

An *in vitro* Comparison of Efficacy of Four Remineralizing Agents on Dental Erosive Lesions: An Atomic Force Microscopic Study

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Abstract

Aims: The aim of the study is to comparatively evaluate the remineralization potential of different agents on erosive lesions produced by the consumption of carbonated drinks. **Materials and Methods:** Hundred extracted human anterior teeth were selected and divided randomly and equally into four study groups: Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), CPP-ACP with fluoride (CPP-ACPF), β -tri calcium phosphate (β -TCP), bioactive glass (BG) and one control group. Samples were prepared by grinding 50–100 μ m of enamel at the cervical third of the labial surface and were subjected to demineralization with a carbonated drink (cola-flavored). Samples in all study groups (except control) were treated with respective remineralizing agents for 7 days and were evaluated with an atomic force microscope for mean surface roughness (R_{rms}) at 3 stages: Baseline, demineralization, and remineralization cycle. Statistical analysis was performed at an alpha level of 5%. **Results:** All samples displayed a significant increase in R_{rms} values after the demineralization procedure followed by a reduction in surface roughness after the remineralization phase which was highest with β -TCP followed by BG, CPP-ACPF, and CPP-ACP remineralizing agent. **Conclusion:** Carbonated cola drinks produce demineralization of the exposed tooth surface. β -TCP remineralizing paste has comparatively better remineralizing capacity than BG and significantly better than CPP-ACPF and CPP-ACP.

Keywords: Bioactive glass, Carbonated drink, Demineralization, Dental erosion, Remineralization, Surface roughness, Tri-calcium phosphate.

INTRODUCTION

Dental erosion is a damaging process that occurs when tooth structure is lost due to acidic disintegration in the absence of microorganisms.^[1] The non-carious erosion of mineralized hard tissues has become more common in modern societies after dental caries.^[2] Non-carious dental erosion alters the composition, crystallography, microstructure, and mechanical properties, resulting in a hypo-mineralized tooth surface, which causes an irreversible loss of tooth structure, weakening the tooth and ultimately fracturing it.^[3] Other effects of tooth erosion include tooth sensitivity, poor esthetics, loss of vertical dimension, functional issues, and in chronic cases, pulp exposure and abscess.^[4]

The oral environment is constantly demineralizing and remineralizing. The process of demineralization advances the degradation of tooth surfaces if this equilibrium is

upset.^[5] Saliva's almost neutral pH gives it a built-in buffering ability. Saliva is supersaturated with fluorapatite and hydroxyapatite (HAP) in a healthy state containing a variety of chemicals, including buffering agents, fluoride, calcium, and phosphate ions, which work together to reverse the natural demineralization of teeth at an early stage.^[6] Subsurface HAP dissolves in a carious lesion, and fluorhydroxyapatite forms on the surface, causing partial enamel disintegration.^[7] Fluorhydroxyapatite dissolved during erosion, causing a lack of

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surface layer and the eventual layer-by-layer disintegration of enamel along with the loss of crystals. Long-term exposure to supersaturated solution is believed to promote remineralization.

It has been demonstrated that certain agents can remineralize, including fluoride, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and CPP-ACP with fluoride (CPP-ACPF). β -tri calcium phosphate (β -TCP) has lattice defects that allow crystal modification and are biocompatible, bioactive, and a precursor to the synthesis of HAP.^[8] Lately, a breakthrough in remineralization, bioactive glass (BG) balances the body's natural mineralizing characteristics by functioning as a biomimetic mineralizer. In addition, it has an advantageous effect on cell signals that help restore tissue shape and function.^[9]

Given the above observations, the present study was undertaken to determine and compare the remineralizing potential of the CPP-ACP, CPP-ACPF, β -TCP, and BG-containing dentifrices on dental erosion produced by soft drinks in enamel using an atomic force microscope (AFM).

MATERIALS AND METHODS

Approval was obtained from the Institutional Ethics Committee before commencing the study. Hundred freshly extracted maxillary anterior teeth free of caries, cracks, or any developmental defects were procured from the department of exodontia, after calculation of the sample size with G*Power Software version 3.1.9.7, using a one-way analysis of variance model with an effect size of 0.4, alpha error of 0.05, power of 80%, and a two-tailed significance level (α) of 0.05. The effect size was hypothesized based on a pilot study carried out prior.

The teeth were cleaned free of calculus, debris, and soft tissue, and stored in a 10% formalin solution.

Sample preparation

The specimens were sectioned at the cemento-enamel junction (Figure 1a). The crown was sectioned mesiodistally with the help of a diamond disc with water spray. Each sectioned tooth's labial segments were added to an acrylic block using the salt-and-pepper method until the resin firmly encased them (Figure 1b). Each of the enamel specimens was standardized by grinding it with silicon carbide sheets (grading 600, 1000 sequentially) while being continuously irrigated with water to remove about 50–100 μ m of enamel. Following this, all the samples were randomly divided into five groups as Group I: CPP-ACP dentifrice (GC Tooth Mousse, Recaldent, GC Corporation, Tokyo, Japan), Group II: CPP-ACPF dentifrice (GC Tooth Mousse Plus, Recaldent, GC Corporation, Tokyo, Japan), Group III: β -TCP Dentifrice (Clinpro™ Tooth Creme, 3M ESPE, Australia), Group IV: BG containing dentifrice (SHY-NM, Group Pharmaceuticals, India), Group V: Control Group: No treatment.

Demineralization

For demineralization, a carbonated drink with a pH of 2.24 was used (a cola-flavored drink). 6 mL of soft drink was pipetted in a



Figure 1: Procedural steps in sample preparation and testing. (a) sectioned tooth specimen at the cemento-enamel junction, (b) labial surface of teeth embedded in acrylic resin, (c) specimens immersed in carbonated drink and placed in a centrifuge machine, (d) application of the respective remineralizing agents with a powered toothbrush, (e) storing of specimen in artificial saliva, (f) atomic force microscope

test tube per specimen, and the specimens were properly dipped in the test such that the entire surface of the specimen was covered with the liquid, for attaining even demineralization. After immersion, the samples were centrifuged for 2 min at 120 rpm (Figure 1c). Immersions were carried out at 4 regular intervals of 6 h, to simulate the exposure occurring at meal times. Carbonated drink was centrifuged at a constant speed of 120 rpm, to reconstruct the sipping action during drinking. In between exposures to demineralizing cycles, samples were stored in deionized water which was changed every week.

Remineralization

Remineralization was carried out in treatment groups at 0 h and subsequently at every 12-h interval for 7 days. Thus, a total of 15 remineralization treatment cycles were rendered during these 7 days. In all 4 experimental groups, for remineralization, respective remineralizing agents were applied with the help of a power-rotated toothbrush (Colgate 360 sonic power battery-operated toothbrush, Colgate Palmolive, India) for 30 s. In addition, the remineralizing agent was allowed to remain on the surface for 3 min (Figure 1d). After each brushing treatment, the specimens were washed with deionized water for 10 s.

Preparation of artificial saliva

Artificial saliva was prepared in the department of biochemistry of an adjacent medical college. The pH was measured with a digital pH meter both during and after the solution was prepared. The equipment was calibrated every time using a pH 7.0 phosphate buffer solution before measuring pH. The following was the chemical composition of the artificial saliva: (a) 2 g/L methyl-p-hydroxybenzoate; (b) 10 g/L sodium carboxy methyl cellulose; (c) 625 g/L of potassium chloride; (d) 0.059 g/L magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$); (e) 0.166 g/L calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$); (f) 0.804 g/L

potassium dihydrogen phosphate (K_2HPO_4); (g) 0.326 g/L monopotassium phosphate (KH_2PO_4).

The final pH was adjusted to 7.00 at 37 °C with 85% lactic acid.

After carrying out the remineralization, of all the samples (except in the control group), the specimens were placed in artificial saliva (Figure 1e) to assess the surface roughness of each specimen at 3 phases: Baseline or unexposed, after demineralization, and after completion of the remineralization procedure, with the help of Veeco AutoProbe CP 100 AFM (Figure 1f). Arbitrarily ten different film areas were scanned to obtain topography on a $30\ \mu m \times 30\ \mu m$ scanning area using a 1 Hz scan rate and 256×256 resolution. SPM Manager V2.11 software (Shimadzu Corporation, Japan) was used to process measured topographical data. The data were obtained by averaging all ten evaluated areas' root-mean-square roughness (R_{rms}).

Statistical analysis

The collected data were analyzed using the IBM SPSS Statistics for Windows, Version 27.0. (Armonk, NY: IBM Corp). Shapiro–Wilk test analysis adjudicated the data to be normally distributed, thereby intra-group comparisons were carried out with a repeated-measures-way analysis of variance (ANOVA) and inter-group comparisons were analyzed by the one-way ANOVA to compare the surface roughness between the study groups. The $P \leq 0.05$ was considered as the level of significance.

RESULTS

The AFM images obtained at baseline, after demineralization, and after remineralization have been illustrated in Figure 2.

Pertaining to the R_{rms} values, at the baseline, there was no significant difference in the surface roughness between the groups ($P = 0.98$), implying that the specimens were matched, nullifying any effect of biased sample preparations. Intra-group comparisons were carried out to compare the surface roughness between the baseline and after remineralization, which yielded a significant increase for each of the study groups ($P < 0.001$), which gradually decreased to 7 days after remineralization in all four study groups ($P < 0.001$) (Tables 1 and 2). This demonstrates the effectiveness of the four remineralizing agents employed in this investigation in remineralizing artificial erosive lesions.

After remineralization, the change in surface roughness was found in the following order of decrease: Group V (259.9 ± 14) > Group I (189.25 ± 15.5) > Group II (185.7 ± 15.61) > Group IV (174.35 ± 15.91) > Group III (166.8 ± 17.6). Thus, the maximum decrease in the surface roughness post-remineralization was evident in Group III (92.57%). Inter-group comparisons revealed that the surface roughness after remineralization of the erosive lesions was significantly different between all the study groups ($P < 0.001$), except for Groups I and II ($P = 0.95$) and Groups III and IV ($P = 0.56$). Figure 3 illustrates the mean surface roughness

values of four remineralizing agents and one control group at baseline, after demineralization, and after remineralization with the help of a line graph showing the increase in surface roughness after demineralization and a decrease in it post-remineralization.

DISCUSSION

The prevalence of dental degradation differs significantly among age groups. Dental erosion has started to get greater attention as a result of lifestyle changes throughout time. Dental erosion is difficult to diagnose since new technologies and altered lifestyles can generate lesions that are invisible or unclear, especially in the early stages. The age range where erosion is most prevalent spans from the young to the old.^[10] Elderly individuals have an accelerated tendency to tooth sensitivity, uneven edges on the teeth, shiny spots on the teeth, increased tooth decay, and tooth fracture in severe cases.^[11] To curtail the side effects of dental erosion, remineralization has been advocated universally, as it contributes toward restoring strength and function within the tooth structure.

This study was thus conducted to evaluate the remineralizing capacity of several contemporary agents on demineralized lesions caused by beverage consumption, to prescribe and apply clinically a more successful erosive lesion treatment regimen using remineralizing agents.

Because permanent anterior teeth are the second most impacted by erosion after maxillary molars, they were chosen for this investigation. They have sufficient enamel thickness, which can be subjected to remineralization, after subjecting into a demineralization phase in a cola-flavored drink and evaluation by an AFM. Ehlen *et al.*^[12] found more erosive wear on enamel specimens by cola-flavored drinks when compared to regular sodas, sports drinks, and fruit juices. Yuan *et al.*^[13] found a reduction of microhardness of enamel after demineralization by the latter.

AFM is a valuable method for obtaining novel structural information since it may create images with great contrast and resolution.^[14] The primary benefit of AFM is that it does not require sample preparation and can deliver quantitative data at the nanoscale level in all three dimensions together with the corresponding pictures. Moreover, non-dehydrated enamel surfaces can be studied.^[15]

In this study among the tested remineralizing agents β -TCP proved to be most effective followed by BG, CPP-ACPF, and finally, CPP-ACP. The agents showed a 92.57%, 86.51%, 76.61%, and 72.05% reduction in the surface roughness after the demineralization phase, respectively. It was discovered that β -TCP produced remineralization that was comparable to BG, but statistically higher than that of CPP-ACPF and CPP-ACP. The structure of β -TCP is comparable to that of apatite, and it has special calcium environments that can react with enamel and fluoride. The results of the present study align with the studies of Patil *et al.*^[16] and Hegde *et al.*^[17] who found β -TCP

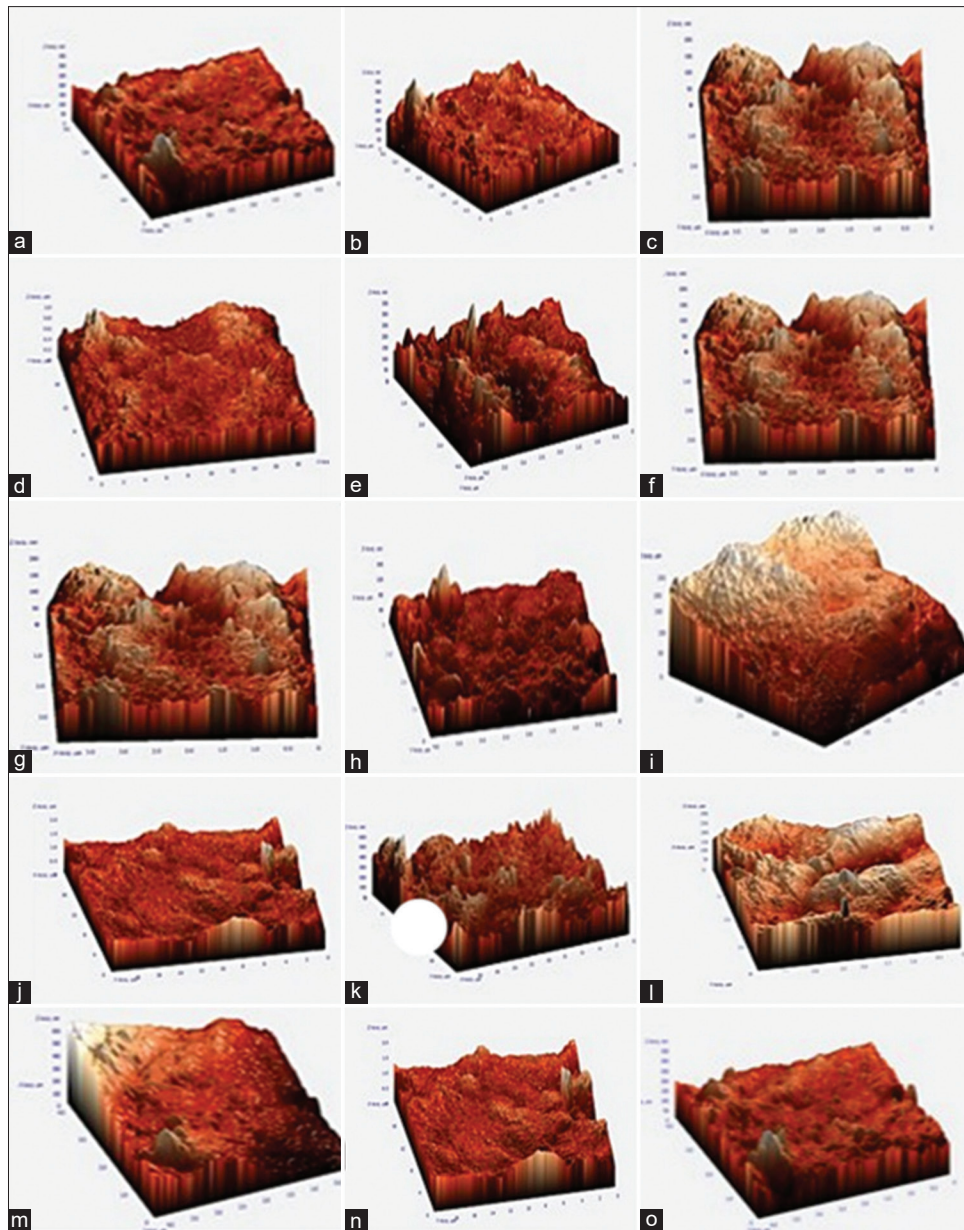


Figure 2: Atomic force microscope images of four remineralizing agents and one control group in $30\ \mu\text{m} \times 30\ \mu\text{m}$ (a) Group I: At baseline, (b) Group I: After demineralization, (c) Group I: After remineralization, (d) Group II: At baseline, (e) Group II: After demineralization, (f) Group II: After remineralization, (g) Group III: At baseline, (h) Group III: After demineralization, (i) Group III: After remineralization, (j) Group IV: At baseline, (k) Group IV: After demineralization, (l) Group IV: After remineralization, (m) Group V: At baseline, (n) Group V: After demineralization, (o) Group V: After remineralization, μm : micrometers

to outperform CPP-ACPF and CPP-ACP. Abdullah *et al.*,^[17] in their study also could not demonstrate any significant rehardening or protection of erosive enamel specimens with CPP-ACP, attributing the effect to be time-dependent.

In the current investigation, BG produced equally promising results as β -TCP, which is similar to the findings of Palaniswamy *et al.*^[18] and Narayana *et al.*,^[19] who found better remineralizing capacity than CPP-ACP because of the formation of a stronger silicate bond with tooth calcium, as BG releases hydroxycarbonate apatite directly, without the intermediate ACP phase. On the contrary Balakrishnan *et al.*^[20]

found CPP-ACP to possess better-remineralizing potential than β -TCP and BG when tested for a longer period.

Taranath *et al.*^[21] found better remineralizing potential with CPP-ACPF as compared to CPP-ACP. They attributed this finding to, the presence of fluoride in CPP-ACPF that probably stabilizes the HAP remineralized by casein phosphor-peptide to compound such as fluorapatite, which is resistant to acid dissolution.^[9] The present study also witnessed similar results although there was no statistically significant difference between the remineralization efficacy of CPP-ACPF and CPP-ACP.

Table 1: Mean surface roughness and standard deviations (R_{rms}) of human enamel specimens as a function of stage and treatment groups

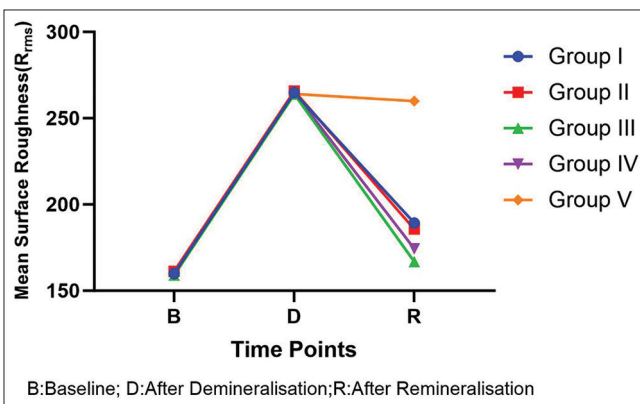
Groups (dentifrices)	n	Baseline	After demineralization	After remineralization	ΔB (%)	ΔD (%)	P-value [‡]
Group I	20	159.9±14.99 ^{a,A}	264.9±15.4 ^{b,B}	189.25±15.5 ^{c,A}	65.67	72.05	<0.001*
Group II	20	161.25±15.14 ^{a,A}	265.8±15.38 ^{b,B}	185.7±15.61 ^{c,A}	65.11	76.61	<0.001*
Group III	20	159±15.8 ^{a,A}	264±16 ^{b,B}	166.8±17.6 ^{c,B}	66.04	92.57	<0.001*
Group IV	20	160.25±14.87 ^{a,A}	264.8±14.98 ^{b,B}	174.35±15.91 ^{c,B}	65.24	86.51	<0.001*
Group V	20	159±12.83 ^{a,A}	264.1±13.28 ^{b,B}	259.9±14 ^{c,C}	66.10	4.00	<0.001*
P-value [§]		0.98NS	0.99NS	<0.001*		-	

n: Number of specimens per group, ΔB (%): Percentage increase in surface roughness relative to Baseline, ΔD (%): Percentage decrease in surface roughness relative to demineralized phase, [‡]: Intra-group comparisons (between the periods within each study group) analyzed by repeated measures ANOVA test yielding $P < 0.001$ between each time point for each group, [§]: Inter-group comparisons (between the study groups) analyzed by one-way ANOVA test, NS: not statistically significant ($P > 0.05$), *: statistically significant ($P \leq 0.05$), Different lowercase letters indicate a significant difference between the follow-up period in each study group, Different UPPERCASE letters indicate a significant difference between the study groups in each follow-up period

Table 2: Pair-wise comparisons between the groups after remineralization

Groups	Mean difference	Adjusted P value
I versus II	3.550	0.95 ^{NS}
I versus III	22.45	0.0002*
I versus IV	14.90	0.03*
I versus V	-70.65	<0.0001*
II versus III	18.90	0.0024*
II versus IV	11.35	0.05 *
II versus V	-74.20	<0.0001*
III versus IV	-7.550	0.56 ^{NS}
III versus V	-93.10	<0.0001*
IV versus V	-85.55	<0.0001*

NS: Not statistically significant ($P > 0.05$), *: Statistically significant ($P \leq 0.05$)

**Figure 3:** Line graph showing the mean surface roughness (R_{rms}) values of four remineralizing agents and one control group at baseline, after demineralization, and after remineralization

Despite several strengths, our study had some potential limitations. First, it is possible that the artificial lesions made on the specimens do not accurately depict clinical white spot lesions. Nonetheless because white lesions are not uniformly characterized, this issue frequently arises in *in vitro* investigations. Second, to simulate the remineralization capacity in clinical settings, artificial saliva is used in laboratory research. It is crucial to remember that it is challenging to

completely duplicate the dynamics of the saliva-enamel interaction in lab studies.

CONCLUSION

Within the limitations of the present study, it can be concluded that cola-flavored carbonated beverages produce demineralization of the exposed tooth surface. CPP-ACPF, CPP-ACP, β -TCP, and BG are effective when used as remineralizing agents. However, β -TCP is a more effective remineralizing agent than BG, CPP-ACPF, and CPP-ACP, BG is a more effective remineralizing agent than CPP-ACPF and CPP-ACP while CPP-ACPF is a more effective remineralizing agent than CPP-ACP. However, further long-term *in situ* and *in vivo* studies should be conducted to determine the clinical validity of these agents, their potential to cause sub-surface remineralization, and their bioactivity and durability.

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