ACTA ODONTOLOGICA SCANDINAVICA, 2018
VOL. 76, NO. 3, 188–194
https://doi.org/10.1080/00016357.2017.1401658

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Effect of dentifrice containing fTCP, CPP-ACP and fluoride in the prevention of enamel demineralization

Suzana Carvalho Teixeira Pinto de Souza[a], Kaline Cassiano de Araújo[a], Joseane Ribeiro Barbosa[b], Viviane Cancio[b,d], Anderson Araújo Rocha[c] and Mônica Almeida Tostes[d]

[a]School of Dentistry, Fluminense Federal University, Niterói, RJ, Brazil; [b]School of Chemistry, Fluminense Federal University, Niterói, RJ, Brazil; [c]Analytical Chemistry Department, Institute of Chemistry, Fluminense Federal University, Niterói, RJ, Brazil; [d]Pediatric Dentistry Department, School of Dentistry, Fluminense Federal University, Niterói, RJ, Brazil

ABSTRACT

Objective: To evaluate the effect of different fluoride- and calcium- and/or phosphate-containing products on their ability to prevent enamel demineralization under pH cycling conditions.

Material and methods: Enamel bovine specimens were assigned to the following groups: G1-MPP (MI Paste Plus, 0.2% NaF, Recaldent™, GC Corporation Tokyo, Japan); G2-FD (Crest™ Cavity Protection, 0.243% NaF, Procter & Gamble, USA); G3-CLP (Clinpro™ 5000, 1.1% NaF, 3M ESPE, USA); and G4-CO (Control without fluoride, Silica-based dentifrice; Daudt Ltda, Brazil). The specimens were soaked in demineralizing solution for 6 h and remineralizing solution for 18 h alternatively for 10 days. The toothpaste was prepared with deionized water in a 1:3 ratio (w/v) for three minutes daily. The solutions were renewed every 48 h. After cycling, enamel changes were analysed by percentage change of SMH (%SMH) and energy-dispersive X-ray spectroscopy (EDS). The %SMH value observed for G3-CLP (2.9 ± 39.2) was higher than that found in G4-CO (-13.0 ± 20.7), G1-MPP (-8.9 ± 20.9) and G2-FD (-3.9 ± 27.1). The %SMH was similar for all treatment groups (one-way ANOVA and Tukey's HSD; $p < .05$). The pH, Ca^{2+} and P_{total} in the remineralization solutions were not different among all groups (Kruskal–Wallis; $p < .05$). At 24 h, the Ca^{2+} concentration in the demineralization solution was significantly lower in G1-MPP. Ca^{2+} concentration increased in all groups after 48 h, except for G3-CLP. The EDX quantitative analysis showed that the atomic % of elements is lower level at G4-CO.

Conclusions: The Clinpro™ 5000 demonstrated having the most protective effect against demineralization; however, the % SMH was similar for all groups.

ARTICLE HISTORY
Received 20 August 2017
Revised 4 October 2017
Accepted 31 October 2017

KEYWORDS
Calcium phosphate systems; bovine enamel; microhardness; remineralization

Introduction

Fluoride is the cornerstone of the non-invasive management of non-cavitated caries lesions, but its ability to promote net remineralization is limited by the availability of calcium and phosphate ions [1,2]. The combination of fluoride and a source of bioavailable calcium and phosphate ions have been proposed as an effective treatment for the early stages of caries disease [2,3]. The casein phosphopeptide, amorphous calcium phosphate, is reported to have topical anticariogenic effects due to its ability to stabilize calcium and phosphate in an amorphous state. CPP not only increased fluoride incorporation into plaque, but it also increased the incorporation of fluoride into subsurface enamel and substantially increased remineralization of subsurface lesions of enamel compared with fluoride alone [2,3]. Despite this, *in vitro* enamel remineralization models have been widely used for prediction of the anti-caries efficacy of CPP-ACP treatment, but the results remain inconsistent [4–11]. The combination of CPP-ACP and fluoride has demonstrated better results in demineralization prevention than CPP-ACP alone [5,8,10].

Tricalcium phosphate products (functionalized β-tricalcium phosphate; fTCP) are prepared with silica, which may provide linking opportunities with hard-tissue defects under acidic conditions. Silica can permeate throughout enamel without attacking the inter-prismatic organic material, which may encourage greater calcium, phosphate and fluoride uptake in demineralized lesions [12]. It is an agent that works in synergy with fluoride to create a stronger, more acid-resistant mineral relative to that achievable with fluoride, β-TCP or fTCP alone [12,13]. Some initial reports have shown that Clinpro™ 5000 tooth Crème anti-cavity toothpaste containing fTCP, is useful for reducing white spot lesions [14]. fTCP is produced by milling TCP with sodium lauryl sulphate. This process prevents undesirable interactions between calcium and fluoride, which could render both inactive.

Based on the above considerations, this *in vitro* study evaluated the influence of the CPP-ACPF (MI Paste Plus- MPP) crème and fTCP on demineralization prevention of bovine enamel. Surface and chemical changes after the toothpaste treatment were evaluated by microhardness tests and energy dispersive X-ray spectroscopy (EDS). Considering that enamel

CONTACT Monica Almeida Tostes matostesuff@yahoo.com.br Universidade Federal Fluminense/Faculdade de Odontologia, Rua Mário Santos Braga, nº 30 – Campus Valonguinho, Centro, Niterói, RJ, CEP 24020-140, Brazil

surfaces are thought to face a high caries risk situation when exposed in the cariogenic condition, the evaluation of a possible additional cariostatic benefit of toothpastes containing fluoride in concentrations above the standard, appears to be particularly useful [6,15]. The second objective was to evaluate the calcium and phosphate concentrations available in the remineralizing and demineralizing solutions after each cycle of 24 and 48 h.

In this study, it was hypothesized that toothpastes containing a high fluoride concentration and calcium (Clinpro™ 5000) could provide additional protection against dental demineralization when compared to CPP-ACPF (MPP) and regular fluoride toothpaste (NaF, 1100 ppm F).

Material and methods

Sample preparation

Enamel specimens were prepared from 120 freshly extracted bovine permanent mandibular incisors. The teeth were cleaned to remove soft tissue and were stored in a 0.1% thymol solution (pH 7.0). Buccal surfaces were separated using a water-cooled diamond-impregnated low-speed saw. An enamel block was obtained from each tooth. After embedding the blocks in acrylic resin, the buccal surfaces of the enamel specimens (4 mm x 4 mm x 2mm) were ground with SiC paper (400, 600 and 1.200 grit sizes) to obtain flat surfaces.

The specimens were then polished using a 1μm diamond polishing suspension with a polishing cloth. The surface hardness was measured using a 2001 MicroMet micro-hardness tester with a Knoop type indenter, and with a static load of 50 g for 15 s. The Knoop hardness number (KHN) is calculated from the length of the indentation and the applied load. An increase in length in μm indicates a softening of the enamel due to demineralization. Five indentations separated by a distance of 100 μm were made in the central region of each block. The average of the five indentations made on each specimen was used as the SMH baseline value ($SMH_{baseline}$). Eighty blocks with a mean surface microhardness between 278.8 and 396.6 KHN were randomly divided into four groups of 20: G1-MPP (MI Paste Plus 0.2% sodium fluoride for NaF, Recaldent™, GC Corporation Tokyo, Japan); G2-FD (Crest™ Cavity Protection; 0.243% NaF, Procter & Gamble); G3-CLP (Clinpro™ 5000; 1.1% NaF, 3M ESPE, USA); and G4-CO (Control without fluoride, Silica-based dentifrice; Daudt Ltda, Rio de Janeiro, RJ, Brazil)

pH cycling and treatment with dentifrice

Before pH cycling, each enamel slab was immersed in 10 mL of artificial saliva for 24 h (0.67 gL^{-1} NaCl; 0.1168 gL^{-1} $CaCl_2$; 8 gL^{-1} CMC; 0.0408 gL^{-1} $MgCl_2$; 0.96 gL^{-1} KCl; 1 gL^{-1} $C_8H_8O_3$; 24 gL^{-1} $C_6H_{14}O_6$; 964.938 mLL^{-1} H_2O; 0.274 gL^{-1} KH_2PO_4) (Figure 1). Following this, the enamel specimens were submitted to pH cycle for 10 days at 37 °C. The specimens were immersed separately in 10 mL of the demineralizing solution (8 – 10 h, 12 – 14 h, 16 – 18 h), and in the remaining hours (18 h day) and they were transferred to a remineralizing solution (10 mL). The demineralization stage used an acid buffer containing 2 mM Ca^{2+} ($Ca(NO_3)_2$), 2 mM PO_4^{3-} (KH_2PO_4) and 75 mM acetate at pH 4.8. The remineralizing solution contained 1.5 mM Ca^{2+}, 0.9 mM PO_4^{3-}, 130 – 150 mM KCl, 0.5 mg F mL^{-1}, 100 mM TRIS buffer, pH 7.0. Standard pH-cycling conditions were used in a daily schedule of three exposures of two hours of demineralization and two exposures of two hours of remineralization. The specimens were exposed to the dentifrice slurries [1:3 toothpaste to deionized water ratio (w/v)] once a day, every day, for 3 minutes after the first demineralization. A standardized volume (60 mL) was used for each group and all specimens were immersed in fresh solution. The solutions of each agent were inserted into a pipette to standardize the volume of product applied to the enamel surface. After, 0.4 mL of each agent was applied to the enamel surface. After the last demineralization, the specimens were immersed in remineralizing solution until the next day (14 h). The solutions were renewed every 48 h. At each transfer between the different solutions, all specimens were rinsed in distilled water for 1 min before and after any solution change or dentifrice slurry application and they were wiped dry with a soft paper towel.

Microhardness analysis and energy-dispersive X-ray spectroscopy (EDS)

After completing 10 days of cycling, the post-treatment measurements ($SMH_{treated}$) were conducted with the same static load and time applied for the baseline measurements. Five indentations spaced 100 μm from the baseline indentations were made with a Knoop diamond indenter under a 50 g load for 15 s (Micromet 2001, Buehler, IL). The percentage change of SMH (%SMH) was calculated [%SMH =100 (SMH_baseline – SMH_treatment)/SMH_baseline]

Following the SMH measurement, the samples were left to air-dry at room temperature for 24 h. Five specimens in each group were randomly selected and analysed using EDS (SEM-HITACHI TM3000, Hitachi High Technologies Corporation, Japan). Five baseline slabs were also evaluated. The EDS examination was performed using a scanning electron microscope. Elemental analysis and precise chemical characterization of the samples were performed at 15,000 kV.

Determination of calcium (Ca^{2+}) and phosphate (P_{total}) in solutions

The experimental groups were tested for Ca^{2+} and P_{total} release. The solutions were changed every 48 h; so they were analyzed at 1, 3, 5, 7 and 9 days (the first 24 h after each exchange of solutions) and at 2,4,6,8 and 10 days (48 h after each exchange of solutions). The aliquot was removed at the end of each 24-h cycle (18 h of remineralization and 6 h of demineralization) and 48-h cycle (36 h of remineralization and 12 h of demineralization in the same solution). The aliquots of 0.5 mL were taken and replaced by fresh solution. The aliquots of the samples were diluted 20-fold in ultrapure water (resistivity >18 $M\Omega cm^{-1}$; Millipore, Bedford, MA).

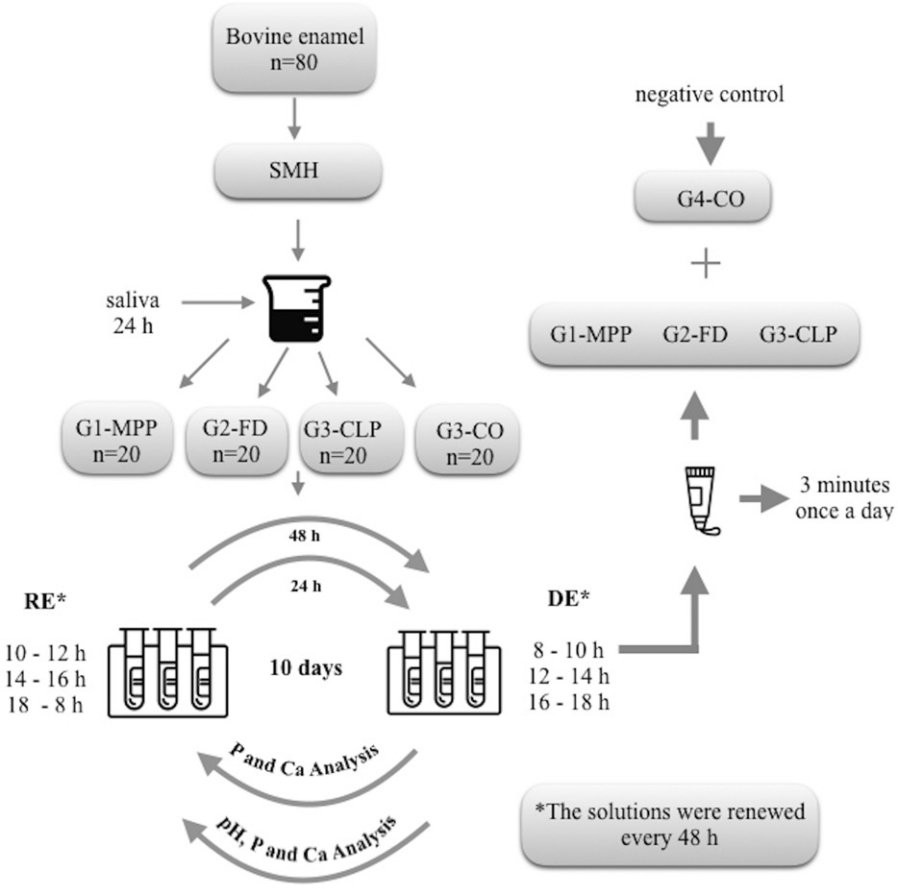

Figure 1. Schematic illustration of the procedure used in the pH cycling and remineralization treatment. MPP (MI Paste Plus); FD (Fluoride Dentifrice 1100 ppm F); CLP (Clinpro™ 5000 ppm F) and CO (no treatment).

A microwave plasma optical emission spectrometry instrument (MIP OES, 4200 MP-AES Agilent Technologies Inc., Santa Clara, CA) was used in the determination of Ca^{2+} and P_{total}. The plasma generator is based on microwave excitation that can maintain a nitrogen plasma (5000 K). The orientation of the plasma was vertical from an axial view. Sample introduction to the MIP OES was pneumatic using a concentric nebulizer and cyclonic spray chamber. Emission line isolation and detection is sequential using a Czerny–Turner monochromator and charge-coupled device (CCD) detector.

An external calibration method was used for the determination used for the determination of the analytes. Monoelement aqueous stock solutions containing 1000 mgL^{-1} of Ca^{2+} and P_{total} (VHG-Labs, Manchester, NH) were used to prepare standard reference solutions. A calibration curve (0.5 – 5.0 mgL^{1}) was prepared with the analytes diluted in ultrapure water. The correlation coefficients obtained were 0.99991 ($y = 814588x + 15.1$) and 0.99994 ($y = 419.52x - 0.4$) for Ca^{2+} and P_{total}, respectively. Limits of detection (LOD=3.σ_{10_blank} a^{-1}) results in 0.002 mgL^{-1} for Ca^{2+} and 0.300 mgL^{-1} for P_{total}. A test with a matrix-matching external calibration method showed that the presence of salinity did not affect the sensitivity of analytical curves.

The pH measurement was performed using the automatic titrator Methrom 808 Titrando, using Tiamo 2.3 software (Herisau, Switzerland). The pH was measured at the end of cycle (48 h). The electrode used was the Metrohm Unitrode with Pt 1000 (order number 6.0258.000), using 3 mol L^{-1} KCl aqueous solution (Gehaka, Rio de Janeiro, Brazil) as the reference electrolyte. The calibration was performed using the following Gehaka aqueous buffers: pH 4.01 ± 0.05 (biphthalate buffer), 7.01 ± 0.05 (phosphate buffer) and 10.01 ± 0.05 (bicarbonate buffer), yielding a slope of 91% with pH (0) 7.8 at 30 °C.

Statistical analysis

The data were analysed using SPSS statistical software package for Windows, version 20.0 (IBM Corporation, NY). Initially, all the data ($SMH_{baseline}$ and $SMH_{treated}$) were checked with Shapiro–Wilk's test. Based on these preliminary analyses, the $SMH_{baseline}$ and $SMH_{treated}$ data were submitted to one-way analysis of variance and Tukey's HSD *post hoc* test. Comparison before and after treatment in the same group was analysed with a paired *t*-test. The data of pH, Ca^{2+} and P concentrations were analysed using the Kruskal–Wallis test and Mann–Whitney. All analyses were performed at a significance level of α=.05.

Results

The mean $SMH_{baseline}$ was not significantly different among the groups (Table 1; ANOVA, $p = 0.65$). The G1-MPP, G2-FD, G3-CPL and G4-CO groups demonstrated a decrease in

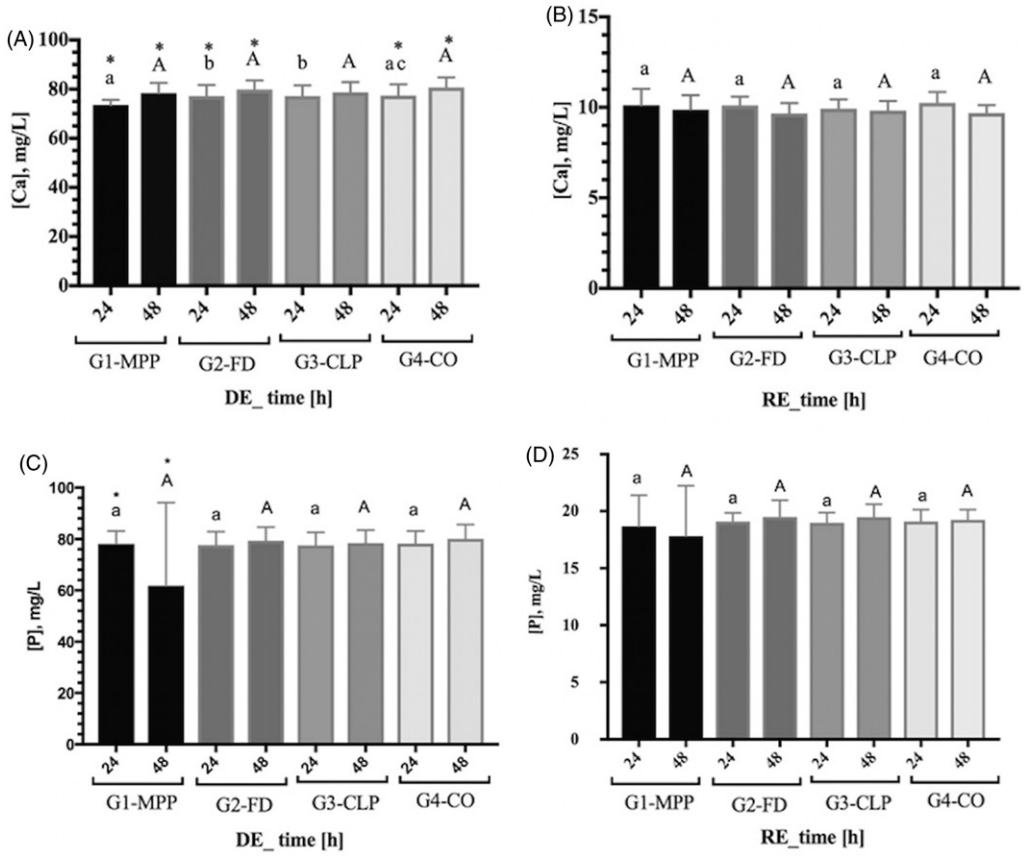

Figure 2. Mean of the [Ca], mg/L^{-1} (Figure 2A and B) and [P], mg/L^{-1} (Figure 2C and D) values for the four groups in the study at 24 and 48 h after pH cycling in demineralizing (DE) and remineralizing (RE) solutions. Results are expressed as means and standard deviations (SD). Different lowercase letters indicate significant differences among groups at 24 h. Different capital letters indicate significant differences at 48 h (unpaired *t*-test; $p < .05$). (*) indicates differences between 24 and 48 h (paired *t*-test; $p < .05$).

Table 1. Surface microhardness and %SMHC (mean ± SD) of bovine enamel specimens according to the different groups.

Groups $N = 20$	SMH_baseline	SMH_treated	% SMH
G1-MPP	264.9 ± 48.2^{a}	241.2 ± 36.5^{aA}	-8.9^{A} (20.9)
G2-FD	275.4 ± 42.2^{a}	264.5 ± 39.8^{aAB}	$-3,9^{A}$ (27.1)
G3-CLP	279.8 ± 39.5^{a}	288.0 ± 41.1^{aB}	2.9^{A} (39.2)
G4-CO	274.4 ± 47.9^{a}	238.6 ± 37.0^{bA}	$-13,0^{A}$ (20.7)

Mean of the SMH baseline and SMH-treated values for the four groups in the study. Results are expressed as means, standard deviations (SD). Different lowercase letters indicate significant differences in enamel SMH before and after treatment. Different capital letters indicate significant differences in enamel SMH and %SMH among the groups after treatment.

Table 2. Atomic percentages of calcium (Ca), phosphorus (P), oxygen (O) and Carbone (C) content of enamel according to the different treatment (mean ± SD) ($n = 5$).

	Ca Mean ± SD	P Mean ± SD	O Mean ± SD	C Mean ± SD
Baseline	18.51 ± 3.36	13.04 ± 2.08	60.67 ± 8.21	23.29 ± 1.56
G1-MPP	16.07 ± 1.60	10.93 ± 1.02	48.70 ± 5.26	22.20 ± 5.85
G2-FD	18.29 ± 3.16	11.84 ± 1.00	54.53 ± 1.31	18.21 ± 3.76
G3-CLP	16.16 ± 1.85	11.37 ± 0.76	51.95 ± 1.06	17.91 ± 2.97
G4-CO	15.05 ± 1.29	10.14 ± 0.59	47.70 ± 3.26	23.50 ± 1.42

microhardness after pH cycling, but a significant difference was found only G4 (Paired *t*-test; $p < .05$). Thus, mean $SMH_{treated}$ was higher in G3-CLP (288.0 ± 41.1) and G2-FD (264.5 ± 39.8) than in G1-MPP (241.2 ± 36.5) and G4-CO (238.6 ± 37.0) (Table 1). G3-CLP was significantly higher than G1-MPP ($p < .05$) and G4-CO ($p < .01$). The other groups did not differ ($p > .05$). Measurements were performed just before and after the treatment. Table 1 shows that all groups had a reduction of %SMH after the pH cycling except G3-CLP (2.9 ± 39.2). However, G4-CO (−13.0 ± 20.7) had a lower % SMH than G1-MPP (−8.9 ± 20.9) and G2 (−3.9 ± 27.1). The percentage change of SMH (%SMH) was similar for all treatment groups (one-way ANOVA and Tukey's HSD; $p < .05$)

Various structural chemical elements were detected in enamel –calcium (Ca), carbon (C), oxygen (O) and phosphorus (P) (Table 2). The toothpaste treatment had an almost negligible effect on concentrations of Ca, P, O and C regardless of the toothpaste applied. However, concentrations of these elements remained lower level at G4-CO, except for carbon ($p > .05$).

The inorganic calcium availability data (mean ± SD) collected in the DE solutions after 24 and 48 h periods for the four groups are presented in Figure 2(A). The baseline calcium in this solution was 63.0 mgL^{-1}. At 24 h, the calcium available in the DE solutions was lower than at 48 h in G1-MPP, G2-FD and G4-CO (paired *t*-test; $p < .05$), but not in G3-CLP. The concentration of calcium was lower in G1 than in

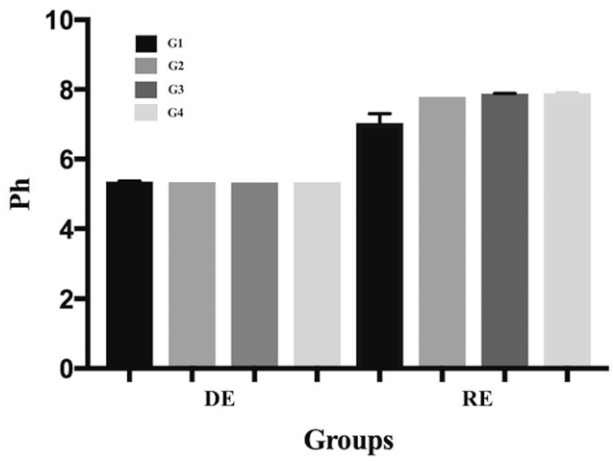

Figure 3. Mean of the pH values for the four groups in the study after pH cycling in remineralizing (RE) and demineralizing (DE) solutions. Results are expressed as means and standard deviations (SD). (Kruskal–Wallis; $p > .05$).

G2, G3 and G4 only after 24 h (unpaired *t*-test; $p < .05$). G1 and G4 were similar ($p > .05$).

The inorganic phosphate availability data (mean ± SD) collected in the DE solutions after each 24- or 48-h period for the four groups are presented in Figure 2(C). The baseline phosphate in this solution was 64.8 mgL^{-1}. In the 24 h and 48 h periods, the phosphate available in the DE solutions was similar in all groups (unpaired *t*-test; $p > .05$). The concentration of phosphate was lower in G1 after 48 h (paired *t*-test; $p < .05$).

In the RE solution, the concentration of calcium and phosphate was similar among groups (Figures 2(B,D); $p > .05$). The baseline concentrations of calcium and phosphate in this solution were 9.37 mgL^{-1} and 15.6 mgL^{-1}, respectively.

The pH of the baseline solutions was 4.8 and 7.0 for demineralizing and remineralizing solutions, respectively. The data of pH was showed in Figure 3. The mean of pH was not significantly different among the groups (Kruskal–Wallis; $p > .05$) and solutions.

Discussion

This study initially sought to investigate whether enamel demineralization could be prevented by CPP-ACP crème, fTCP dentifrice treatment, and fluoride dentifrice. It was demonstrated that fTCP (G3-CLP) treatment could increase resistance to demineralization. While this laboratory model could offer useful information about potentially condition [4,5,16], which is different from the *in vivo* situation, the results should be interpreted with caution. It is important to clarify that the design of the present study was not focused on showing the impact of the products on the remineralization of pre-formed enamel caries lesions, but on the prevention of demineralization. The change of the enamel was evaluated by microhardness and EDS. Microhardness analyses have been widely used to assess changes occurring in enamel after treatment with toothpaste and can provide indirect evidence of mineral loss or gain [4,5,9,11,17–20]. The standardization of enamel KHN in artificial caries allowed establishing the %SMH among the groups after treatment (4,5,19). As an indicator of enamel mineral loss or gain, surface hardness has been widely employed to assess changes occurring in enamel after treatment with toothpaste [17]. The remineralization and demineralization processes are dependent on the mineral changes in the structure of dental hard tissues [18,21]. The high levels of Ca, P elements in enamel represent an indication of the rate of remineralization [21].

In the present study, the application of fTPC-containing toothpaste (G3-CLP) resulted in a significantly prevented demineralization. The %SMH was positive only in G3-CLP, but did not differ in comparison with the other groups ($p < .05$). The %SMH results were consistent with the concentration of fluoride in the product (G3-CLP), which is an indicator of the effect of fluoride in demineralization and remineralization. This is evidenced by a clear dose response between G3-CLP and G4-CO; therefore, this outcome helps validate its applicability in assessing the *in vitro* remineralization potential of toothpaste fTCP. The possible mode of action of fTCP can be attributed to high fluoride and fTPC concentrations [22]. The soluble calcium, phosphate, and fluoride ions facilitate to remineralization with fluorapatite, which is more resistant to demineralization. In current study, at 48h, all groups showed a greater concentration of Ca^{2+} in the DE solution, except for the G3-CLP group; so, the lower Ca^{2+} availability can be relatively smaller dissolution in this group. As observed in previous studies [15,20,22,23], the fTPC/F combination increased the capacity of toothpaste to reduce the demineralization of the enamel. These results can be explained by the synergy with fluoride to create stronger, more acid-resistant mineral relative to that achievable with fluoride or fTCP alone [19]. Another studies have also been performed whereby 5000 ppm F plus fTCP provided significantly greater remineralization benefits when compared to control [14,15,22–24].

On the other hand, in this current study, bovine enamel treatment with fTCP (G3-CLP) was more resistant to the demineralization but was not different when compared to the NaF 1100-ppm F (G2-FD) group at the end of 10 days of cycling. There is reasonable evidence that high fluoride dentifrices significantly increase the fluoride concentration in saliva during the day and the fluoride concentration in plaque compared to traditional F toothpaste [25]. Due to the fact that only few randomized clinical trials have been conducted, there is not conclusive evidence at the highest level that high fluoride toothpaste plus fTCP performs better than traditional fluoridate toothpaste in the prevention. However, data from the few randomized clinical trials in this area showed no significant difference in remineralization between Clinpro Tooth Crème (950 ppm) and toothpaste with 0.1% or 0.15% w/v fluoride ion [3,13]. Recently, a report found no statistical differences in the remineralization of erosion lesions in bovine enamel using a 1100, 5000 or 5000-ppmF plus fTCP toothpaste *in vitro* [13]. In addition, despite the increase in fluoride levels, which was found especially with Clinpro™ 5000 toothpaste, all fluoridated products protected the dental substrates against erosion in a similar fashion when compared to placebo, but Clinpro™ 5000 toothpaste improved F retention on the tooth surfaces. [13]. However, the difference between placebo and treatment may not be detected because of the more severe demineralization [13]. Although,

in our study, prevention of demineralization was not significantly different among 5000-ppmF plus fTCP and 1100-ppm F dentifrices with respect to SMH data, we observed there was an advantage of 5000-ppm F plus fTCP dentifrice over 1100-ppm F dentifrices. The interaction of F with tooth surfaces may be saturated at some point, beyond which no further protection can be observed [13].

CPP-ACP has the capacity to stabilize calcium and phosphate on the tooth surface, thereby maintaining high concentration gradients of inorganic calcium and of phosphate ions, the latter of which promote remineralization of hard tissues [1,2]. However, the present study showed that MPP was less effective in reducing mineral loss than fTCP dentifrice, as shown by SMH and % SMH. In addition, the remineralizing of enamel with MPP has been previously studied with conflicting results. The conflicting results of these studies may be related to the different methods of specimen preparation, treatment period, additional application of fluoride, and different solutions for pH cycling. In this current study, to minimize differences between methodologies, all products were applied for three minutes, once a day, as per the manufacturer's instructions of MPP, but despite this, the results were not satisfactory. This result is in accordance with Karlinsey et al. [11] and Souza et al. [4]. These results may be due to the presence of the fluoride ion in MPP that could interact with the ACP component of the casein complex, rendering both inorganic components ineffective [11]. In the current study, the Ca^{2+}, P_{total} and pH were lower in the G1-MPP group, which could justify the low performance of this product in the model studied. In addition, the MPP showed lower ability to reduce mineral loss, which was similar to the control group (G4-CO). Probably, a greater amount of CaF in the medium negatively influenced the level of available fluoride [11]. Instead, CPP-ACPF provides superior remineralization effects and greater resistance to acid softening as compared to artificial saliva [5,9,24]. In the oral environment, only the Tooth Mousse Plus (TMP) product significantly increased salivary calcium and inorganic phosphate concentrations [3]. Thus, the presence of dental plaque seems to be crucial for the synergistic effect of CPP-ACP with fluoride [2,3]. The MPP product increased the concentration of calcium, phosphate and fluoride ions in saliva, which prevents spontaneous precipitation and allows penetration of the ions deep into subsurface lesions [2,3,24]. It has been suggested that CPP-ACP crème leads to more mineral deposits in the body the lesion than in the outerlayer [3,24]. Therefore, demineralization depth was not determined in this study. In addition, it might be necessary to have a longer application time to be able to detect some deposition of calcium and phosphate in a remineralized lesion [4,7,9,24,26].

The negative control group (G4-CO) showed the smallest hardness values after pH cycling ($p < .05$). The subsaturation condition can lead to the dissolution of hydroxyapatite and the diffusion of calcium and phosphate ions towards the enamel surface, reducing the SMH after the pH-cycling. These may be due to the limited availability of calcium, phosphate and fluoride ions in the solutions that did favor demineralization. It should be emphasized that to eliminate the influence of the minerals present in the products, the specimens were rinsed with deionized water after slurry exposure. We performed this procedure to standardize the amounts of residual toothpaste in the solutions and to eliminate the influence of the minerals and also to focus only on the ions adhered to the enamel. In this condition, the G4-CO showed the smallest %SMH value after the pH-cycling.

The Ca, P, O and C content was lower after treatment in all groups than at baseline (higid enamel); however, in G4-CO a lower level was found. This indicates the demineralization potential of the ph cyclic model used in the study and the protection against the demineralization of the enamel following treatment with dentifrice. Another aspect that may be observed in relation to the EDS results is that, based on the amounts of calcium, phosphorus, oxygen and carbon, the mineral contents of the three treatment groups are similar, a fact that is not in line with the obtained microhardness values.

The principle result of the present study is the demonstration of a dose response effect of the Clinpro™ 5000 containing functionalized tricalcium phosphate. The prevention of lesions may be speeded up by an exogenous Ca and P supply, but the clinical significance of the remineralization should be better evaluated. In addition, MPP might provide some benefits as an adjunctive therapy in children and adults at higher risk of developing caries [27].

Conclusions

The Clinpro™ 5000 demonstrated having the most protective effect against demineralization; however, the % SMH was similar for all groups. So, the Clinpro™ 5000 clearly does not provide additional protection against dental demineralization when compared to regular fluoride toothpaste (NaF, 1100 ppm F).

Acknowledgements

The authors acknowledge the technical support provided by MiCRON (Department of Geosciences) for performing the SEM-EDS analysis. The authors are grateful to NAB (Nucleous of Biomass and Water Research) at the logistic for its laboratorial assistance. They also thank the National Council for Scientific and Technological Development (CNPq) for Support and concession of scholarship to the second author (IC166552).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico.

ORCID

Viviane Cancio http://orcid.org/0000-0001-6932-7077
Anderson Araújo Rocha http://orcid.org/0000-0001-5179-1146
Mônica Almeida Tostes http://orcid.org/0000-0002-6878-7482

References

[1] Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res. 1997;76:1587–1595.

[2] Reynolds EC, Cai F, Cochrane NJ, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res. 2008;87:344–348.

[3] Shen P, Manton DJ, Cochrane NJ, et al. Effect of added calcium phosphate on enamel remineralization by fluoride in a randomized controlled *in situ* trial. J Dent. 2011;39:518–525.

[4] Souza CC, Cury JLM, Coutinho TCL, et al. Effect of different application frequencies of CPP-ACP and fluoride dentifrice on demineralized enamel: A laboratory study. Amer J Dent. 2014;27:215–219.

[5] Oliveira PR, Fonseca AB, Silva EM, et al. Remineralizing potential of CPP-ACP crèmes with and without fluoride in artificial enamel lesions. Aust Dent J. 2016;61:45–52.

[6] Oliveira GMS, Ritter AV, Heymann HO, et al. Remineralization effect of CPP-ACP and fluoride for white spot lesions *in vitro*. J Dent. 2014;42:1592–1602.

[7] Pulido MT, Wefel JS, Hernandez MM, et al. The inhibitory effect of MI paste, fluoride and a combination of both on the progression of artificial caries-like lesions in enamel. Oper Dent. 2008;33:550–555.

[8] Kumar VL, Itthagarun A, King NM. The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: An *in vitro* study. Aust Dental J. 2008;53:34–40.

[9] Elkassas D, Arafa A. Remineralizing efficacy different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions. J Dent. 2014;42:466–474.

[10] Hamba H, Nikaido T, Inoue G, et al. Effects of CPP-ACP with sodium fluoride on inhibition of bovine enamel demineralization: A quantitative assessment using micro-computed tomography. J Dent. 2011;39:405–413.

[11] Karlinsey RL, Mackey AC, Stookey GK, et al. *In vitro* assessments of experimental NaF dentifrices containing a prospective calcium phosphate technology. Am J Dent. 2009;22:180–184.

[12] Karlinsey RL, Pfarrer AM. Fluoride plus functionalized β-TCP: A promising combination for robust remineralization. Adv Dent Res. 2012;24:48–52.

[13] Scaramucci T, Borges AB, Lippert F, et al. *In vitro* effect of calcium-containing prescription-strength fluoride toothpastes on bovine enamel erosion under hyposalivation-simulating conditions. Am J Dent. 2015;28:18–22.

[14] Karlinsey RL, Mackey AC, Walker TJ, et al. *In vitro* remineralization of human and bovine white-spot enamel lesions by NaF dentifrices: A pilot study. J Dent Oral Hyg. 2011;3:22–29.

[15] Vanichvatana S, Auychai P. Efficacy of two calcium phosphate pastes on the remineralization of artificial caries: a randomized controlled double-blind *in situ* study. Int J Oral Sci. 2013;5:224–228.

[16] Montasser MA, El-Wassefy NA, Taha M. In vitro study of the potential protection of sound enamel against demineralization. Prog Orthod. 2015;16:12.

[17] Arends J, ten Bosch JJ. Demineralization and remineralization evaluation techniques. J Dent Res. 1992;71:924–928.

[18] Savas S, Kavrìk F, Kucukyìlmaz E. Evaluation of the remineralization capacity of CPP-ACP containing fluoride varnish by different quantitative methods. J Appl Oral Sci. 2016;24:198–203.

[19] Fernández CE, Tenuta LM, Del Bel Cury AA, et al. Effect of 5,000 ppm fluoride dentifrice or 1,100 ppm fluoride dentifrice combined with acidulated phosphate fluoride on caries lesion inhibition and repair. Caries Res. 2017;51:179–187.

[20] Mensinkai PK, Ccahuana-Vasquez RA, Chedjieu I, et al. In situ remineralization of white-spot enamel lesions by 500 and 1,100 ppm F dentifrices. Clin Oral Investig. 2012;16:1007–1014.

[21] Nakata K, Nikaido T, Ikeda M, et al. Relationship between fluorescence loss of QLF and depth of demineralization in an enamel erosion model. Dent Mater J. 2009;28:523–529.

[22] Karlinsey RL, Mackey AC, Walker ER, et al. Surfactant-modified b-TCP: structure, properties, and in vitro remineralization of subsurface enamel lesions. J Mater Sci: Mater Med. 2010;21:2009–2020.

[23] Amaechi BT, Karthikeyan R, Mensinkai PK, et al. In situ remineralization of a new high-fluoride dentifrice. Gen Dent 2012;60:186–192.

[24] Kucuk EB, Malkoc S, Demir A. Microcomputed tomography evaluation of white spot lesion remineralization with various procedures. Am J Orthod Dentofacial Orthop. 2016;150:483–490.

[25] Ekstrand KR, Ekstrand ML, Lykkeaa J, et al. Whole saliva fluoride levels and saturation indices in 65+ elderly during use of four different toothpaste regimes. Caries Res. 2015;49:489–498.

[26] Baroni C, Marchionni S, Bazzocchi MG, et al. A SEM and non-contact surface white light profilometry *in vivo* study of the effect of a crème containing CPP-ACP and fluoride on young etched enamel. Scanning. 2014;36:36270–36277.

[27] Tostes MA. Remineralizing potential of CPP-ACP creams with and without fluoride in artificial enamel lesions: Authors' reply. Aust Dent J. 2016;61:391.