Effect of CPP-ACPF and Nano-hydroxyapatite Preventive Treatments on the Susceptibility of Enamel to Erosive Challenge

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Purpose: The purpose of this *in vitro* study was to evaluate the effect of two preventive clinical treatments on bovine enamel susceptibility after erosive challenge induced by a soft drink.

Materials and Methods: Eighteen sound bovine incisors were used for this study. Three experimental groups were assigned as follows: Group 1 was the control group; Group 2 specimens received a CPP-ACPF paste treatment; and Group 3 specimens received a treatment with a product containing 1% nano-hydroxyapatite and 455 ppmF-. The specimens were submitted to erosive challenge using a common soft drink (Coca Cola). The erosive activity on the enamel was evaluated by measuring surface microhardness change (Vickers method), surface roughness alterations and surface loss (vertical scanning interferometry (VSI) method). The data were statistically analysed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test at a level of statistical significance a = 0.05.

Results: The tested treatments exhibited significantly lower decreases in surface microhardness compared to the control group after the erosive challenge ($p < 0.05$). The tested treatment groups did not show statistically significant lower increases in surface roughness compared to the control group ($p > 0.05$). In addition, the treatment groups exhibited statistically significant lower surface loss than the control group ($p < 0.05$).

Conclusion: Although the tested treatments reduced erosive activity, none of them provided complete protection against the development of enamel erosion, which means that they should be only used as a part of an individually tailored preventive programme.

Key words: enamel erosion, nano-hydroxyapatite, CPP-ACPF, soft drink

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Dental erosion is of increasing concern as an important cause of long-term tooth wear due to non-carious intrinsic or extrinsic erosive factors, which means that oral bacteria are not involved. In addition, there is evidence that dental erosion is constantly spreading. The aetiology of dental erosion is multifactorial.[14] In particular, various chemical (ie, pH and buffering capacity of the diet), biological (ie, saliva, pellicle, composition and structure of the teeth) and behavioural factors (ie, number of meals, dental hygiene, sports, alcohol, soft drinks, and so on) play a main role in pathogenesis of this condition and their different interaction explains why some individuals present more severe erosive lesions than others, even if they are exposed to exactly the same acid challenges in their diet.[16,29]

The mechanism of dental erosion includes attack of hydrogen cations (H^+) derived from the acidic agents to dental surfaces.[5] More specifically, the H^+ form chemical bonds with carbonate (CO_3^{-2}) and phosphate (PO_4^{-3}) anions of hydroxyapatite crystals, leading to demineralisation of the surface of dental tissues and as a result softening and loss of tooth volume.[8] In the past, it was believed that dental surface loss took place by a constant layer-by-layer dissolution which increased and accelerated as the erosive activity was repeated more often and lasted for longer.[17] Nowadays, new concepts have been proposed for the mecha-

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Table 1 The technical characteristics of the materials used in the present study

Product	Active agent (form)	Manufacturer	Composition	Lot number
GC MI Paste Plus	Recaldent + NaF (paste)	GC, Tokyo, Japan	Pure water, glycerol, CPP-ACP, D-sorbitol, CMC-Na, propylene glycol, silicon and titanium dioxide, xylitol, phosphoric acid, flavour, sodium fluoride (900 ppmF), sodium saccharin, ethyl-, propyl-, butyl-, p-hydroxybenzoate	150710S
Unident Dental Conditioner	n-HAp + NaF (foam)	InterMed, Kifissia, Greece	Aqua, sodium lauryl sarcosinate, n-HAp (1%), xylitol, panthenol, allantoin, CPC, krameria triandra extract, commiphora myrrha extract, chamomilla recutita flower extract, sodium saccharin, sodium hydroxyl-methyl-glycinate, NaF (455 ppmF-), aroma, limonene, citric acid	1401
Coca Cola	Phosphoric acid, carbonate	Coca Cola 3E Company, Marousi, Greece	Water, sugar, carbon dioxide, caramel colour E 150d, phosphoric acid, natural flavours, caffeine, pH = 2.47	–

nism of the erosive wear, which deal with the fact that besides the removal of the surface, erosion shows dissolution of minerals within the softened layer beneath the surface. Additionally, the critical pH value of dental erosion is calculated from the calcium and phosphate concentrations in the erosive solution itself, which means it depends on the composition of the erosive solution.[15]

As a matter of fact, diagnosis of dental erosion at its early stages is of great importance, especially in children and adults. Nevertheless, early diagnosis of erosion of the teeth is difficult due to few signs and symptoms and only clinical appearance may help for this purpose.[14] When dental erosion is diagnosed, an individually tailored preventive programme should be recommended. Preventive measures may include neutralising the effects of the acidic attacks and enhancing the resistance of the teeth to the erosive agents.[29]

In the past various clinical techniques have been suggested to limit or prevent the progress of dental erosion by improving the susceptibility of tooth structures to acidic challenges. Most of those methods work by modifying the tooth surface in a way that it becomes less vulnerable to the destructive activity of the acids. The use of fluoride has been investigated and there is evidence that it can reduce erosion by decreasing the solubility of hydroxyapatite crystals due to formation of fluorapatite and its buffering capacity.[6,19] Monovalent and polyvalent fluorides have been used for different vehicles such as mouthrinses, toothpastes, gels and varnishes. The most effective formulations were the highly concentrated, acidic and polyvalent fluorides.[13]

Recently, bioactive agents such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)[30] and nano-hydroxyapatite (n-HAp)[18] have been introduced in various forms for prevention and remineralisation of erosive lesions of the teeth. The last decades due to changes in lifestyle a rapid increase in daily consumption of soft drinks has been observed. Usually, soft drinks are very acidic (pH < 3) and it is well documented that they can frequently cause dental erosion.[26] Excessive consumption of soft drinks could result in decreased enamel hardness[25] as a consequence of dissolution of the enamel minerals, reduction of the enamel surface volume[20] and increased enamel surface roughness.[31]

Therefore, the aim of this *in vitro* study was to evaluate the effect of two preventive clinical surface treatments using CPP-ACPF paste and n-HAp foam on enamel susceptibility after erosive challenge induced by a common soft drink. The novelty of the present study was the evaluation of a novel clinical treatment with 1% n-HAp formula. Three null hypotheses were formulated prior to the study: the first null hypothesis was that there were no statistically significant differences in decrease in surface microhardness among the experimental groups after the erosive challenge; the second null hypothesis was that there were no statistically significant differences in increase in surface roughness among the experimental groups after the erosive challenge; the third null hypothesis was that there were no statistically significant differences in surface loss among the experimental groups after the erosive challenge.

MATERIALS AND METHODS

Preparation of the Specimens

Eighteen sound bovine incisors were carefully extracted and stored in a 0.5% chloramine-T solution at 6°C for up to 1 month. The crowns were separated from the roots, and each crown was sectioned into two halves using a water-cooled diamond disc (Isomet, Buehler, Lake Bluff, IL, USA). Each enamel specimen was approximately 4 mm long, 4 mm wide and 1.5 mm high. The 36 tooth fragments were not allowed to be dehydrated, and were examined by means of optical microscope under ×10 magnification for any sur-

Table 2 Means and standard deviations of surface microhardness (VHN) of the experimental groups of the study before and after the erosive challenge. Same uppercase superscripts in columns indicate no statistically significant differences among treatments ($p > 0.05$). Same lowercase superscripts in rows indicate no statistically significant differences between before and after erosive challenge values ($p > 0.05$)

Groups	Treatments	Before erosive challenge	After erosive challenge	Mean ΔVHN	Mean %VHN decrease
1	Control	277.2 ± 19.5^{Aa}	157.4 ± 21.7^{Ab}	119.8 ± 24.4^{A}	43.1%
2	CPP-ACPF (MI Paste Plus)	280.2 ± 14.6^{Aa}	174.2 ± 13.6^{Bb}	106.0 ± 7.9^{B}	37.9%
3	n-HAp (Unident Dental Conditioner)	266.2 ± 19.8^{Aa}	181.0 ± 15.2^{Bb}	85.2 ± 15.8^{C}	31.7%

ΔVHN: reduction of surface microhardness in VHN after the erosive challenge, %VHN decrease: % decrease of surface microhardness after erosive challenge.

Table 3 Means and standard deviations of surface roughness (Sq) of the experimental groups of the study before and after the erosive challenge. Same uppercase superscripts in columns indicate no statistically significant differences among treatments ($p > 0.05$). Same lowercase superscripts in rows indicate no statistically significant differences between before and after erosive challenge values ($p > 0.05$)

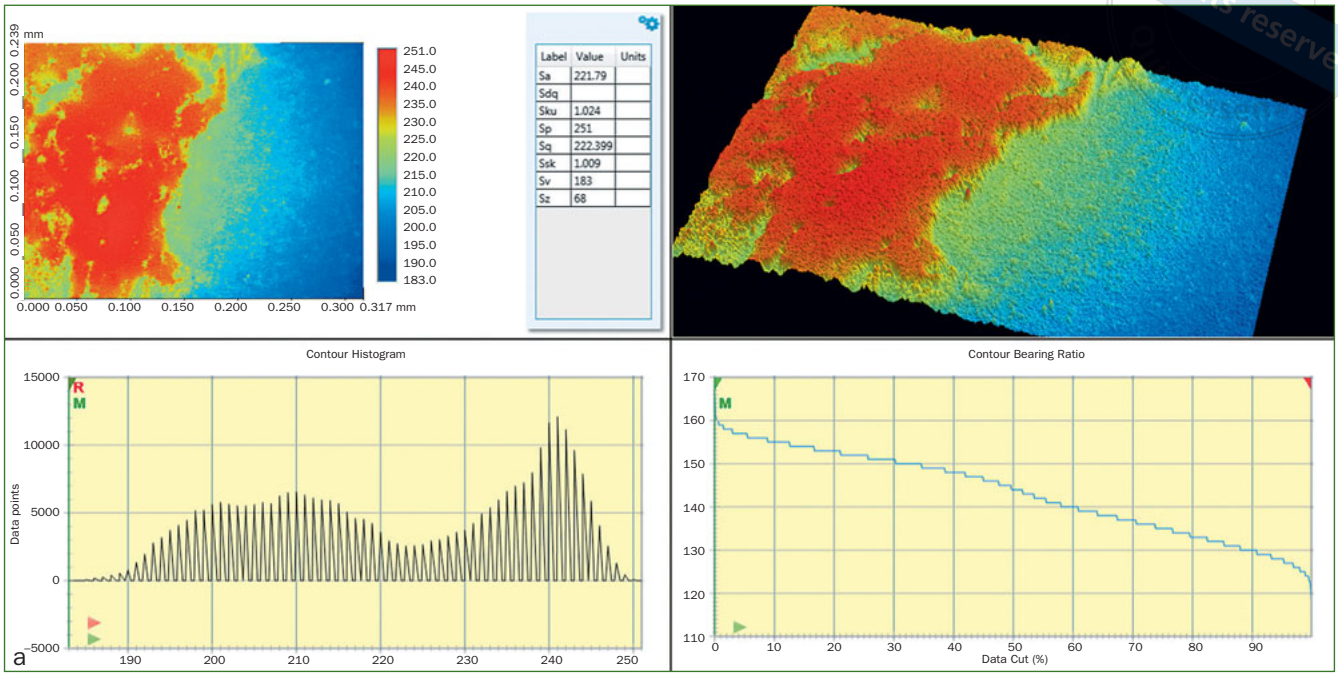
Groups	Treatments	Before erosive challenge	After erosive challenge	Mean ΔSq (μm)	Mean %Sq increase
1	Control	0.205 ± 0.010^{Aa}	0.221 ± 0.006^{Ab}	0.016 ± 0.010^{A}	7.2%
2	CPP-ACPF (MI Paste Plus)	0.201 ± 0.013^{Aa}	0.222 ± 0.006^{Ab}	0.021 ± 0.011^{A}	9.5%
3	n-HAp (Unident Dental Conditioner)	0.197 ± 0.001^{Aa}	0.214 ± 0.007^{Ab}	0.017 ± 0.007^{A}	7.9%

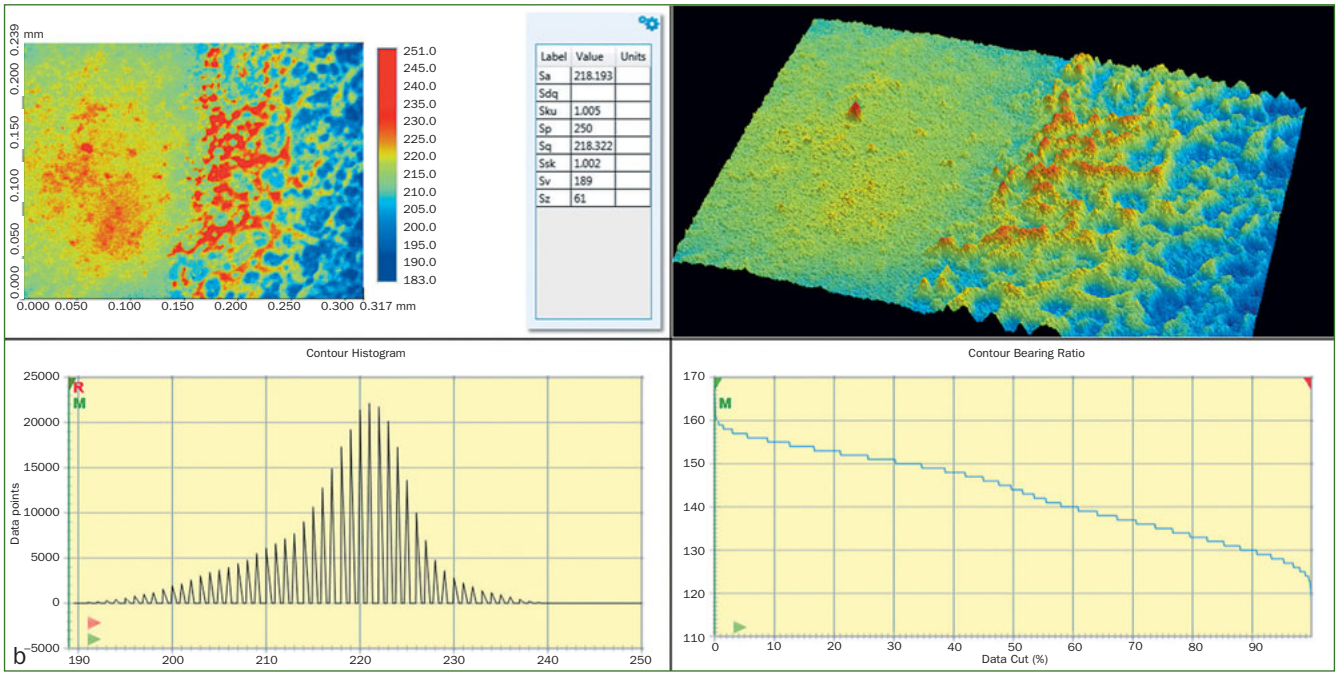
ΔSq: increase of surface roughness in Sq after the erosive challenge, %Sq increase: % increase of surface roughness after erosive challenge.

Table 4 Means and standard deviations of surface loss (μm) of the experimental groups of the study after the erosive challenge. The decrease in surface loss of the treatment groups compared to the control group is also presented. Same uppercase superscripts in columns indicate no statistically significant differences among treatments ($p > 0.05$)

Groups	Treatments	Mean surface loss (μm)	% decrease loss compared to control
1	Control	65.8 ± 9.0A	–
2	CPP-ACPF (MI Paste Plus)	58.7 ± 6.5B	10.8%
3	n-HAp (Unident Dental Conditioner)	56.5 ± 12.3B	14.1%

face structural damage or defection. The enamel specimens were randomly distributed into three groups (n = 12) and were embedded in epoxy resin (EpoFix Resin, Struers, Copenhagen, Denmark) with the facial or lingual surface facing up. The enamel surfaces were ground and polished on a polishing machine (Jean Wirtz TG 250, Dusseldorf, Germany) using up to 1200 grit silicon carbide abrasive papers (Struers) and a 0.4 μm alumina polishing suspension, in order to form parallel planar surfaces. After polishing, the specimens were checked for the absence of dentine on the polished surfaces and immersed in an ultrasonic bath (Euronda, Montecchio Precalcino, Vicenza, Italy) to remove any impurities and stored in a remineralising solution for 24 h at 37°C before the experiment. The composition of the remineralising solution was, according to Dionysopoulos et al[7], as follows: 0.103 g/L of $CaCl_2$, 0.019 g/L $MgCl_2*6H_2O$, 0.544 g/L KH_2PO_4, 2.24 g/L KCl and buffer (TCP-KOH) was added to adjust the pH to 7. The specimens were stored in fresh remineralising solution throughout the experimental period.





Experimental Groups of the Study

Three experimental groups (n = 12) of the study were submitted to one of the following treatments:

- Group 1 specimens (control group) did not receive any treatment during the experimental period.
- Group 2 specimens, before each erosive challenge, were smeared with GC MI Paste Plus (GC, Tokyo, Japan), which contains Recaldent (GC, Tokyo, Japan), and 900 ppmF- (CPP-ACPF) for 3 min, rinsed with distilled water for 10 s using a squeeze bottle, and then submitted to the erosive challenge. The composition of this product is shown in Table 1.
- Group 3 specimens, before each erosive challenge, were smeared with Unident Dental Conditioner (InterMed, Kifissia, Greece), which contains 1% nano-hydroxyapatite (n-HAp) crystals, and 455 ppmF- for 3 min, rinsed with distilled water for 10 s using a squeeze bottle, and then submitted to the erosive challenge such as Group 2

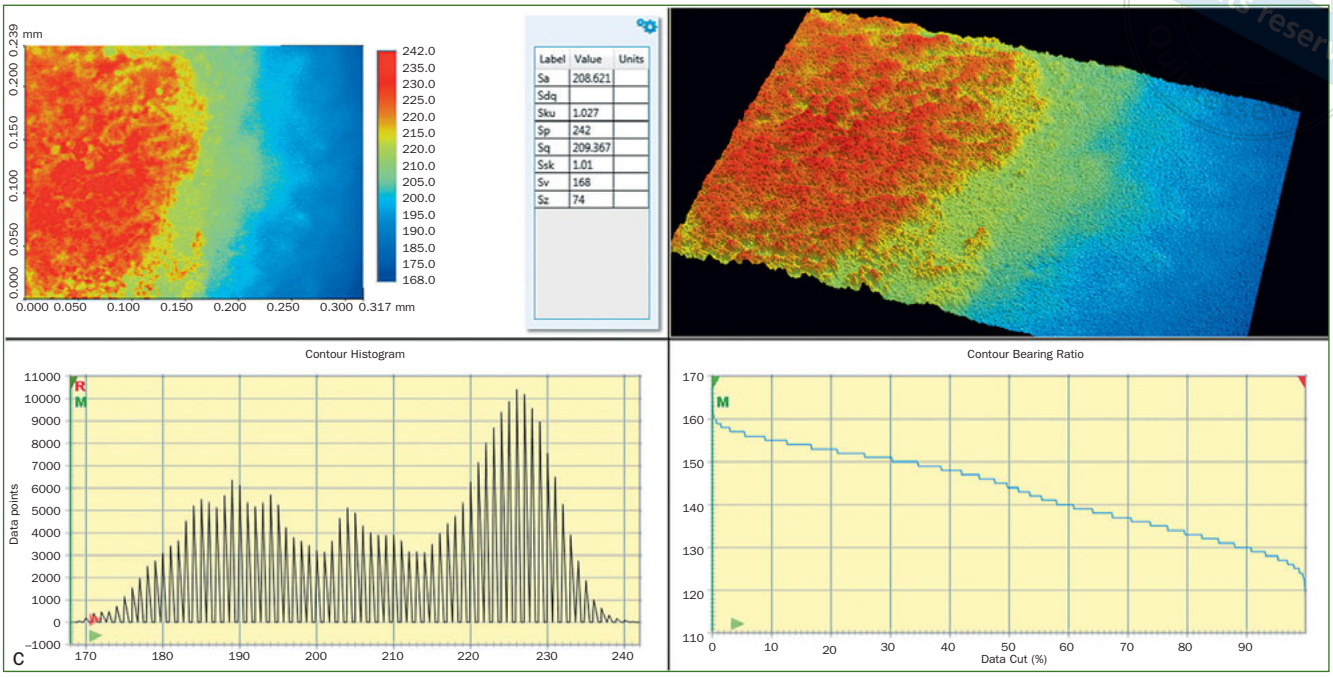


Fig 1 (A–C) Representative topographic surface maps and surface analysis of enamel specimens of the experimental groups of the study (×20 magnification) at the centre of the specimens indicating the depth of the erosive lesions. The S values are also presented as well as the contour histogram and the contour bearing ratio. A: Group 1, B: Group 2 and C: Group 3.

specimens. The composition of this product is also presented in Table 1.

Erosive Challenge

In the present investigation, a common soft drink (Coca Cola, 3E Company, Greece) was used as an erosive agent (Table 1). Each enamel specimen was rinsed with distilled water for 10 s and immediately immersed into 6 ml of fresh soft drink in a plastic container for four intervals of 2 min and then rinsed again with distilled water and stored in fresh remineralising solution. The treatments were applied at 0, 12, 24, 36, 48 and 60 h. This cycling treatment was conducted according to Wang et al.[26] The application of the tested dental products was carried out before of each erosive challenge and during all cycling treatments of the experiment. The pH of the soft drink was measured using a digital pH meter (Orion Star Series ISE Meter, Thermo Scientific, Beverly Hills, CA, USA) and was stable (pH = 2.47 ± 0.09) for at least 15 min at room temperature (23 ± 1°C).

Surface Microhardness Measurements

Surface microhardness of each enamel specimen was evaluated prior to and after the erosive challenge. The method used for the assessment of surface microhardness was the Vickers method with a hardness tester (HMV-2000, Shimadzu, Tokyo, Japan) at a load of 200 g and indentation time of 10 s. Five indentations were made on the top surface of each enamel specimen, one in the centre and one in every quadrant (500 μm apart). Data were independently averaged and reported in Vickers Hardness Numbers (VHN).

Surface Roughness Measurements

Surface roughness analysis of the enamel specimens was performed according to ISO 25178 (non-contact type) which is related to the analysis of 3D areal surface textures. The measurements were carried out before and after the erosive challenge. Surface roughness measurements were performed using a vertical scanning interferometry (VSI) microscope (Bruker, ContourGT, Berlin, Germany). Three images were obtained (×20 magnification) from each specimen in the four quadrants of the enamel surface which correspond to a surface of 0.317 × 0.238 mm^2. Vision64 software (Bruker, ContourGT, Berlin, Germany) was used to acquire the data and compute the mean surface roughness in square units (Sq) of each image. The values of the 12 images of each specimen were averaged and the mean value was calculated. Surface roughness baseline was recorded prior to erosive challenge.

Surface Loss Measurements

Before the erosive challenge, half of each enamel surface of the specimens was covered with one-sided silver adhesive tape (Wonder Tape, PVC Electrical Tape). Measurements were carried out using VSI method following the erosive challenge. After removal of the adhesive tape four images were obtained in the centre of each specimen's surface. The enamel surface loss was calculated after su-

perimposing the baseline and post-erosion profiles. The depth of the eroded area for each specimen was calculated based on the subtraction of the two profiles.

Statistical Analysis

The sample size for each test was calculated considering 80% power and a statistical significance level of 0.05. The data were statistically analysed using SPSS Statistics 20.0 software (IBM, Chicago, IL, USA). Data were preliminary tested for normality and homogeneity using Shapiro–Wilk test and Levene test, respectively. Surface hardness, surface roughness and surface loss data of the enamel specimens were statistically analysed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test was used to detect statistical differences at a level of statistical significance a = 0.05.

RESULTS

Surface Microhardness Outcomes

Means and standard deviations of surface microhardness expressed in VHN of the experimental groups of the study before and after the erosive challenge are shown in Table 2. Surface microhardness was significantly reduced in the tested experimental groups after the erosive challenge ($p < 0.05$). The tested treatments exhibited significantly lower decrease in surface microhardness compared to the control group ($p < 0.05$). The lowest decrease presented in the Group 3 specimens ($p < 0.05$).

Surface Roughness Outcomes

Means and standard deviations of surface roughness expressed in Sq of the experimental groups of the study before and after the erosive challenge are shown in Table 3. Surface roughness was significantly increased in all experimental groups after the erosive challenge ($p < 0.05$). The tested treatment groups did not show a statistically significant lower increase in surface roughness compared to the control group ($p > 0.05$).

Surface Loss Outcomes

Means and standard deviations (μm) of surface loss from the study's experimental groups after the erosive challenge are shown in Table 4. Representative topographic surface maps (×20 magnification) and surface analysis of the experimental groups of the study are illustrated in Figure 1 (A–D). Surface loss was detected in all experimental groups after the erosive challenge. The tested treatment groups exhibited statistically significant lower surface loss than control group ($p < 0.05$). However, no statistically significant differences were detected in surface loss decrease between the two tested treatments ($p = 0.654$).

DISCUSSION

Based on the outcomes of the present investigation, the first null hypothesis of the study – which stated there were no statistically significant differences in decreased surface microhardness among the experimental groups after the erosive challenge – was rejected. After the erosive challenge, all the experimental groups exhibited lower microhardness values than those obtained before. A decrease in surface hardness was perhaps explained by the loss of minerals from the enamel surface due to acidic activity of the soft drink's ingredients. This is in agreement with previous reports.[4,26] The tested surface treatments reduced the surface microhardness drop compared to the control group. Most effective was the surface treatment with n-HAp.

Coca Cola is a very common acidic (pH < 3) soft drink and its erosive activity on tooth enamel has been documented. It is able to decrease pH on the tooth surface leading to mineral dissolution and statistically significant erosive lesions when it is consumed excessively.[25] Yuan et al[28] reported that a single intake of 100 ml Coca Cola could significantly decrease enamel microhardness and initiate erosion; this decrease reaches its lowest point at 2–8 min, and starts to recover after 10 min. Additionally, the authors claimed that enamel surface microhardness could not fully recover 30 min after the intake without using a remineralisation treatment. In another study,[1] it was demonstrated that after erosive challenge (immersion time: 60 min) using a similar soft drink (orange juice, pH = 3.85 ± 0.5) the micromechanical properties of the eroded enamel were influenced at 30, 40 and 50 μm depths from the enamel surface, but at 100 μm depth no changes were detected.

Bioactive materials, such as n-HAp and CPP-ACP, are broadly investigated for many applications in dentistry in recent years. Synthetic n-HAp is composed of apatite crystals in size of 50–1000 nm and constitutes a perfect substitute for enamel microstructure, especially in an acidic environment. It is a source of Ca and P ions and forms a strong bond with the enamel surface.[12] In the past, the addition of n-HAp to soft drinks has been suggested, due to its anti-erosive effects. Previous investigations reported lower decreases in enamel surface hardness after the consumption of soft drinks including n-HAp, which means that it prevented dissolution of surface minerals by acting as a remineralising agent.[18,23] Wang et al[27] observed that the use of a nano-fluorapatite (n-FAp) paste after erosive challenge enhanced the surface hardness of enamel. In the present investigation, application of n-HAp as a foam onto enamel reduced any decrease in surface microhardness compared to the control group. This treatment has never been investigated before, and as a result further studies are needed to confirm its clinical effectiveness.

In previous studies, CPP-ACP has been well documented for its effectiveness on the prevention of enamel erosion by evaluating changes in surface microhardness.[9,26,30] This is in agreement with the results of the present investigation. The mechanism of the anti-erosive action of CPP-ACP comprises an increase in the number of potential calcium-binding sites

on an enamel surface, leading to reduction of the constant diffusion of calcium. Additionally, ACP buffers the free calcium and phosphate ion activities on enamel surface, helping to maintain a state of supersaturation that reduces demineralisation and enhances remineralisation.[22] Another explanation may be that the application of CPP-ACP on enamel surface facilitates the formation of a crystal layer, which fills the interprism voids and partially covers the apatite prisms protecting them from the acidic activity.[20] Based on previous studies that reported a synergistic effect of fluoride with CPP-ACP regarding the protective effect against dental erosion,[24] various dental products contain a combination of these anti-erosive agents in a form of CPP-ACPF and are recommended for dental erosion therapy. In the current study, MI Paste Plus (which contains CPP-ACPF) increased resistance of enamel to erosive activity of the tested soft drink.

As per the results of statistical analysis, the second null hypothesis of the study (which stated that there were no statistically significant differences in increase in surface roughness among the experimental groups after the erosive challenge), was accepted. All the experimental groups presented the same behaviour regarding the increase in enamel surface roughness after erosive challenge. Previous studies have described the morphological changes of enamel following acidic attack resulting from the collapse of demineralised apatite crystals, which lead to increased surface roughness.[10]

Although in the experimental groups of the study, surface roughness increase was statistically significant after the erosive challenge, this increase may not be clinically significant for enamel integrity. Also, it is well documented that surface roughness of the tooth tissues is important for the formation of dental biofilms and bacterial adhesion.[11] Bollen et al[2] demonstrated that the critical mean surface roughness value (Ra) for bacterial colonisation of restorative materials is considered to be 0.2 μm. In the present investigation, the mean Sq values of the enamel specimens were approximately 0.2 μm after erosive challenge with a soft drink.

According to the results of the present study, the third null hypothesis (which stated that there were no statistically significant differences in surface loss among the experimental groups after the erosive challenge) was rejected. The tested anti-erosive treatments of the study significantly reduced surface loss after the erosive challenge compared to the control group. This evidence coincides with previous reports regarding similar treatments against enamel demineralisation.[4,18] Enamel surface loss occurs by a continuous layer-by-layer dissolution of the HAp crystals, which results to a permanent decrease of tooth volume. Surface loss evaluation after the erosive challenge is the most important measurement to estimate quantitatively the destructive effect of an erosive agent on enamel surface. However, surface hardness and roughness changes after the erosive challenge are also useful for providing qualitative information for the induced erosive lesions.[17]

Despite the reduction of enamel surface loss following the tested treatments, none of them prevented enamel erosion. This reduction ranged between 10.8% and 14.1%, meaning that the tested treatments may improve resistance to enamel erosion but there is still a need for additional preventive measures in order to minimise the destructive action of the erosive agents. In addition, it is questionable if this protective action of the anti-erosive agents is clinically important.

The protective action of the tested anti-erosive agents is mainly attributed to their remineralising and buffering properties. Previous studies reported morphological changes on enamel surface after CPP-ACP treatment.[4] Carvalho et al[4] demonstrated that after the use of CPP-ACP a protective layer was formed on the enamel surface. Nevertheless, this layer was non-homogeneous with adherent irregularities, appearing as globular structures which fill the interprismatic cavities and partially cover the prisms.

In vitro studies present various limitations, so clinical studies must be followed to confirm their outcomes. The use of human saliva in laboratory studies is very difficult due to its variable composition and lack of stability outside the oral cavity. An additional problem is the colonisation of human saliva by bacteria that may lead to alterations in its chemical composition, and the sterilisation of the liquid may result in the degradation of organic salivary components. Thus, the main purpose of using synthetic preparations is to obtain a stable environment and standardised procedures in laboratory tests across a wide range of biological studies.[21] In the current study we used a formula of artificial saliva that was also used in previous *in vitro* studies.

Another limitation of the present *in vitro* study is the use of bovine teeth. Human teeth can be regarded as the most appropriate source from the perspective of clinical relevance. Nevertheless, their composition is variable due to genetic influences, environmental conditions and age. These discrepancies may lead to large variations in their response under erosive challenges. In contrast, bovine teeth are more readily available and have a more uniform composition when compared to human teeth, thus providing a less variable response to erosive treatments. Moreover, bovine teeth have larger surfaces, which make them advantageous for experimental manipulation. Despite bovine enamel being more porous than human enamel, which results in faster demineralisation and remineralisation processes, these differences are mainly quantitative and not qualitative in behaviour. Furthermore, the erosive lesions produced from bovine teeth have a mineral distribution and structure that resembles lesions produced from human teeth, both for enamel and dentine. Thus, bovine teeth can be considered an acceptable alternative to human teeth and may offer some advantages in comparison with them.[3]

CONCLUSIONS

Within the limitations of this *in vitro* study, it can be concluded that the tested treatments may be promising for the limitation of enamel erosion induced by excessive consumption of soft drinks and may also be useful for prevention of

dental erosion attributed to different erosive factors. However, none of these treatments provided complete protection against the development of erosion, which means that they should be used as a part of an individually tailored preventive programme including measures such as diet modification, oral hygiene, fluoride use and regular dental supervision. Further studies are needed to clarify whether the protective effect of the tested anti-erosive agents is clinically important and if it is possible to enhance the effectiveness of the treatments.

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REFERENCES

1. Bakry AS, Marghalani HY, Amin OA, Tagami J. The effect of a bioglass paste on enamel exposed to erosive challenge. J Dent 2014; 42:1458–1463.
2. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. Dent Mater 1997;13:258–269.
3. Buzalaf MAR, Hannas AR, Magalhaes AC, Rios D, Honorio HM, Delbem ACB. pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. J Appl Oral Sci 2010;18:316–334.
4. Carvalho FG, Brasil VLM, Filho TJS, Carlo HL, Santos RL, Lima BASG. Protective effect of calcium nanophosphate and CPP-ACP agents on enamel erosion. Braz Oral Res 2013;27:462–470.
5. Derceli JR, Faraoni JJ, Pereira-da-Silva MA, Palma-Dibb RG. Analysis of the early stages and evolution of dental enamel erosion. Braz Dent J 2016;27:313–317.
6. Dionysopoulos D, Koliniotou-Koumpia E, Tolidis K, Gerasimou P. Effect of fluoride treatments on bleached enamel microhardness and surface morphology. Oral Health Prev Dent 2017;15:169–175.
7. Dionysopoulos D, Strakas D, Koliniotou-Koumpia E, Koumpia E. Effect of Er,Cr:YSGG laser irradiation on bovine enamel surface during in-office tooth bleaching ex vivo. Odontology 2017;105:320–328.
8. Featherstone JD, Lussi A. Understanding the chemistry of dental erosion. Monogr Oral Sci 2006;20:66–76.
9. Haghgoo EH, Haghgoo R, Roholahi MR, Ghorbani Z. Effect of casein phosphopeptide-amorphous calcium phosphate and three calcium phosphate on enamel microhardness. J Contemp Dent Pract 2017;18:583–586.
10. Hara AT, Livengood SV, Lippert F, Eckert GJ, Ungar PS. Dental surface texture characterization based on erosive tooth wear processes. J Dent Res 2016;95:537–542.
11. Hosoya N, Honda K, Iino F, Arai T. Changes in enamel surface roughness and adhesion of Streptococcus mutans to enamel after vital bleaching. J Dent 2003;31:543–548.
12. Huang S, Gao S, Yu H. Remineralization potential of nano-hydroxyapatite on initial enamel lesions: an in vitro study. Caries Res 2011;45:460–468.
13. Huysmans MC, Young A, Ganns C. The role of fluoride in erosion therapy. Monogr Oral Sci 2014;25:230–243.
14. Lussi A. Erosive tooth wear – a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 2006;20:1–8.
15. Lussi A, Carvalho TS. Erosive tooth wear: a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 2014;25:1–15.
16. Lussi A, Hellwig E, Cans C, Jaeggi T. Dental Erosion. Oper Dent 2009;34: 251–262.
17. Lussi A, Schlueter N, Rakhmatullina E, Ganss C. Dental erosion – an overview with emphasis on chemical and histopathological aspects. Caries Res 2011;45:2–12.
18. Min JH, Kwon HK, Kim BI. Prevention of dental erosion of a sports drink by nano-sized hydroxyapatite in situ study. Int J Paediatr Dent 2015;25:61–69.
19. O'Toole S, Bartlett DW, Moazzez R. Efficacy of sodium and stannous fluoride mouthrinses when used before single and multiple erosive challenges. Aust Dent J 2016;61:497–501.
20. Poggio C, Lombardini M, Dagna A, Chiesa M, Bianchi S. Protective effect on enamel demineralization of a CPP-ACP paste: an AFM in vitro study. J Dent 2009;37:949–954.
21. Pytko-Polonczyk J, Jakubik A, Przeklasa-Bierowiec A, Muszynska B. Artificial saliva and its use in biological experiments. J Physiol Pharmacol 2017;68:807–813.
22. Ranjitkar S, Kaidonis JA, Richards LC, Townsend GC. The effect of CPP-ACP on enamel wear under severe erosive conditions. Arch Oral Biol 2009;54:527–532.
23. Rezvani MB, Rouhollahi MR, Andalib F, Hamze F. Nano-hydroxyapatite could compensate the adverse effect of soft carbonated drinks on enamel. J Contemp Dent Pract 2016;17:635–638.
24. Srinivasan N, Kavitha M, Loganathan SC. Comparison of the remineralization potential of CPP-ACP and CPP-ACP with 900 ppm fluoride on eroded human enamel: an in situ study. Arch Oral Biol 2010;55:541–544.
25. Tantbirojn D, Huang A, Ericson MD, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. J Dent 2008;36: 74–79.
26. Wang CP, Huang SB, Liu Y, Li JY, Yu HY. The CPP-ACP relieved enamel erosion from a carbonated soft beverage: an in vitro AFM and XRD study. Arch Oral Biol 2014;59:277–282.
27. Wang X, Megert B, Hellwig E, Neukaus KW, Lussi A. Preventing erosion with novel agents. J Dent 2011;39:163–170.
28. Yuan M, Zhang Q, Gao XJ. Influence of Coca Cola on early erosion and surface microhardness of human enamel: an in situ study. Zhonghua Kou Qiang Yi Xue Za Zhi 2016;51:357–361.
29. Yip HK, Smales J, Kaidonis JA. Management of tooth tissue loss from erosion. Quintessence Int 2002;33:516–520.
30. Zawaideh FI, Owais AI, Mushtaha S. Effect of CPP-ACP or a potassium nitrate sodium fluoride dentifrice on enamel erosion prevention. J Clin Pediatr Dent 2017;41:135–140.
31. Zheng J, Xiao F, Qian LM, Zhou ZR. Erosion behavior of human tooth enamel in citric acid solution. Tribol Int 2009;42:1558–1564.