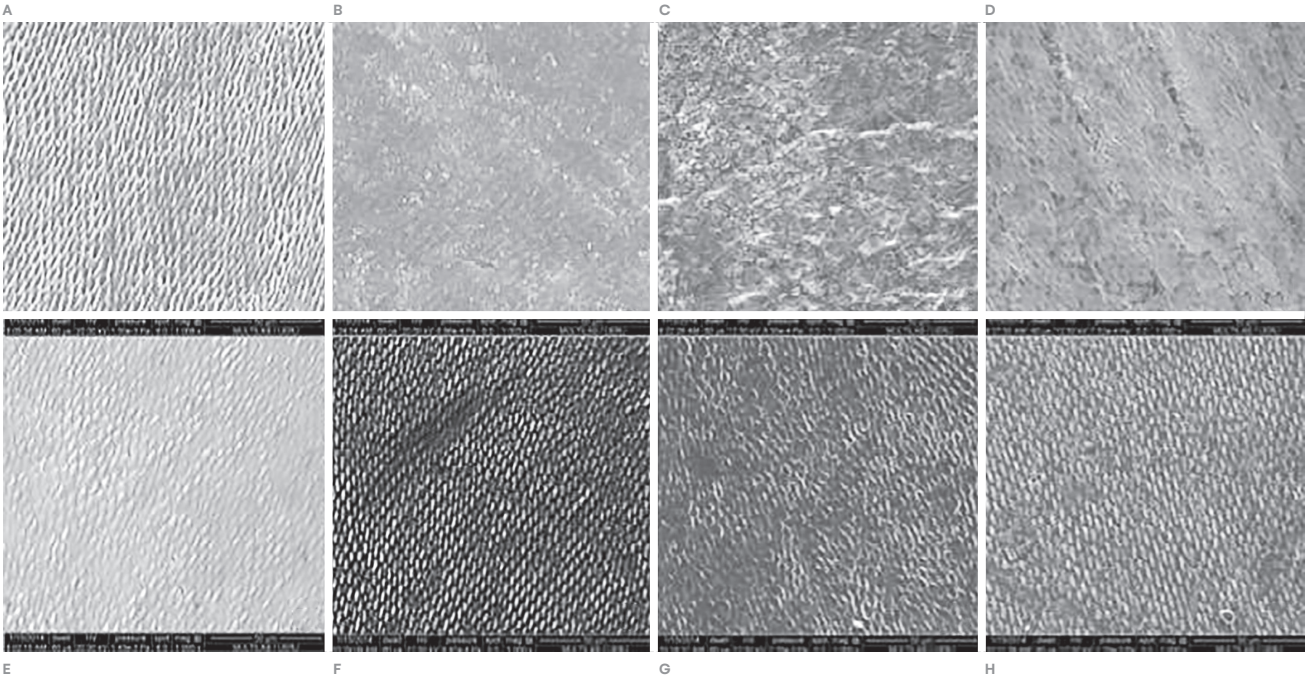

Figure 3:

SEM micrographs (**1000X**) of surfaces after treatments: (**A**) erosion in control group; (**B**) G2 after nano-HAp treatment; (**C**) G3 after nano-HAp and fluoride 9000 ppm treatment; (**D**) G4 after CPP-ACP treatment; (**E**) G5 after CPP-ACP and fluoride 900 ppm treatment; (**F**) G6 after sodium fluoride (0.05%) solution treatment; (**G**) G7 after nano-HAp (0.375%) aqueous solution treatment; (**H**) G8 after nano-HAp (0.375%); and sodium fluoride (0.05%) aqueous solution.

SEM evaluation

Figure 3 shows the different patterns after treatments in groups 1 to 8, observed in SEM.

DISCUSSION

There are some bioactive materials that are commercially available for dentists and patients, but they are very expensive for most of population or need to be used in office by a dentist in periodic appointments. Usually, these products are less used than fluoride, which is not the ideal situation, since fluoride, in some studies evaluating dental erosion treatments modalities, is not the material with best results.[14-20] So, this study was designed to evaluate the effect of an experimental mouth rinse with nano-HAp, associated or not with fluoride, to offer a home treatment modality, easily applied and accessible for most patients with high risk of dental erosion.

This experiment was adapted to identify the differences between the various remineralizing agent formulations and allow detection of loss of minerals. It should be emphasized that this study, similar to most *in vitro* studies, was not designed to consider only the clinical conditions. It should also be considered as a test procedure that isolates certain factors. It should also be asserted that, at least in the case of enamel, *in vitro* results should be interpreted with caution, because mineral dissolution may be influenced by the presence of acquired enamel pellicle in actual clinical condition.[22,23] Finally, it is important to note that, in *in vitro* studies, the erosive potential is overestimated and the role of products is underestimated, as they do not cover all intraoral events.[24,25]

The methodologies for evaluation of mineral deposition chosen for this study were the XRF, VM and SEM.

The X-ray fluorescence was chosen because it is a method based on quantitative and qualitative measurement of intensity (number of X-rays detected per unit time) of the characteristic X-rays emitted by elements constituting a sample[26], which allows the evaluation of all the stages of study in the same teeth. Not being a destructive method, it allows the use of the same sample at different times of the study, thus reducing the variables.[27] The multi-element analysis by SEM/EDS is popularly used as it simultaneously results in microscopic images and elemental distribution images. But for analysis in SEM/EDS, the sample must have electrical conductivity (an electrically conductive coating) and should be maintained at high vacuum during observation,[28] which would not

allow observation in different stages of the study. The multi-elemental analysis with X-ray fluorescence (XRF) by energy dispersion used in this study use characteristic X-rays emitted under irradiation of high-energy X-rays, and have some advantages over EDS. The XRF analysis may be performed in air, without the need of vacuum; there is no need for pretreatment of the surface such as inclusions, dehydration, or electrically conductive coating, and it doe not destroy the samples[28].

The surface microhardness was performed to evaluate the mineral loss that occurred in the enamel surface. These indentation techniques have been extensively used to investigate the erosion of enamel, by measuring the surface hardness of that tissue. As initially, the erosive process promotes the dissolution of enamel, which is associated with the "softening" of the surface due to the weakening of the enamel structure, hardness is a simple observation method of the early stages of enamel erosion[29]. Variations on the enamel surface can be observed even after a few minutes of exposure to an erosive agent. It should be remembered that there are limitations when highly eroded substrates do not have a clear limit, which can lead to inaccurate measurements, and when there is deposition of material on the surface, which can lead to non-representative measurements.[23,29] Thus, analyses of the indentations before and after the erosive processes have been used to evaluate only the initial losses.[23,29]

The images obtained by scanning electron microscopy (SEM) have been essential to study ultrastructural changes associated with erosion of enamel and dentin, according to a systematic review[23] on methods of measurement and characterization of erosion on enamel and dentin.

The development of bioactive products based on nano-hydroxyapatite and/or CPP-ACP with or without fluoride, for remineralizing and desensitizing the dental tissue, able to promote mineral deposition on tooth surface, and promote a quality seal, brought the initial challenge to the development of this study. The authors used professional-use substances that have high concentrations of bioactive compounds. In addition, experimental solutions were used, which have lower concentrations of these compounds, to simulate the home and daily use of mouth rinses and compare them to the substances for professional use. It should also be remembered that, as it was an *in vitro* study, the factors were isolated, so that it was possible to analyze only the action of these products; as in real clinical conditions treatment would be associated with the use of various toothpastes.

Regarding the effective action of fluoride, it is believed that the action of this agent is due to the formation of a mechanical barrier by depositing calcium fluoride on the tooth surface. When there is sufficient fluoride in the oral environment, fluoridated hydroxyapatite, which makes enamel less soluble to the next acid etching, will form.[8]

In this study, the result of the effect of sodium fluoride (0.05%) showed relative efficacy regarding gain of P and Ca, but the hardness was not sufficiently increased to statistically match the initial values. And there was no statistically significant difference between the control group and the treatment with fluoride solution, regarding microhardness ($p > 0.05$). These results agree with other studies[30] that have added fluoride to soft drinks and found benefits, but with lower magnitude than the benefit of also embedding calcium in soft drinks. The results also agree with other experiments[31] that found benefits of fluoride use only associated with metal ions such as Sn in experimental solutions and lower benefit of sodium fluoride. Furthermore, another *in situ* study[32] evaluating the enamel surface microhardness found remineralizing effect of toothpastes with or without fluoride associated with mouth rinses and with and without fluoride in enamel subjected to erosive challenge. This study found similar results to the present study with respect to hardness, in a group with dentifrice without fluoride, associated with mouth rinse with sodium fluoride 450 ppm. Other authors[33] also found a slight increase in hardness when using fluoride solutions; however, they asserted that this increase was significant, particularly when compared to other solutions that are fluoride-free, when used in schemes similar to mouth rinses. SEM images of teeth that were treated with 0.05% sodium fluoride solution showed a uniform surface and a greater exposure of enamel prisms, when compared to groups that received other treatments.

Agents based on milk derivatives have been studied for many years. Nowadays, several formulas are available as GC Tooth Mousse (GC Tokyo, Japan) or Topacal C-5 (NSI Dental, Hornsby, Australia).[5] A technology based in casein phosphopeptide and amorphous calcium phosphate (CPP-ACP) [Recaldent™] states that the CPP stabilizes high concentrations of calcium and phosphate ions with fluoride ions on the tooth surface, binding to the acquired enamel pellicle and to biofilm. Calcium, phosphate, and fluoride ions are freely bioavailable to spread at low-concentration gradients of subsurface enamel lesions, and being stabilized by the CPP, does not promote dental calculus and thus promotes *in vivo* remineralization[17,34].

The results of this study with respect to the effect of GC Tooth Mousse and GC Tooth Mousse Plus with the bioactive compound CPP-ACP, casein phosphopeptides, and amorphous calcium phosphate, and with compound associated with fluoride, was effective regarding gain of P and Ca, but the microhardness did not increase enough to statistically match the initial amount. There was a statistically significant increase in microhardness compared to G1 (control) ($p > 0.05$). The results of another *in vitro* study demonstrated that CPP-ACP compounds used with or without combination of NaF 250 ppm provide little protection to enamel erosion, when compared to high concentrations of acidic AmF.[35] Likewise, Weishaupt et al[36] found a lower effect of the CCP-ACP compounds protection against erosion/abrasion in daily application when compared to daily applications of NaF or AmF gel. On the other hand, Wang et al[37] found that all groups, including those with CCP-ACP compounds, had decreased hardness when they underwent erosive challenge, but different from this study, no group had statistical difference compared to control group. Other authors[38-40] found synergistic effects in association of fluoride with CPP-ACP, which was not found in this study. SEM images in G4 and G5, where teeth were treated with CPP-ACP dentifrices containing no fluoride and fluoride, respectively, showed a uniform surface with partial exposure of enamel prisms in G4 and a slightly higher exposure of prisms in G5.

Hydroxyapatite (HAp) has excellent biocompatibility with soft tissues such as skin, muscle, and gums, thus making it an ideal candidate for implants or components of orthopedic and dental implants. Synthetic HAp has been widely used in the repair of hard tissue and common uses including the repair and augmentation of bones, as well as implant coating or acting as fillers for bones or teeth.[13,41] However, the low strength of normal HAp generally restricts its use to low load-bearing applications.[13,41] With recent developments in nanoscience and nanotechnology, however, it has been suggested that the nano-HAp can be an ideal biomaterial because of its good biocompatibility and bone integration capacity. [13,41] This study found positive results with nano-Hap groups, in pastes with or without fluoride, as well as in the experimental solutions designed for this study. The products were effective regarding the gain in P and Ca concentrations, but the microhardness did not sufficiently increase to statistically match the initial amount. There was a statistically significant increase in microhardness between groups treated with the nano-HAp paste and solutions with nano-HAp and the control group (G1) ($p > 0.05$). In G2 and G3, which were treated with pastes containing

nanoparticles of calcium hydroxyapatite without and with fluoride, respectively, SEM images showed a uniform surface without exposure of enamel prisms. In G7 and G8, which were treated with a solution of calcium hydroxyapatite nanoparticles (0.375%) and a solution of calcium hydroxyapatite nanoparticles (0.375%) and sodium fluoride (0.05%), respectively, SEM images showed a uniform surface and a partial exposure of enamel prisms. In another study[42] evaluating the tooth erosion and the demineralization potential of a sports drink containing nano-hydroxyapatite (nano-HAp) as additive, dental erosion was effectively prevented with increasing concentration and addition of nano-HAp. The authors[42] asserted that sports drink containing nano-HAp (0.25%) could prevent tooth erosion. Tschoppe et al[43] found that toothpastes containing nano-HAp showed higher remineralizing effects, compared to fluoride dentifrices, on the bovine dentin and comparable results were obtained for enamel. Comar et al,[44] who evaluated *in vitro* the potential of experimental pastes containing nanoparticles of hydroxyapatite (10% and 20%) with or without fluoride, in preventing tooth demineralization, found that the experimental pastes with nano-HAp, regardless of the presence of fluoride, were able to reduce tooth demineralization. Other authors,[45] evaluating mouth rinses containing nano-HAp compared to other desensitizing mouth rinses in the occlusion of dentinal tubules, concluded that the mouth rinse with nano-HAp was able to occlude dentinal tubules and reduce the measurement of fluid flow. Further studies, especially with nano-HAp solutions should be performed, not only *in vitro*, but also *in situ* and *in vivo*, since these materials seem to be very promising, as shown in this study and the others mentioned before.

In all treatments in this study, there was a loss of microhardness that has not matched the initial values, even after the use of remineralizing agents. Analyzing the SEM images, it can be seen that the surface has particle deposition and that this deposition may not have been fully incorporated in the enamel and also may not be stable. As asserted before, it must be remembered that there are limitations when using microhardness tests when highly eroded substrates do not have a clear boundary, which could cause inaccurate measurements[23].

The experimental solutions with nano-HAp, associated or not with fluoride, revealed to be a promising option of mouth rinse for daily use in patients with high risk of dental erosion, replacing the sodium fluoride solution (0.05%), which is actually indicated as standard treatment option. Obviously, several aspects must be taken into account when considering the development of a substance like this. The main one is the effectiveness, but the availability and cost-effectiveness are essential. It is expected that companies have an interest in manipulating home-use solutions with the studied substances, so that we have more options for treatment and prevention of dental erosion in addition to what already exists on the market.

CONCLUSION

The treatments carried out in groups with experimental solutions (0.375% nano-HAp and fluoride) were able to promote mineral deposition, with results similar to professional application pastes with bioactive materials, and superior to the sodium fluoride solution.

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