Original Article

Remineralization Effect of Calcium Glycerophosphate in Fluoride Mouth Rinse on Eroded Human Enamel: An *In Vitro* Study

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Received : 09-02-23
Revised : 19-06-23
Accepted : 23-06-23
Published : 30-08-23

Abstract

Aims and Objectives: The aim of this *in vitro* study was to evaluate the remineralization effect of calcium glycerophosphate (CaGP) in fluoride mouth rinse on permanent enamel eroded by a soft drink. **Materials and Methods:** Forty sound permanent premolars were embedded in self-curing acrylic resin and immersed in Coca-Cola to create erosive lesions. The teeth were divided into four groups (n = 10): Group I artificial saliva; Group II sodium fluoride; Group III sodium fluoride + sodium monofluorophosphate; and Group IV sodium monofluorophosphate + CaGP. The specimens in the assigned groups underwent pH cycling for ten days. The baseline, after erosion, and after remineralization surface microhardness (SMH) values were determined. The data were analyzed by one-way analysis of variance (ANOVA). The mean SMH value between groups and one-way repeated measures ANOVA for the mean SMH value within each group and Bonferroni's for multiple comparisons at a 95% confidence level were determined. The average SMH was used and calculated as the percentage recovery of SMH. **Results:** After being eroded by the cola soft drink, the mean SMH values in all groups were significantly decreased. After remineralization, Group I had the lowest %SMHR. The %SMHR of Groups II, III, and IV were significantly higher than Group I ($P < 0.001$). However, there were no significant differences among Groups II, III, and IV ($P > 0.05$). **Conclusions:** Fluoride mouth rinse with and without CaGP showed similar efficacies in remineralizing eroded permanent enamel.

Keywords: *Calcium glycerophosphate, microhardness, permanent teeth, remineralization, erosion*

Introduction

Dental erosion is one of the most frequently occurring dental pathologies.[1] Erosion occurs due to an increased intake of soft drinks over time.[2,3] Erosion is the chemical loss of mineralized tissue caused by acid dissolution that is not involved with bacteria. Demineralization by erosion is caused by frequent contact between the tooth surface and acids.[2] Several systematic reviews have found there is a distinct correlation between the intake of carbonated beverages and erosion.[1,2]

Fluoride is one of the most common substances used to remineralize lesions to prevent and treat tooth erosion.[4] As documented in previous studies, Fluoride's remineralization efficacy can be improved by mixing it with other chemical agents.[5-7] Additionally, numerous studies have shown that the presence of calcium and phosphate in dental products can lead to tooth remineralization, thereby enhancing their anti-erosion

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jpcd

DOI: 10.4103/jispcd.JISPCD_23_23

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How to cite this article: Torsakul P, Rirattanapong P, Prapansilp W, Vongsavan K. Remineralization effect of calcium glycerophosphate in fluoride mouth rinse on eroded human enamel: An *in vitro* study. J Int Soc Prevent Communit Dent 2023;13:327-32.

effect.[8-10] Manaswini *et al*. reported that calcium glycerophosphate (CaGP), a calcium-phosphate compound, exhibited anti-erosive properties on teeth.[11]

CaGP is an organic calcium phosphate salt that has anti-cariogenic effects.[12] It is hypothesized that CaGP strengthens the enamel by promoting plaque pH buffering by enhancing calcium and phosphate levels in plaque and in the hydroxyapatite in enamel.[12] Research has indicated that incorporating CaGP in soft drinks may aid in preventing enamel acid breakdown.[11,13] Puig-Silla *et al.* discovered that adding CaGP to sodium monofluorophosphate mouth rinse improved remineralization more than fluoride mouth rinse alone, despite having the same concentration of fluoride in both mouth rinses.[14]

Mouth rinses used for remineralization typically contain fluoride or calcium alone because fluoride, one of the most electronegative ions, rapidly combines with calcium to generate calcium fluoride (CaF_2), which is insoluble in water. This unfavorable interaction reduces the bioavailability of calcium and fluoride in products.[4,15,16] CaGP is the current form in which fluoride-containing calcium mouth rinses are available.

There are currently no data available on the remineralization effect of CaGP in a fluoride mouth rinse on eroded permanent enamel. Therefore, the aim of this *in vitro* study was to evaluate the effect of a fluoride mouth rinse with CaGP on the surface microhardness (SMH) of eroded permanent enamel.

Materials and Methods

The study method was approved by the Ethics Committee of Mahidol University (COE.No.MU-DT/PY-IRB2020/057.0211). The sample size determination was done according to the study by Rirattanapong *et al*.[9] The sample size was calculated using G*power version 3.1.2 with one-way analysis of variance (ANOVA) with $\alpha = 0.05$ and $\beta = 0.20$, which determined that 10 teeth per group were required.

Specimen preparation

Forty sound-extracted premolars were collected and stored in normal saline, with the radicular portion of each tooth removed. This study used teeth with sound enamel. Teeth with cracks, fluorosis, hypoplasia, white or brown lesions, or tetracycline staining were excluded. The specimens were embedded in self-curing acrylic resin (Ortho-Acylic P/L, Homedent Group Co. Ltd, Bangkok). To obtain a clean flat surface on the labial surfaces, the specimens were wet ground using 400, 600, 1200, 2,000, and 2,500 grit silicon carbide paper. A 3 mm x 4 mm test window was defined with scalpel cuts. The Baseline microhardness of the sound enamel was determined on the labial surface using a Vickers indenter (FM-700e Type D, Future-tech, Tokyo) with a 100 *g* force for 15 s. Each specimen received four indentations per test during each step.[9] The average SMH was used to calculate the percentage recovery of SMH (%SMHR) = 100 X (SMH value after remineralization – SMH value after demineralization)/(SMH value at baseline – SMH value after demineralization).[17]

Erosion procedure

The pH of Coca-Cola soft drink (Coca-Cola, ThaiNamthip, Bangkok, Thailand), artificial saliva[17] containing 0.65 g/L KCl, 0.058 g/L $MgCl_2$, 0.165 g/L $CaCl_2$, $K_2[HPO_4]_2$, $KH_2[PO_4]_3$, 2 g/L $NaCO_2CH_3$ cellulose, and deionized water to make one liter were measured by a pH meter (Thermo Scientific Orion 3 star RDO portable pH meter, Massachusetts).

The specimens were immersed in Coca-Cola for 5 s, then in artificial saliva for 5 s, for 10 cycles at room temperature.[9] The procedure was repeated three times at 6-h intervals. Between each test, the specimens were stored in artificial saliva.[9] After the erosion process, the specimens were rinsed with deionized water and blotted dry.

Demineralizing and Remineralizing Solution Preparation

Demineralization (D) and remineralization (R) solutions were prepared. D and R solutions were used to simulate apatitic mineral supersaturation in saliva. D consisted of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M acetic acid, and the pH was adjusted to 4.7 using 1 M KOH. R consisted of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCI with the pH adjusted to 7.0 using 1 M KOH.[17]

Specimen groups

After the erosion process, the specimens were randomly divided into four groups ($n = 10$): the teeth in the groups received the following treatment: Group I artificial saliva (no treatment), Group II sodium fluoride (NaF – 220ppmF) (Listerine Total Care Zero Alcohol), Group III sodium fluoride + sodium monofluorophosphate (NaF+SMFP – 241ppmF) (Fluocaril Zero Alcohol Double Mint Mouthwash), and Group IV sodium monofluorophosphate + CaGP (SMFP+CaGP – 224ppmF+75ppmCa) (Fluor Kin Mouthwash) [Table 1].

pH-cycling

The pH-cycling procedure reproduced the pH change in the oral environment by rinsing the teeth twice daily for ten days. Each tooth was placed in D for 3 h,

Table 1: Mouth rinses used in this study

GrouP	Active ingredients	Tradename	Other ingredients	Manufacturers
NaF	Sodium Fluoride 220ppm F	Listerine Total Care Zero Alcohol	Water, Sorbitol, Propylene Glycol, Paloxamer407, Sodium Lauryl Sulfate, Flavor, Eucalyptol, Zinc Chloride, Benzoic Acid, Sodium Benzoate, Sodium Saccharin, Thymol, Methyl Salicylate, Menthol, Aroma, Sucralose, Cl 16035, Cl 42090	Johnson & Johnson, Thailand
NaF+SMFP	Sodium Monofluorophosphate Sodium Fluoride Total 241 ppmF	Fluocaril Zero Alcohol Double Mint Mouthwash	Water, Glycerin, Sodium benzoate, PPG-26 Buteth-26, PEG-40 Hydrogenated Castor oil, Flavour, Cetylpyridinium Chloride, Sodium Saccharin, Citric acid, Cl47005, Cl42051	Meiyume Manufacturing, Thailand
SMFP+CaGP	Sodium Monofluorophosphate 224 ppm F Calcium Glycerophosphate 75 ppm Ca	Fluor Kin Mouthwash	Aqua, Glycerin, Sorbitol, Propylene Glycol, PEG-40 Hydrogenated Castor Oil, Xylitol, Aroma, Sodium Methylparaben, Citric Acid, Cetylpyridinium Chloride, Sodium Propylparaben, Sodium Saccharin, Potassium Acesulfame, CI 14720	Industria Farmaceutica Andromaco, Mexico

Table 2: Microhardness value (mean±SD) at baseline, after erosion, after remineralization, and the %SMHR

	Baseline	After erosion	After remineralization	%SMHR	*P* Value
Control	388.56±4.04	323.23±7.39	336.99±5.75	20.66±8.63	<0.001*
NaF	386.43±5.85	323.37±8.04	375.12±7.45	83.56±15.69	<0.001*
NaF+SMFP	384.67±5.62	328.59±12.57	374.73±8.80	87.41±29.73	<0.001*
SMFP+CaGP	387.65±7.63	314.09±18.09	381.17±13.62	90.67±17.87	<0.001*
P Value	0.497	0.083	<0.001*	<0.001*	

% SMHR = % Recovery of Surface Microhardness after remineralization

Data are presented as mean ± SD

P Values in the lowest row were determined by One-way ANOVA

P Values in the right column were determined by Repeated measure ANOVA

*Statistically significant at the 0.05 level (α = 0.05)

in R for 2 h, in D for 3 h, and then in R overnight at 37°C in an incubator. Each tooth was treated twice for one minute with its assigned group: once before the first demineralization of the day and once following the second demineralization.[18] After 10 days of pH cycling, the specimens' SMH was measured using the Vickers indenter test protocol.

Statistical analysis

We performed one-way repeated measures ANOVA to examine the SMH values at baseline, after erosion, and after remineralization within each group. For the differences in SMH values between the groups at each stage, one-way ANOVA was performed. The Bonferroni method was used for multiple comparisons at the 95% confidence level. All the analyses were performed by SPSS version 27.0.

Results

The mean SMH values at baseline, after erosion, and after remineralization are presented in Table 2. The mean SMH values at baseline were not significantly different among the groups ($P = 0.497$).

After the erosion process, the mean SMH value decreased with a 16.68% mean reduction from baseline. This decrease represented a significant decrease in the mean SMH value. In addition, there were no significant differences among the groups ($P = 0.083$).

After remineralization, the mean SMH values and %SMHR in each group significantly increased. However, the no treatment group had the lowest mean SMH values and %SMHR. Furthermore, there were no significant differences in the mean SMH value or %SMHR among the three treatment groups [Table 3] ($P < 0.001$).

Discussion

This study investigated the effect of a fluoride mouth rinse with CaGP on the SMH of eroded permanent tooth enamel. The mean SMH value in our study at Baseline was 386.83±10.36 VHN, which was

Table 3: Multiple comparison (*post hoc* test) of the microhardness in the different groups

	NaF	NaF+SMF*P*	SMFP+CaG*P*
After remineralization			
Control	38.13* ($P < 0.001$)	37.74* ($P < 0.001$)	44.19 *($P < 0.001$)
NaF	–	0.39 ($P = 1.000$)	6.06 ($P = 0.943$)
NaF+SMFP	–	–	6.45 ($P = 0.798$)
% SMHR			
Control	62.90* ($P < 0.001$)	66.76* ($P < 0.001$)	70.01* ($P < 0.001$)
NaF	–	3.86 ($P = 1.000$)	7.11 ($P = 1.000$)
NaF+SMFP	–	–	3.25 ($P = 1.000$)

Multiple comparison performed using the Bonferroni test

*The mean difference is significant at the 0.05 level ($\alpha = 0.05$)

higher than the values reported by Rirattanapong *et al*. and Shetty *et al*.[9,19] Their mean SMH value at the baseline was 342.10±21.90 and 357.82±25.04 VHN, respectively. These differences could be related to differences in specimen preparation methods, the degree of enamel mineralization, or local variations resulting from enamel rods and tufts.[20] However, there were no significant differences in the mean SMH value among the groups at Baseline.

The erosion process that we used in this study was adapted from Rirattanapong *et al*.[9] and was designed to simulate saliva's washing effect when drinking acidic beverages. After the tooth samples were eroded by Coca-Cola, the mean SMH values significantly decreased compared with Baseline. The mean microhardness percent reduction from Baseline was 16.68%. This finding was consistent with Panich *et al*. and Rirattanapong *et al*., whose percent reduction was 14.30% and 14.20%, respectively.[9,21]

After remineralization, the mean SMH value and %SMHR in every group significantly increased. However, the mouth rinse groups showed greater remineralization compared with the no treatment group. This finding was consistent with other studies that showed that fluoride remineralized erosive lesions as well as calcium-phosphate-containing materials.[4,8,10,22] The remineralization effect in the control group may be because the artificial saliva used in our study included chemical substances that promote remineralization.[23]

The fluoride mouth rinse groups (NaF and NaF+SMFP group), contained two different types of fluoride, NaF and SMFP. There were no significant differences in remineralization between these two fluoride groups. However, whether NaF and SMFP have a higher efficacy compared with each other is unresolved.[24] Several studies have shown that NaF and SMFP had the same remineralization effect and no significant difference existed between them.[25,26] Furthermore, a study found that combining NaF and SMFP had the same remineralizing effect as NaF alone when both had the same fluoride concentration.[27] These findings are supported by Faller *et al*. and Eversole *et al*., who found no significant differences in the ability of NaF, SMFP, and NaF+SMFP to protect against enamel surface loss during the Erosion process.[28,29]

The SMFP+CaGP treatment also had a remineralization effect, which was in line with the results of other research that discovered that calcium-phosphate-containing products increased the microhardness of eroded enamel.[8-10,21] The remineralization in the SMFP+CaGP group may be the result of a synergistic effect between SMFP and CaGP. There is evidence that CaGP enhances the remineralization effect of sodium monofluorophosphate, resulting in an increase in enamel remineralization. It is believed that this is the consequence of increased fluoride uptake in a non-alkali-soluble form at the expense of calcium fluoride in its alkali-soluble form.[12]

In this study, the remineralization by CaGP was caused by CaGP acting directly on enamel.[12] Within the CaGP molecule, calcium is ionically bound to phosphate, whereas glycerol is covalently bonded to phosphate.[11] Studies indicated that CaGP increased calcium and phosphate levels, which are important for the mineral structure of teeth.[12,30] This allows CaGP to have a direct effect on enamel and decrease the acid solubility of hydroxyapatite.[12] Additionally, CaGP may also prevent the plaque pH from dropping and reduce plaque mass.[12]

There was no significant difference in remineralization between the SMFP+CaGP, NaF, and NaF+SMFP groups after Remineralization, which contrasted with the results in Puig-Silla *et al*.[14] These researchers found that fluoride mouth rinse combined with CaGP had a significantly greater remineralization effect than fluoride mouth rinse alone at the same fluoride concentration.[14] This could be because our study used erosion as the demineralization process, whereas

Puig-Silla used initial caries as the demineralized specimens.

There are differences between caries and erosion lesions; for caries, the primary site is the enamel subsurface, whereas for erosion, the primary site is the outermost surface of the enamel. These differences result in a subsurface lesion with an intact outer enamel layer for caries and a softening of the surface mineral for erosion.[31] The enamel surface layer may be necessary for allowing calcium and phosphate ions to be absorbed into the enamel surface during remineralization after erosion.[32]

Manaswini *et al*. showed that CaGP has a dose-dependent effect, with concentrations of 2mM or higher significantly reducing enamel loss. The protective effect of CaGP is directly proportional to its concentration.[11] However, in our study, the concentration of CaGP was calculated to be 1.8mM, which is lower than the concentration found to be effective in a previous study.[11] The degree of remineralization may differ. This could explain why there was no significant difference in remineralization among the NaF, NaF+SMFP, and SMFP+CaGP groups.

Rirattanapong *et al*., Emamieh *et al*., Carvalho *et al*., and Valian *et al.* found that adding fluoride to calcium-phosphate-containing dental products had no additional effect on eroded enamel remineralization.[9,33-35] Their results were similar to ours in that the SMFP+CaGP group did not have a significant remineralizing effect compared with the NaF and NaF+SMFP groups.

Our study used a pH-cycling model to simulate the dynamics of mineral loss and gain in the oral cavity. However, this pH-cycling has some disadvantages, e.g., no microorganisms were present. Although our study did not accurately simulate the complex intraoral environment, we designed the study's methodology based on prior studies that have successfully shown a remineralization effect.[9,36]

Fluoride mouth rinse containing CaGP can be an alternative mouth rinse for remineralizing eroded permanent tooth enamel, particularly in patients with a calcium or phosphate deficiency in saliva, such as irradiated or salivary gland dysfunction patients. Additional laboratory research on CaGP in remineralized eroded teeth may be necessary to determine its true mechanism of action.

Conclusion

Fluoride mouth rinse with CaGP and fluoride mouth rinses showed similar efficacies in remineralizing eroded permanent tooth enamel. However, further clinical studies with a larger sample size are needed to validate this finding and evaluate the effectiveness and efficiency of CaGP in dental products for remineralizing eroded permanent enamel.

Acknowledgements

The authors thank Miss Chayada Teanchai and the senior staff of the Research Service Center of the Faculty of Dentistry, Mahidol University.

Financial support and sponsorship

None.

Conflicts of interest

Nil.

Authors contributions

PR formulated the research question and designed the framework with WP and KV. PT conducted the experiments, and all authors participated in data analysis. PT and PR wrote and revised the manuscript. All authors approved the final version of the manuscript.

Ethical policy and institutional review board statement

This study was approved by the Ethics Committee of Mahidol University, Thailand (MU-DT/PY-IRB2020/057.0211).

Patient declaration of consent

Nil.

Data availability statement

The additional data of this study are available on request from Dr. Pannaros Torsakul at pannaros.tor@gmail.com.

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