©Operative Dentistry, 2021, **46-6**, E296-E306

Effects of Remineralizing Agents Based on Calcium Phosphate, Sodium Phosphate, or Sodium Fluoride on Eroded Cervical Dentin

WG Escalante-Otárola • GM Castro-Núñez • TP Leandrim • CM Alencar
FF de Albuquerque Jassé • MC Kuga

Clinical Relevance

Calcium phosphate-based remineralizing agents can prevent the harmful effects of erosion challenges on the dentin collagen matrix. In addition, they may be a promising and viable alternative for treatment of dentin hypersensitivity—and prevent the harmful impacts of erosion challenges on the collagen matrix.

SUMMARY

Objectives: To evaluate the effect of remineralizing agents on collagen matrix pattern, precipitate formation, and dentinal tubule obliteration in eroded cervical dentin.

Methods and Materials: One hundred bovine cervical dentin specimens were previously eroded (0.6% hydrochloric acid, pH 2.3, 5 minutes) and then randomized into five groups (n=20): G1, control (without treatment); G2, Desensibilize Nano P (FGM); G3, MI Paste Plus (Recaldent); G4, Regenerate (NR-5); and G5, Desensibilize KF 2% (FGM). These treatments were applied in four sessions with 7-day intervals. During this period, the samples were subjected to an erosive

Wilfredo Gustavo Escalante-Otárola, DDS, MS, PhD, NCCL Research Group, School of Dentistry, Faculty of Health Sciences, Jorge Basadre Grohmann National University – UNJBG, Tacna, Perú; Department of Restorative Dentistry, Araraquara School of Dentistry, Paulista State University – UNESP, Araraquara, SP, Brazil

Gabriela Mariana Castro-Núñez DDS, MS, PhD, School of Dentistry, Faculty of Health Sciences, Jorge Basadre Grohmann National University – UNJBG, Tacna, Perú

Thaís Piragine Leandrim DDS, MS, PhD student, Department of Restorative Dentistry, Araraquara School of Dentistry, Paulista State University – UNESP, Araraquara, SP, Brazil

Cristiane de Melo Alencar DDS, MS, PhD student, Department of Restorative Dentistry, Araraquara School of Dentistry, Paulista State University – UNESP, Araraquara, SP, Brazil

Fernanda Ferreira de Albuquerque Jassé DDS, MS, PhD, School of Dentistry, Federal University of Para, Belém, PA, Brazil

*Milton Carlos Kuga DDS, MS, PhD, Department of Restorative Dentistry, Araraquara School of Dentistry, Paulista State University – UNESP, Araraquara, SP, Brazil

*Corresponding author: Humaitá Street 1680, Araraquara, SP, 14801-903, Brazil; e-mail: kuga@foar.unesp.br

http://doi.org/10.2341/20-209-L

challenge with orange juice (pH 3.8, 5 minutes). The specimens were analyzed by polarized light microscopy with picrosirius red staining, scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX).

Results: The G3 showed a higher concentration of type I collagen than G2 and G5 (p<0.05). The G3 showed greater formation of surface precipitates than that of G1 and G5 (p<0.05). In addition, G4 and G5 showed a greater number of open dentinal tubules than that of G3 (p<0.05).

Conclusions: Calcium phosphate-based remineralizing agents have shown to be a promising alternative treatment for preventing deleterious effects on the eroded dentin collagen matrix. In addition, they promoted precipitate formation and dentinal tubule obliteration on the eroded dentin.

INTRODUCTION

A wide variety of products based on different technologies containing phosphate and calcium are commercially available, with the aim of providing greater resistance to demineralization of dental substrates or even recovering their mineralization. Among them, calcium- and sodium phosphate-based ones have been launched, focusing on erosion prevention and treatment. However, most of them are limited to their role on enamel and not on dentin.[1,2] With regard to dentin treatment, greater complexity is involved, due to the participation and dynamics of organic content. In addition, the available evidence in this regard is limited.[3] According to the best knowledge of the authors, to date, no evidence has been found regarding the effect of different remineralizing treatments on the profile of the eroded dentin collagen matrix.

One of the most frequent causes of dental tissue loss and exposure of cervical dentin is dental erosion.[4] A previous study showed that gastroesophageal reflux (GR) of hydrochloric acid is directly related to dental erosion.[5] At the same time, acidic foods and drinks have been increasingly included in current diets.[6] Prevalence studies have shown that erosion caused by both intrinsic[7] and extrinsic[8] factors have high incidences. However, there are few materials on the market specifically indicated for the prevention and treatment of dental erosion. For this reason, alternative materials with remineralizing potential have been investigated.

Materials containing calcium phosphate nanoparticles in the form of nanohydroxyapatite (n-HAP) facilitate the deposition of precipitates on dentin and are recommended for dental remineralization in patients with dentin hypersensitivity (DH).[9] This action mechanism is related to the potential of stabilizing calcium and amorphous phosphate ions in dental structures.[10] Another material studied is casein phosphopeptide-amorphous calcium phosphate associated with sodium fluoride (CPP-ACPF). The remineralizing mechanism of CPP-ACPF is related to the supply of calcium and phosphate ions on the tooth surface. In addition, it facilitates the stabilization of mineral precipitates that contain fluoroapatite.[11,12] Its action mechanism is similar to n-HAP, and CPP-ACPF has also been recommended for the treatment of DH.[11-16]

Calcium silicate and sodium phosphate (mono and trisodium phosphate) have been developed for the prevention and treatment of dental demineralisation.[15,16] The probable action mechanism of this product is related to the deposition of calcium silicate particles and induction of hydroxyapatite formation on the dental substrate.[17] This material can reduce dentin permeability and, therefore, it is a promising alternative for DH treatment.[18,19] Potassium nitrate associated with sodium fluoride (NaF) is another material widely studied in the current literature, but its reported effects on DH have been controversial.[20,21] Potassium ions (K^+) are the active component of this material and promote a reduction in the activity of dentinal sensory nerves due to the depolarizing activity of K^+. On the other hand, NaF acts on tooth sensitivity by blocking exposed dentinal tubules and reducing the flow of fluid to the pulp.[22]

Therefore, the aim of the present study was to evaluate the effect of remineralizing materials containing calcium phosphate nanoparticles (Desensibilize Nano P, FGM, Joinville, SC, Brazil), CPP-ACPF (MI Paste Plus Recaldent CPP-ACP, GC Company, Japan), calcium silicate associated with sodium phosphate (Regenerate NR-5, Unilever, Wirral, United Kingdom), and potassium nitrate associated with NaF (Desensibilize KF 2%, FGM) on the presence of collagen in eroded cervical dentin, the formation of precipitates, and obliteration of dentinal tubules. The null hypotheses tested were: H01—There are no differences in the presence of collagen in eroded cervical dentin under the different treatments tested; H02—There are no differences in the formation of precipitates and obliteration of dentinal tubules under the different treatments tested.

METHODS AND MATERIALS

Sample Preparation

One hundred bovine incisors with anatomically similar crowns and roots were stored in 0.1% thymol solution at 4°C until use. One hundred specimens (10 mm long × 10 mm wide × 5 mm thick) were obtained from the cervical-third of the teeth using a cutting machine (Isomet 100, Buehler, Lake Bluff, IL) under constant refrigeration. The buccal surface of the fragments was polished on a polishing machine (DP-10; Panambra, Struers, Ballerup, DI) using a sequence of #600 and #1200 granulation sandpapers, for 20 seconds each, to expose the cervical dentin.

The dentin surface was previously eroded with 0.6% HCl and pH 2.3 (Arte & Ciência, Araraquara, São Paulo, Brazil) by immersion for 5 minutes. Then, the specimens were rinsed with distilled water and stored in artificial saliva (0.375 g/l $CaCl_2 2H_2O$, 0.125 g/l $MgCl_2 6H_2O$, 1.2g/l KCl, 0.85g/l NaCl, 2.5g/l $NaHPO_4 12H_2O$, 1g/l sorbic acid, 5 g/l hydroxyethyl cellulose-sodium, and 43 g/l sorbitol solution) (Arte & Ciência) at 37°C until use.

Treatments and Erosive Challenges

The specimens were removed from the artificial saliva and slightly dried with soft paper. After that, the samples still wet received the application of the treatment materials. Table 1 shows the chemical composition of the remineralizing agents used in this study. Twenty specimens were used per treatment protocol (n=20), 10 specimens for polarized light microscopy, and 10 specimens for SEM/ energy-dispersive X-ray spectroscopy (EDX) analysis. The evaluated protocols were as follows:

- (G1): Specimens were kept in deionized water for 10 minutes.
- (G2): The product containing calcium phosphate nanoparticles (Desensibilize Nano P) was applied with a microbrush (KG Sorensen, São Paulo, SP, Brazil) and rubbed on the dentin surface with a rubber bowl (KG Sorensen) in low-speed rotation (500 rpm for 10 seconds). Then, the material was left in contact with the dentin for 5 minutes and was rinsed with 10 mL of deionized water, according to the manufacturer's recommendations.
- (G3): The material containing CPP-ACPF (MI Paste Plus Recaldent) was applied with a microbrush (KG Sorensen) and left in contact with the dentin surface for 3 minutes. The residues were then rinsed with 10 mL of deionized water.
- (G4): The material containing calcium silicate and sodium phosphate (Regenerate NR-5) was previously manipulated according to the manufacturer's guidelines, and subsequently applied with a microbrush (KG Sorensen) and left in contact with the dentin for 3 minutes. The residues were then rinsed with 10 mL of deionized water.
- (G5): The material containing potassium nitrate and 2% NaF (Desensibilize KF 2%) was applied to the dentin surface with a microbrush (KG Sorensen), without friction, and left in contact with the dentin for 10 minutes, according to the manufacturer's recommendations. The residues were then rinsed with 10 mL of deionized water.

Remineralizing treatments and erosion challenges were performed for 28 days. The specimens were

Table 1: *Composition of Materials Used in the Study*

Group	Composition	Manufacturer
G1: Control	Deionized water	—
G2: Desensibilize Nano P	Calcium phosphate (nano-HAP), potassium nitrate, and 9000 ppm NaF	FGM, Joinville, Brazil (Lot: 270417)
G3: MI Paste Plus Recaldent	CPP-ACPF, glycerol, D-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavoring, sodium saccharin, ethyl p-hydroxybenzoate, propyl p-hydroxybenzoate, butyl p-hydroxybenzoate, 900 ppm fluoride	GC, Tokyo, Japan (Lot: 1401221)
G4: Regenerate NR-5	Glycerin, calcium silicate, PEG-8, hydrated silica, trisodium phosphate, sodium phosphate, aqua, PEG-60, sodium lauryl sulfate, sodium monofluorophosphate, synthetic fluorphlogopite, sodium saccharin, polyacrylic acid, tin oxide, limonene, CI 77891, 1450 ppm fluoride	Unilever, Wirral, United Kingdom (Lot: 4175000)
G5: Desensibilize KF 2%	5% Potassium nitrate, 2% sodium fluoride (NaF), deionized water, glycerin, neutralizing and thickening agent	FGM, Joinville, Brazil (Lot: 131014)

Abbreviations: CPP-ACPF, casein phosphopeptide-amorphous calcium phosphate fluoride; NaF, sodium fluoride; PEG, polyethylene glycol.

submitted to four remineralizing treatment sessions, with 7-day intervals. Every day, the specimens were subjected to acid challenge by immersion in orange juice (Minute Mais, Coca Cola, SP, Brazil) at pH 3.80 for 5 minutes. After the erosive challenge, the samples were rinsed with 10 mL of deionized water and kept individually immersed in artificial saliva at 37°C. After each remineralizing treatment session, the specimens were subjected to the erosive challenge in the same way as the other days.

Polarized Light Microscopy Analysis

After undergoing the test protocols, the specimens were stored for 24 hours in artificial saliva. Then, they were placed in a 10% ethylenediaminetetraacetic acid (EDTA) solution (Merck, Darmstadt, Germany) at pH 7.0. This solution was changed twice a week for 90 days. After that, the specimens were rinsed again with distilled water, dehydrated in alcohol, diaphanized in xylol, and included in paraffin (Histosec, Merck, Darmstadt, Germany). The paraffin blocks were subjected to semiserial histological sectioning of 5-μm thickness and submitted to the picrosirius red staining technique.[23]

After histological processing, each sample was analyzed using a microscope (Olympus BX51, Tokyo, Japan) equipped with filters to provide circularly polarized illumination. All image-acquisition parameters were standardized, and the intensity of illumination was calibrated by adjusting only the microscope's condenser opening. Digital images were obtained at 20× magnification (DP-71, Olympus, Tokyo, Japan). The spectra of green, yellow, and red colors were defined following RGB values (red, green, blue) that were standardized for all images.

According to the orientation of the collagen fibers, the color of the spectrum can vary from greenish (type III collagen) to reddish (type I collagen).[24] To quantify only the greenish and reddish fibers, the images were analyzed using Affinity Photo Software (Serif Europe Ltd, Nottingham, United Kingdom), and the number of pixels for each color was calculated. In the sequence, the averages of the values obtained were calculated and expressed as percentages.

Scanning Electron Microscopy (SEM) Analysis

After completing the treatments described, the specimens were stored in artificial saliva for 24 hours. Then, they were dehydrated in a closed chamber with colloidal silica for 7 days. After assembling the specimens in metal stubs, they were metallized with colloidal gold (simple cycle: 120 seconds) under vacuum in a metallization chamber (MED 010, Balzers Union, Balzers, Liechtenstein) and examined by SEM with JEOL 6060 (JEOL Ltd, Tokyo, Japan) operated at 20 kV, as described by Escalante-Otárola and others.[25]

Representative images were obtained from the central region of each specimen. First, a 500× magnification image was obtained to evaluate and quantify the precipitates formed on the dentin surface. Another image was obtained from the same location with 2000× magnification, for counting open dentinal tubules. All images were obtained by a single operator.

Two properly calibrated independent examiners, using the weighted Kappa test (k= 0.82), classified the presence of precipitates formed on the dentin surface according to the modified parameters described by Kuga and others.[26] (Scores from 0 to 4): Score 0, without precipitates; Score 1, any precipitates and all the tubules opened; Score 2, minimum amount of precipitates and > 50% of the dentin surface clean; Score 3, moderate amount of precipitates and < 50% of the dentin surface clean; Score 4, heavy precipitates with almost all tubules obstructed.

Energy-dispersive X-ray (EDX) Spectroscopy Analysis

Four specimens from each protocol were covered with carbon (BalTec SCD 004 Sputter Coater, Balzers) at 15 kV for 180 seconds. Then they were analysed by EDX (JEOL 6060; JEOL Co., Tokyo, Japan) to evaluate the main chemical components present on the dentin surface after performing the experimental protocols.

Statistical Analysis

Initially, all data obtained were subjected to the Shapiro–Wilk test to ascertain the data normality. Data on the dentin organic matrix were subjected to two-way ANOVA and Bonferroni tests. Data on the formation of precipitates and the number of open dentinal tubules (SEM) were subjected to Kruskal–Wallis and Dunn tests. All assessments were performed with a significance level of $\alpha = 0.05$.

RESULTS

Polarized Light Microscopy Analysis

G3 presented more type I (mature) collagen and less type III (immature) collagen than that in G2 and G5 ($p<0.05$). In addition, G2, G3, and G5 did not show significant differences with the other groups ($p>0.05$). Figure 1 shows the percentage of the presence of type I and type III collagen in the dentin substrate for each group. Figure 2 shows the incidence of type I (red

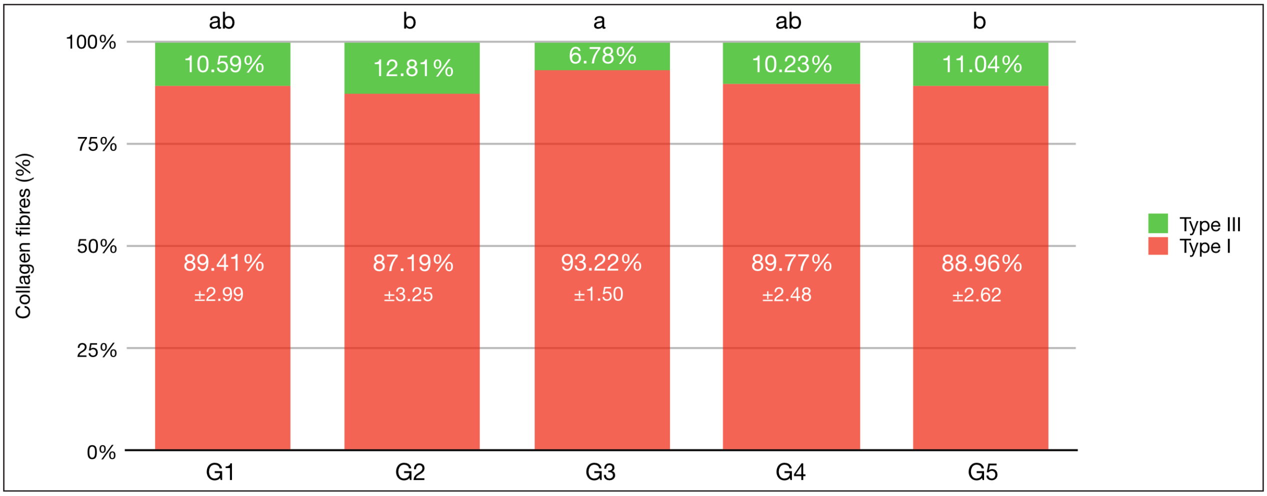

Figure 1. *Results on the dentin organic matrix evaluation. a,b: Different letters indicate significant differences (p<0.05). G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%.*

shade) and type III (green shade) collagen in the dentin substrate after staining with picrosirius red.

SEM Analysis

Table 2 shows the results of the formation of immediate precipitates in eroded cervical dentin and the occlusion pattern of dentinal tubules. Regarding the evaluation of dentin surface, G3 showed a higher incidence of precipitates on the dentin surface than that of G1 and G5 (p<0.05). However, there was no difference in precipitate formation between G2, G3, and G4 (p>0.05). On the other hand, there was no difference between G1 and G5 data compared to G2 and G4 (p>0.05).

Regarding the number of open dentinal tubules, G3 presented the lowest incidence of open dentinal tubules when compared to that of G1, G4, and G5 (p< 0.05). However, there was no significant difference between G2 and G3 (p>0.05). Figure 3 shows a representative image of the formation of precipitates on the surface (500×) and obliteration of dentinal tubules (2000×) for each evaluated group.

EDX Analysis

The EDX analysis showed that G3 presented higher concentrations of Ca/P (atomic ratio: Ca/P=1.93) when compared to the other groups. In addition, it demonstrated the presence of silicon (Si), magnesium (Mg), and sodium (Na). G1, G2, G4, and G5 groups showed the following concentration values between Ca/P atomic ratio: (G1), Ca/P=2.02; (G2), Ca/P=1.78; (G4), Ca/P=1.95; and (G5), Ca/P=1.89.

Figure 4 shows the relationship between Ca/P after the use of the tested materials. On the other hand, Figure 5 shows a representative image of the precipitate composition.

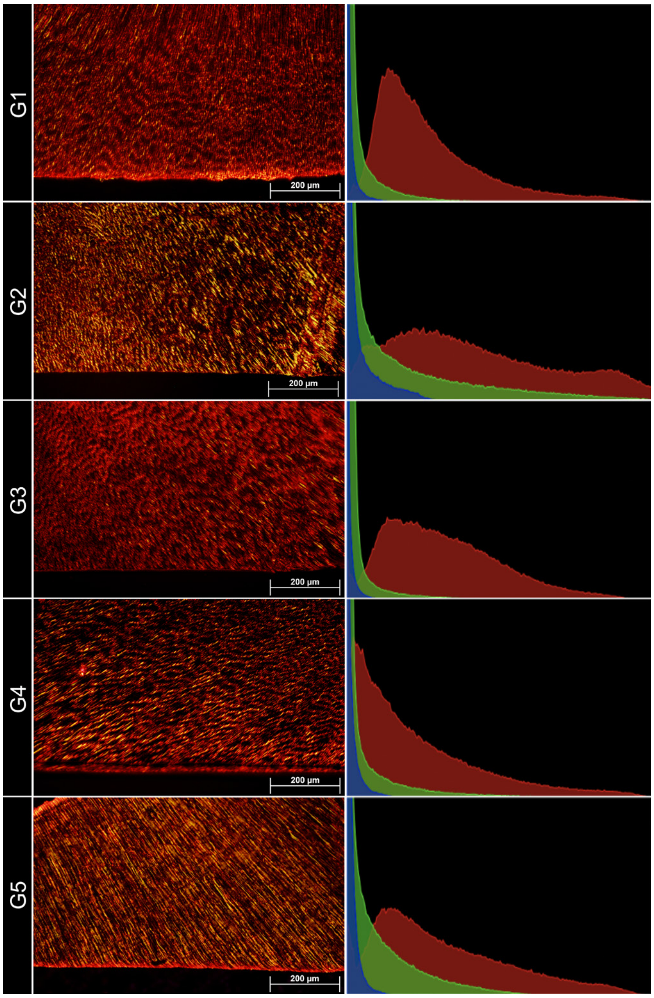

Figure 2. *Representative image of the eroded cervical dentin organic matrix after treatments. G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%. Scale 200 mm.*

Table 2: *Quantitative Determination of Precipitates Formation (Average Scores) and Number of Open Dentinal Tubules*[a]

		G1	G2	G3	G4	G5
Precipitates over dentin (score)	Median	1 b	2 ab	3 a	2 ab	1 b
	Min/Max	1-2	1-3	2-3	1-3	1-2
	1Q/3Q	1-1	2-3	2-3	2-3	1-1
Open dentinal tubules	Mean	61.71 c	26.86 ab	18.57 a	43.14 bc	45.29 bc
	SD	6.90	8.57	4.76	9.12	9.55

Abbreviations: 1Q and 3Q, first and third quartile; Min, minimum value; Max, maximum value; SD, standard deviation; G1, Negative control; G2, Desensitize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensitize KF2%.
[a] *Different lowercase letters on the same line indicate significant differences* ($p<0.05$).

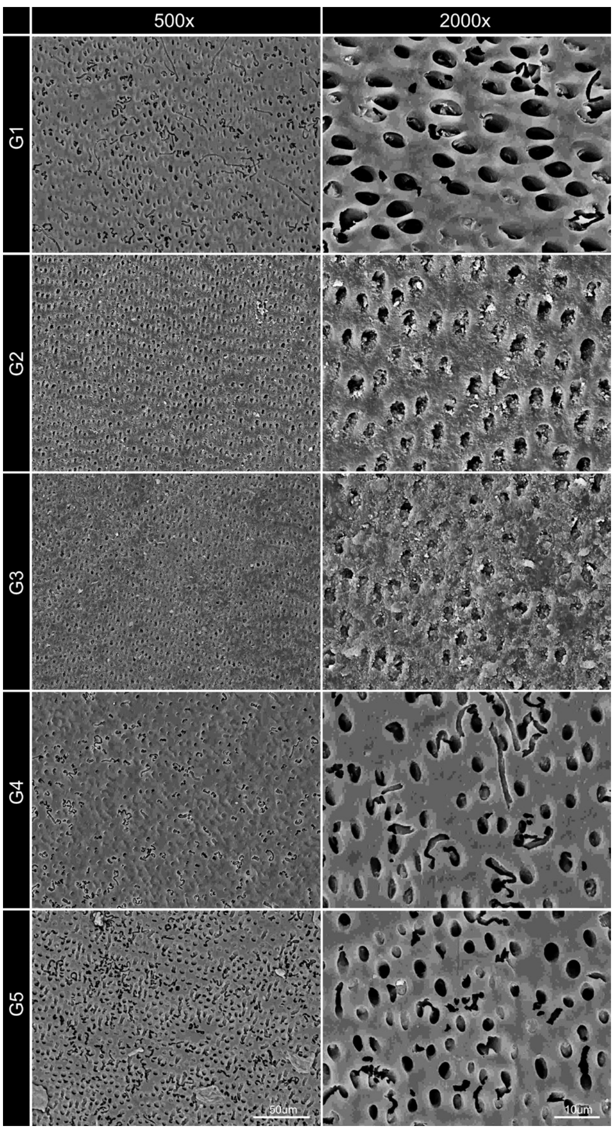

Figure 3. *Representative image of the eroded cervical dentin surface treatments, at 500× and 2000×. G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%. Scales 50 mm and 10 mm.*

DISCUSSION

Currently, the management of dental erosion is a challenge for the clinician, and several forms of treatment and prevention have been investigated.[11-15] The severity of erosive tooth loss depends on several parameters, such as quality and quantity of saliva, type of nutrition, and association with abrasion and mechanical friction.[4] In the present study, remineralizing treatments were performed on previously eroded dentin. This methodology was carried out to simulate the clinical situation of patients with erosion, which is unlike many previous studies that carried out treatment experiments with healthy dentin.

The erosive demineralization of dentin results in the exposure of an outer layer of fully demineralized and often denatured organic matrix.[27] For this reason, the process of remineralization and biological reconstruction of this eroded substrate is crucial for its treatment.[28] The main organic component of dentin is type I collagen, which has a complex structure with four levels of hierarchical organization. One of the levels is in the molecular category formed by fibrils or collagen fibers.[29] These collagen fibrils are arranged both linearly and laterally in a hydrogel network under suitable physical–chemical conditions.[30] The distribution pattern of hydroxyapatite in type I collagen is distinguished in intrafibrillar and extrafibrillar mineralization. In the first case, hydroxyapatite is deposited within the gap area of the collagen fibrils, and, in the second case, it is deposited on the surface of the collagen fibrils.[31,32]

Previous studies have already investigated the use of some materials with remineralizing potential on demineralized dentin; however, the investigations of these treatments on dentin collagen are scarce.[33,34] Although a given material has good remineralizing properties on the dentin surface, it is crucial that it does not cause deleterious effects to the collagen matrix. In case of damage to the matrix, it may compromise the longevity of future restorations.[25,35] In the present

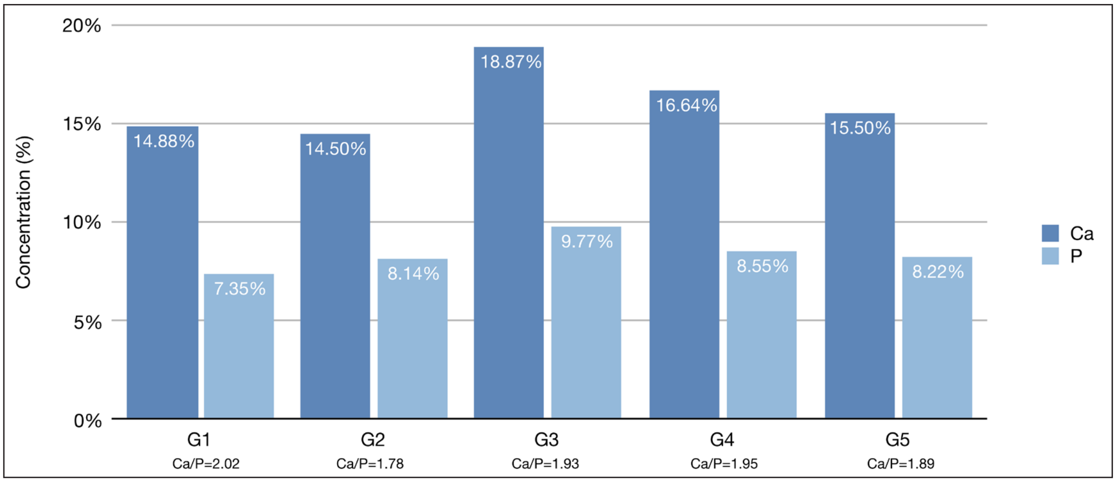

Figure 4. *Images of X-ray energy dispersion [energy-dispersive X-ray spectroscopy (EDX)] analysis. Ca, calcium; Ca/P, atomic ratio; G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%; P, phosphorus.*

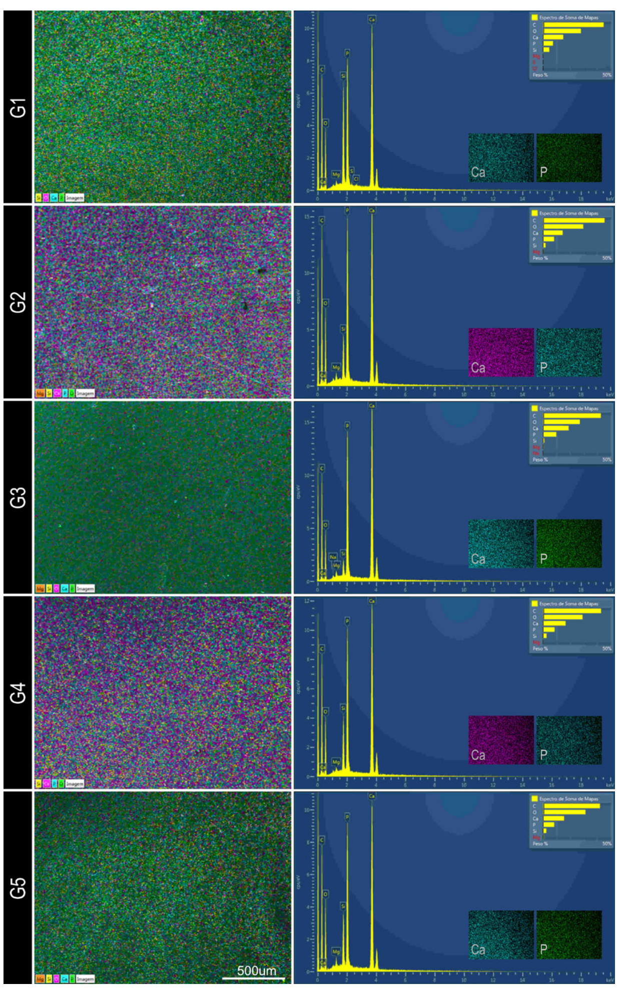

Figure 5. *Image of X-ray energy dispersion [energy-dispersive X-ray spectroscopy (EDX)] analysis of the eroded cervical dentin surface after treatments. G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%. Scale 500 mm.*

study, polarized light microscopy and staining with the picrosirius red system was used to detect collagen, where red–yellow pigmentation and yellow–green pigmentation show type I and type III collagen, respectively. This method has been recommended by previous studies.[23,24]

The results of the present investigation showed that the dentin substrate in G3 showed a higher incidence of type I collagen without abnormalities. However, in G2 and G5, there was a higher incidence of type III collagen, probably due to the presence of potassium ions, which can trigger changes in the organic matrix of the dental substrate.[36] Therefore, the null hypothesis H01 was rejected. A previous study conducted by Osorio and others[33] showed that the use of silver-loaded nanoparticles contributed to improve the interconnection, nature, and secondary structure of collagen in demineralized dentin. These results can be justified due to the use of polymeric nanoparticles, which are materials that sequester calcium and phosphate, facilitating the remineralization of dentin.[37,38] In the present study, only the group treated with CPP-ACPF showed the type I collagen pattern without changes even in face of the erosive challenge. It is possible to hypothesize that the presence of calcium and phosphate ions in this material contributed to an effective remineralization and stabilization of type I collagen fibrils. However, further studies are necessary for a definitive conclusion on this issue.

In addition, G3 protocol demonstrated greater formation of precipitates on the dentinal surface and a lower incidence of open dentinal tubules than those of the G1 and G5. Therefore, the null hypothesis H02 was rejected. This can be explained by the mechanism of action of this material on eroded dentin. G3 contains in its chemical composition the CCP-ACPF and 900 ppm of fluorine,[39,40] and when applied to the dental substrate, it forms fluorapatite, which is a more stable and less soluble mineral than hydroxyapatite.[41,42] In addition, the fluorapatite layer has acid-resistant properties,[43,44] which could justify G3 results in this study.

EDX analysis showed a higher incidence of Ca and P on the dentin surface treated with CCP-ACPF, when compared to that of the other treatments. This finding shows that the presence of these ions is directly related to the presence of a layer of stable precipitates as well as resistance to the acid challenge in the dentin studied in G3. In addition, this material contains the milk protein casein, which could stabilize calcium phosphate in a solution and increase the level of calcium phosphate in enamel and dentin.[45] A previous study conducted by Alencar and others[11] evaluated the antierosive potential of CPP-ACPF on dentin, and concluded that this material is capable of obliterating dentinal tubules and promoting an increase in Ca and P ions on the dental surface. These results corroborate the findings of the present study.

G2 treated with n-HAP associated with 9000 ppm of NaF also showed promising results in the formation of superficial precipitates and the obliteration of dentinal tubules in this study. The nanoparticulate hydroxyapatite is morphologically similar to enamel apatite crystals and has high biocompatibility with dental tissues. Initially, n-HAP was studied as a biomimetic material for the reconstruction of lost enamel because of its high remineralizing potential.[46,47] The phosphate and hydroxide anions released by n-HAP trap the hydrogen cations of the acid, and this reaction may be related to a decrease in the titratable acidity of saliva due to the increase in the concentration of n-HAP.[48] This could explain the antierosive effect of this material on dentin in the present study. However, a recent study showed that although n-HAP shows promising results in preventing dental erosion, it should not be used as the only treatment in the management of erosive tooth loss. It should be part of a preventive program associated with other strategies.[12] Another recent study showed that n-HAP applied only once did not show positive results in the obliteration of dentinal tubules.[11] It is possible that this material needs more applications to show an effective result.

The material used in G2 also contains 9000 ppm of NaF in its composition. It is possible that the remineralizing effect of NaF associated with n-HAP promoted a synergistic effect, potentiating the remineralizing effect of the material. NaF interacts with saliva and forms a layer of calcium fluoride (CaF_2) over the dentin. During an erosive challenge, this layer protects the dental structure from acid-induced degradation. Thus, the presence of NaF in the oral cavity can prevent the acid from causing irreversible damage to dentin.[49,50] On the other hand, this protective layer did not show a significant presence of Ca and P ions when compared to that of G3. It is possible that materials containing n-HAP and NaF are more susceptible to the erosive challenge than CPP-ACPF. This result corroborates with the findings of Alencar and others.[11]

Although the compound with calcium silicate and sodium phosphate used in G4 induced the formation of precipitates on dentin, it demonstrated a high number of open dentinal tubules. This can be explained by the treatment strategy used in this study, as the manufacturer recommends home and daily use of the material.[16,17] Therefore, it is possible that the precipitates formed were constituted by weak chemical bonds and present high sensitivity to the acid challenge. A study conducted by Poggio and others[15] showed that regenerated toothpaste (NR-5) was able to prevent erosion in the face of an erosive challenge with soft drinks. However, that study carried out only one erosive challenge, while this present study subjected the samples to erosion for 28 days. The methodological differences in laboratory erosion studies hinder the interrelation between data presented in the literature.

G5 treated with 5% potassium nitrate associated with 2% NaF showed the worst antierosive results and was similar to the control group. Potassium nitrate acts specifically on the polarity of the nerve fiber stimulated during DH,[22] and there is no evidence that it can form mineralized precipitates on the dental surface. Previous studies have shown that desensitizing toothpastes containing potassium nitrate have shown promising results in the obliteration of dentinal tubules.[3,51] As already stated by some studies, they can promote effective obliteration when associated to other components.[14] For this reason, studies that showed tubular occlusion after the use of materials containing Na^+ ions attributed this effect to the deposition of solid particles in the products and not to the influence of potassium.[3,51] However, further studies must be carried out to clarify this issue.

The materials used in G2 and G3 proved to be remineralizing agents with potential antierosive effects. That is, they provide satisfactory precipitate formation in the intertubular dentin and occlusion of the dentinal tubules. Therefore, they can be an interesting alternative to assist in the prevention and treatment of dentin erosion. However, G2 showed a negative impact on the collagen matrix, unlike that of G3. Therefore, CPP-ACPF seems to be more indicated as an antierosive alternative, as it does not damage collagen. In addition, an evaluation of dentin permeability could complement our results of dentin tubule obliteration, as it was done in preliminary studies on the use of desensitizing agents in eroded dentin.[52,53] Future studies are necessary to corroborate and consolidate the results obtained in this study, as well as to deepen the knowledge about the impact of remineralizing agents on the dentin collagen matrix.

Saliva plays a significant role in the formation of the acquired film on the teeth. This semipermeable membrane makes it difficult for acid to contact the dental surface during an erosive challenge.[54] Therefore, one of the limitations of laboratory erosion studies would be the impact of this protective film on the observed results. To achieve this, all groups studied were subjected to the same conditions. In addition, it was observed that the control group showed significant superficial damage after the erosion challenge. This demonstrates that the saliva used in this study did not prevent erosive damage from occurring.

CONCLUSION

Calcium phosphate-based remineralizing agents showed to be a promising alternative treatment for preventing deleterious effects on the eroded dentin collagen matrix. In addition, they promoted precipitate formation and dentinal tubule obliteration on the eroded dentin.

Conflict of Interest

The authors declare that they have no conflict of interest. The authors of the present study certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in the present article.

Regulatory Statement

This study was conducted in accordance with all the provisions of the guidelines and policies of the Animal Ethics Committee of the Araraquara School of Dentistry, Paulista State University. The approval code issued for this study is No. 30/2018. PROSPERO acknowledgement of receipt is 158813.

Acknowledgements

This study was financed in part by “Fondo Nacional de Desarrollo Científico, Tecnológico y de Innovación Tecnológica” FONDECYT – Perú (N097-2016) and by “Conselho Nacional de Desenvolvimento Científico e Tecnológico” CNPq—Brazil (140152/2019-9).

(Accepted 27 February 2021)

REFERENCES

1. Parker AS, Patel AN, Al Botros R, Snowden ME, McKelvey K, Unwin PR, Ashcroft AT, Carvell M, Joiner A, & Peruffo M (2014) Measurement of the efficacy of calcium silicate for the protection and repair of dental enamel *Journal of Dentistry* **42(Supplement 1)** S21- S29.
2. Leal IC, Costa WKF, & Passos VF (2020) Fluoride dentifrice containing calcium silicate and sodium phosphate salts on dental erosion: *In vitro* study *Archives of Oral Biology* **118** 104857.
3. João-Souza SH, Scaramucci T, Bühler Borges A, Lussi A, Saads Carvalho T, & Corrêa Aranha AC (2019) Influence of desensitizing and anti-erosive toothpastes on dentine permeability: An *in vitro* study *Journal of Dentistry* **89** 103176.
4. Kanzow P, Wegehaupt FJ, Attin T, & Wiegand A (2016) Etiology and pathogenesis of dental erosion *Quintessence International* **47(4)** 275-758.
5. Li W, Liu J, Chen S, Wang Y, & Zhang Z (2017) Prevalence of dental erosion among people with gastroesophageal reflux disease in China *Journal of Prosthetic Dentistry* **117(1)** 48-54.
6. Isaksson H, Birkhed D, Wendt L-K, Alm A, Nilsson M, & Koch G (2014) Prevalence of dental erosion and association with lifestyle factors in Swedish 20-year olds *Acta Odontologica Scandinavica* **72(6)** 448-457.
7. Picos A, Badea ME, & Dumitrascu DL (2018) Dental erosion in gastro-esophageal reflux disease. A systematic review *Clujul Medical* **91(4)** 387-390.
8. Mafla AC, Cerón-Bastidas XA, Munoz-Ceballos ME, Vallejo-Bravo DC, & Fajardo-Santacruz MC (2017) Prevalence and extrinsic risk factors for dental erosion in adolescents *Journal of Clinical Pediatric Dentistry* **41(2)** 102-111.
9. Alencar CM, de Paula BFL, Ortiz MIG, Magno MB, Silva CM, & Maia LC (2019) Clinical efficacy of nano-hydroxyapatite in dentin hypersensitivity: A systematic review and meta-analysis *Journal of Dentistry* **82** 11-21.
10. Souza BM, Comar LP, Vertuan M, Fernandes Neto C, Buzalaf MA, & Magalhaes AC (2015) Effect of an experimental paste with hydroxyapatite nanoparticles and fluoride on dental demineralisation and remineralisation *in situ Caries Research* **49(5)** 499-507.
11. Alencar CM, Leite KLF, Ortiz MIG, Magno MB, Rocha GM, Silva CM, & Maia LC (2020) Morphological and chemical effects of in-office and at-home desensitising agents containing sodium fluoride on eroded root dentin *Archives of Oral Biology* **110** 104619.
12. Dionysopoulos D, Tolidis K, & Sfeikos T (2019) Effect of CPP-ACPF and nano-hydroxyapatite preventive treatments on the susceptibility of enamel to erosive challenge *Oral Health and Preventive Dentistry* **17(4)** 357-364.
13. Soares GG, Magalhães PA, Fonseca ABM, Tostes MA, da Silva EM, & Coutinho TCL (2017) Preventive effect of CPP-ACPF paste and fluoride toothpastes against erosion and erosion plus abrasion *in vitro* - A 3D profilometric comparative study *Oral Health and Preventive Dentistry* **15(3)** 269-277.
14. Wang L, Magalhães AC, Francisconi-Dos-Rios LF, Calabria MP, Araújo D, Buzalaf M, Lauris J, & Pereira JC (2016) Treatment of dentin hypersensitivity using nano-hydroxyapatite pastes: A

randomized three-month clinical trial *Operative Dentistry* **41(4)** E93-E101.

15. Poggio C, Gulino C, Mirando M, Colombo M, & Pietrocola G (2017) Preventive effects of different protective agents on dentin erosion: An *in vitro* investigation *Journal of Clinical and Experimental Dentistry* **9(1)** e7-e12.

16. Hornby K, Ricketts SR, Philpotts CJ, Joiner A, Schemehorn B, & Willson R (2014) Enhanced enamel benefits from a novel toothpaste and dual phase gel containing calcium silicate and sodium phosphate salts Journal of Dentistry **42(Supplement 1)** S39-S45.

17. Sun Y, Li X, Deng Y, Sun JN, Tao D, Chen H, Hu Q, Liu R. Liu W, Feng X, Wang J. Carvell M, & Joiner A (2014) Mode of action studies on the formation of enamel minerals from a novel toothpaste containing calcium silicate and sodium phosphate salts *Journal of Dentistry* **42(Supplement 1)** S30-S38.

18. Joao-Souza SH, Scaramucci T, Buhler Borges A, Lussi A, Saads Carvalho T, & Correa Aranha AC (2019) Influence of desensitizing and anti-erosive toothpastes on dentine permeability: An *in vitro* study *Journal of Dentistry* **89** 103176.

19. Dong Z, Chang J, Deng Y, & Joiner A (2011) Tricalcium silicate induced mineralization for occlusion of dentinal tubules *Australian Journal of Dentistry* **56(2)** 175-180.

20. Marto CM, Paula AB, Nunes T, Pimenta M, Abrantes AM, Pires AS, Laranjo M, Coelho A, Donato H, Botelho MF, Ferreira MM, & Carrilho E (2019) Evaluation of the efficacy of dentin hypersensitivity treatments - A systematic review and follow-up analysis *Journal of Oral Rehabilitation* **46(10)** 952-990.

21. Bae J-H, Kim Y-K, & Myung S-K (2015) Desensitizing toothpaste versus placebo for dentin hypersensitivity: A systematic review and meta-analysis *Journal of Clinical Periodontology* **42(2)** 131-141.

22. Wang Y, Gao J, Jiang T, Liang S, Zhou Y, & Matis BA (2015) Evaluation of the efficacy of potassium nitrate and sodium fluoride as desensitizing agents during tooth bleaching treatment—A systematic review and meta-analysis *Journal of Dentistry* **43(8)** 913-923.

23. Escalante-Otarola WG, Castro-Nunez GM, Jordao-Basso KCF, Guimaraes BM, Palma-Dibb RG, & Kuga MC (2018) Evaluation of dentin desensitization protocols on the dentinal surface and their effects on the dentin bond interface *Journal of Dentistry* **75** 98-104.

24. Kuga MC, So MV, De Faria-junior NB, Keine KC, Faria G, Fabricio S, & Matsumoto MA (2012) Persistence of resinous cement residues in dentin treated with different chemical removal protocols *Microscopy Research and Technique* **75(7)** 982-985.

25. Biguetti CC, Cavalla F, Tim CR, Saraiva PP, Orcini W, De Andrade Holgado L, Renno ACM, & Matsumoto MA (2019) Bioactive glass-ceramic bone repair associated or not with autogenous bone: a study of organic bone matrix organization in a rabbit critical-sized calvarial model *Clinical Oral Investigations* **23(1)** 413-421.

26. Cintra LTA, Ferreira LL, Benetti F, Gastelum AA, Gomes-Filho JE, Ervolino E, & Briso ALF (2017) The effect of dental bleaching on pulpal tissue response in a diabetic animal model *International Endodontic Journal* **50(8)** 790-798.

27. Kinney JH, Balooch M, Haupt DL, Marshall SJ, & Marshall GW (1995) Mineral distribution and dimensional changes in human dentin during demineralization *Journal of Dental Research* **74(1)** 1179-1184.

28. Mei ML, Ito L, Cao Y, Li QL, Lo EMC, & Chu CH (2013) Inhibitory effect of silver diamine fluoride on dentine demineralisation and collagen degradation *Journal of Dentistry* **41(9)** 809-817.

29. O'leary LE, Fallas JÁ, Bakota EL, Kang MK, & Hartgerink JD (2011) Multi-hierarchical self-assembly of a collagen mimetic peptide from triple helix to nanofibre and hydrogel *Nature Chemistry* **3(10)** 821-828.

30. Lutolf MP, Gilbert PM, & Blau HM (2009) Designing materials to direct stem-cell fate *Nature* **462** 433-441.

31. Cölfen HA (2010) Biomineralization: A crystal-clear view. *Nature Materials* **9(12)** 960-961.

32. Kim D, Lee B, Thomopoulos S, & Jun YS (2018) The role of confined collagen geometry in decreasing nucleation energy barriers to intrafibrillar mineralization *Nature Communications* **9(1)** 962.

33. Osorio R, Osorio E, Aguilera FS, Medina-Castillo AL, Toledano M, & Toledano-Osorio M (2018) Silver improves collagen structure and stability at demineralized dentin: A dynamic-mechanical and Raman analysis *Journal of Dentistry* **79** 61-67.

34. Wang Z, Jiang T, Sauro S, Pashley DH, Toledano M, Osorio R, Liang S, Xing W, As Y, & Wang Y (2011) The dentine remineralization activity of a desensitizing bioactive glass-containing toothpaste: an *in vitro* study *Australian Dental Journal* **56(4)** 372-381.

35. Osorio R, Alfonso-Rodríguez CA, Medina-Castillo AL, Alaminos M, & Toledano M (2016) Bioactive polymeric nanoparticles for periodontal therapy *PloS One* **11** e0166217.

36. Toledano-Osorio M, Osorio E, Aguilera FS, Medina-Castillo AL, Toledano M, & Osorio R (2018) Improved reactive nanoparticles to treat dentin hypersensitivity *Acta Biomaterialia* **72** 371-380.

37. Schroeder M, Correa IC, Bauer J, Loguercio AD, & Reis A (2017) Influence of adhesive strategy on clinical parameters in cervical restorations: A systematic review and meta-analysis *Journal of Dentistry* **62** 36-53. 10.1016/j.jdent.2017.05.006

38. Hata R, Sunada H, & Arai K (1984) Reciprocal effects of excess potassium salts in the culture medium on type I and type III collagen synthesis by human skin fibroblasts *Cell Biology International Reports* **8(6)** 433-440.

39. Mehta D, Gowda V, Finger WJ, & Sasaki K (2015) Randomized, placebo-controlled study of the efficacy of a calcium phosphate containing paste on dentin hypersensitivity *Dental Materials* **31(11)** 1298-1303.

40. Komabayashi T, Imai Y, Ahn C, Chow LC, & Takagi S (2010) Dentin permeability reduction by a sequential application of calcium and fluoride-phosphate solutions *Journal of Dentistry* **38(9)** 736-741.

41. Tenuta LM & Cury JA (2010) Fluoride: Its role in dentistry *Brazilian Oral Research* **24(Supplement 1)** 9-17.

42. Carvalho FG, Brasil VL, Silva Filho TJ, Carlo HL, Santos RL, & Lima BA (2013) Protective effect of calcium nanophosphate and CPP-ACP agents on enamel erosion *Brazilian Oral Research* **27(6)** 463-470.

43. Reynolds EC (2009) Casein phosphopeptide-amorphous calcium phosphate: The scientific evidence *Advances in Dental Research* **21(1)** 25-29.

44. Cochrane NJ & Reynolds EC (2012) Calcium phosphopeptides —mechanisms of action and evidence for clinical efficacy *Advances in Dental Research* **24(2)** 41-47.

45. Reema SD, Lahiri PK, & Roy SS (2014) Review of casein phosphopeptides-amorphous calcium phosphate *Chinese Journal of Dental Research* **17(1)** (2014) 7-14.

46. Huang S, Gao S, & Yu H (2011) Remineralization potential of nanohydroxyapatite on initial enamel lesions: An *in vitro* study *Caries Research* **45(5)** 460-468.

47. Vano M, Derchi G, Barone A, Pinna R, Usai P, & Covani U (2018) Reducing dentine hypersensitivity with nano-hydroxyapatite toothpaste: A double-blind randomized controlled trial *Clinical Oral Investigations* **22(1)** 313-320.

48. Min JH, Kwon HK, & Kim BI (2011) The addition of nano-sized hydroxyapatite to a sports drink to inhibit dental erosion: *In vitro* study using bovine enamel *Journal of Dentistry* **39(9)** 629-635.

49. Magalhães AC, Levy FM, Rizzante FA, Rios D, & Buzalaf MA (2012) Effect of NaF and TiF4 varnish and solution on bovine dentin erosion plus abrasion *in vitro Acta Odontologica Scandinavica* **70(2)** 160-164.

50. de Souza BM, Santi LRP, de Souza Silva M, Buzalaf MAR, & Magalhães AC (2018) Effect of an experimental mouth rinse containing NaF and TiF4 on tooth erosion and abrasion *in situ Journal of Dentistry* **73** 45-49.

51. Wang Z, Jiang T, Sauro S, Wang Y, Xing W, Liang S, Sa Y, Zhang C, Shen Y, & Haapasalo M (2012) Nerve-targeted desensitizing toothpastes occlude dentin tubules and induce mineral precipitation *American Journal of Dentistry* **25(1)** 26-30.

52. Machado AC, Rabelo FEM, Maximiano V, Lopes RM, Aranha ACC, & Scaramucci T (2019) Effect of in-office desensitizers containing calcium and phosphate on dentin permeability and tubule occlusion *Journal of Dentistry* **86** 53-59.

53. Calabria M, Porfirio R, Fernandes S, Wang L, Buzalaf M, Pereira JC, & Magalhães AC (2014) Comparative *in vitro* effect of TiF4 to NaF and potassium oxalate on reduction of dentin hydraulic conductance *Operative Dentistry* **39(4)** 427-32.

54. Hara AT & Zero DT (2014) The potential of saliva in protecting against dental erosion. *Monographs in Oral Science* **25** 197-205.