COMPARATIVE EVALUATION ON THE ROLE OF SILVER DIAMINE FLUORIDE AND CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE ON ENAMEL RESISTANCE IN A DENTAL EROSIVE CHALLENGE: AN *EX-VIVO* MICRO-CT ANALYSIS

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Abstract

Aim: Dental erosion is the chronic loss of dental hard tissue by acids without bacterial involvement. Currently, silver diamine fluoride (SDF) is one of the most successful developments in the field of pediatric dentistry against early childhood caries. A lacuna, however, exists in scientific research on the possible role of SDF in dental erosion. The present study aimed to evaluate and compare the possible role of SDF on enamel resistance with Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) in an *ex-vivo* Micro-CT analysis.

Methodology: Twenty-five premolars were sectioned to prepare 50 enamel block samples which were divided into 3 groups; Group 1-acid exposed, Group 2-No acid exposure, and Group 3-Control. The group 1 and 2 samples were further subdivided into 2 sub-groups based on their corresponding intervention; Group A - SDF, and Group B - CPP-ACP. The mineral density values were assessed at baseline, immediately after acid exposure, 14 days post-

treatment, and 6 months post-treatment. A Micro-CT was used to analyze the mineral density values. The results were subjected to statistical analysis. $p \leq 0.05$ was considered significant.
Results: A significant increase in mineral density values post-treatment for all samples was noticed from the baseline value. Higher mineral density values for all samples treated with CPP-ACP were observed over SDF after 14 days of treatment ($p \leq 0.05$). However, the mineral density assessed after 6 months was higher for samples treated with SDF than CPP-ACP, though statistically not significant.
Conclusion: SDF may be as effective as CPP-ACP in the remineralization of dental erosive lesions.
Keywords: *Casein phosphopeptide-amorphous calcium phosphate, CPP-ACP, Tooth erosion, Tooth remineralization, Silver diamine fluoride*

1. Introduction

Dental erosion is the chronic loss of dental hard tissue due to exogenous or endogenous acids without bacterial involvement, and the common sources include acidic beverages, medications, occupational exposure, and lifestyle activities.[1] An estimated overall worldwide prevalence of tooth erosion is 30% in permanent teeth of children and adolescents aged 8–19 years.[2] Indian studies have also reported the prevalence of dental erosion among children ranging from 8.9% to 34.12% in different states.[3,4] It is crucial for doctors to be aware of dental erosion because it appears to be a growing issue in children and teenagers and can result in considerable loss of tooth substance, necessitating time-consuming and expensive restorative procedures.[5] An early diagnosis provides opportunities to influence and change unhealthy habits, diagnose and intercept a possible underlying medical condition, and initiate preventive measures.

Multiple in-vitro studies have shown an erosion-inhibiting effect from different topical fluoride applications.[6,7,8] Currently, products such as CPP-ACP have proved to be more effective than fluoride varnishes in increasing the enamel's resistance to erosion.[9] However, there exist controversies on the potential of CPP-ACP in remineralizing early enamel lesions. Two systematic reviews [10,11] concluded that CPP-ACP alone cannot be established as the best clinical practice in remineralization than when combined with fluoride, and other clinical trials have concluded that the combined effect of CPP-ACP and fluorides have no superiority over fluoride monotherapy.[12,13]

A simple, yet powerful tool in the fight against early childhood caries (ECC) makes SDF one of the most efficient anti-caries agents used in the field of Pediatric dentistry. At a concentration of 38%, SDF contains 44,800 ppm fluoride which is more than any available fluoride agents.[14] A lacunae, however, exists in scientific research on the possible role of SDF in dental erosion. The aim of the present study was to evaluate and compare the possible role of SDF on enamel resistance with CPP-ACP in an ex-vivo dental erosive challenge. The null hypothesis is that there is no difference in remineralizing potential between SDF and CPP-ACP.

2. Methodology

The present study was performed in the Department of Nanotechnology, Amrita Institute of Medical Sciences, Kochi, and the Department of Pediatric and Preventive Dentistry, Amrita School of Dentistry, Kochi. The ethical clearance for the present study was provided by the Ethical Committee of our institute (IRB-AIMS-2020-150; Dated: 20-03-2020).

Sample size

The sample size of 50 blocks was calculated at a 95% confidence interval based on previous research by Punyanirun *et al.*[15] Two blocks each were prepared from 25 premolar teeth. Ten enamel blocks each were subjected to analysis within a group.

Study samples

Twenty-five freshly extracted premolars with no signs of caries, fracture, or visible morphological alterations in enamel were collected from patients who reported for orthodontic purposes and were placed in 0.9% normal saline at 37°C till the experiment was performed.

Sample preparation

The premolars were initially sectioned below the cementoenamel junction. Thin sections were then prepared by slicing the proximal surfaces of the premolar using a slow-speed water-cooled cutting machine (Isomet 1000, USA). The sections were then embedded in acrylic blocks of dimensions 2mm x 2mm x 2mm to prepare a total of 50 enamel blocks (2x25 premolars). The blocks were painted with acid-resistant nail varnish to expose an occlusal window of 1mm x 1mm dimensions of the specimen and then allowed to dry. (Figure 1)

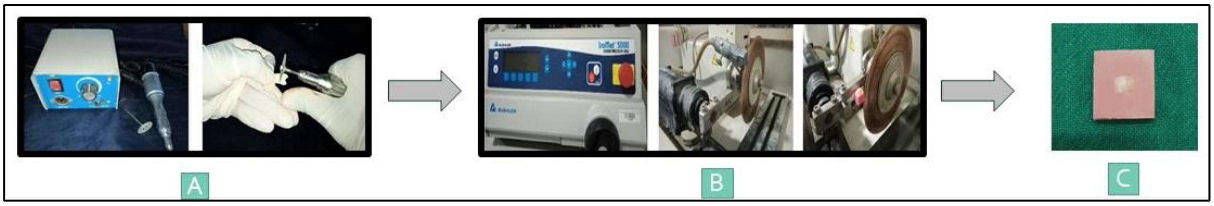

Figure 1: Sample preparation: A) Premolar tooth sectioned at the cementoenamel junction. B)Proximal sections prepared with ISOMET 1000, USA. C) prepared section embedded in acrylic block

Assessment of baseline mineral density

The baseline mineral density value for the samples was determined using a micro-CT prior to exposure to the acidic pH cycle and corresponding interventions. After baseline evaluation, the samples were divided as:

Group 1 - Exposure to acid pH cycle (n = 20)
Group 2 - Non-exposure to acid pH cycle (n = 20)
Group 3 - Control (no intervention) (n = 10)

Acidic pH cycle:

To induce demineralization, freshly opened bottles of Coca-Cola (Hindustan beverages PVTT, Pune, Maharashtra; 50 mL/specimen) were used. The samples were immersed for two minutes interspaced with a duration of one minute in artificial saliva. Artificial saliva was prepared based on the modified Macknight-Hane and Whitford formula by Ammaechi *et al*[16,17] (30 mL/sample). This cycle was repeated thrice for each sample to simulate the ingestion of a carbonated drink. The artificial saliva was replaced every week to avoid oversaturation. A summary of the methodology followed in this study is presented in Figure 2.

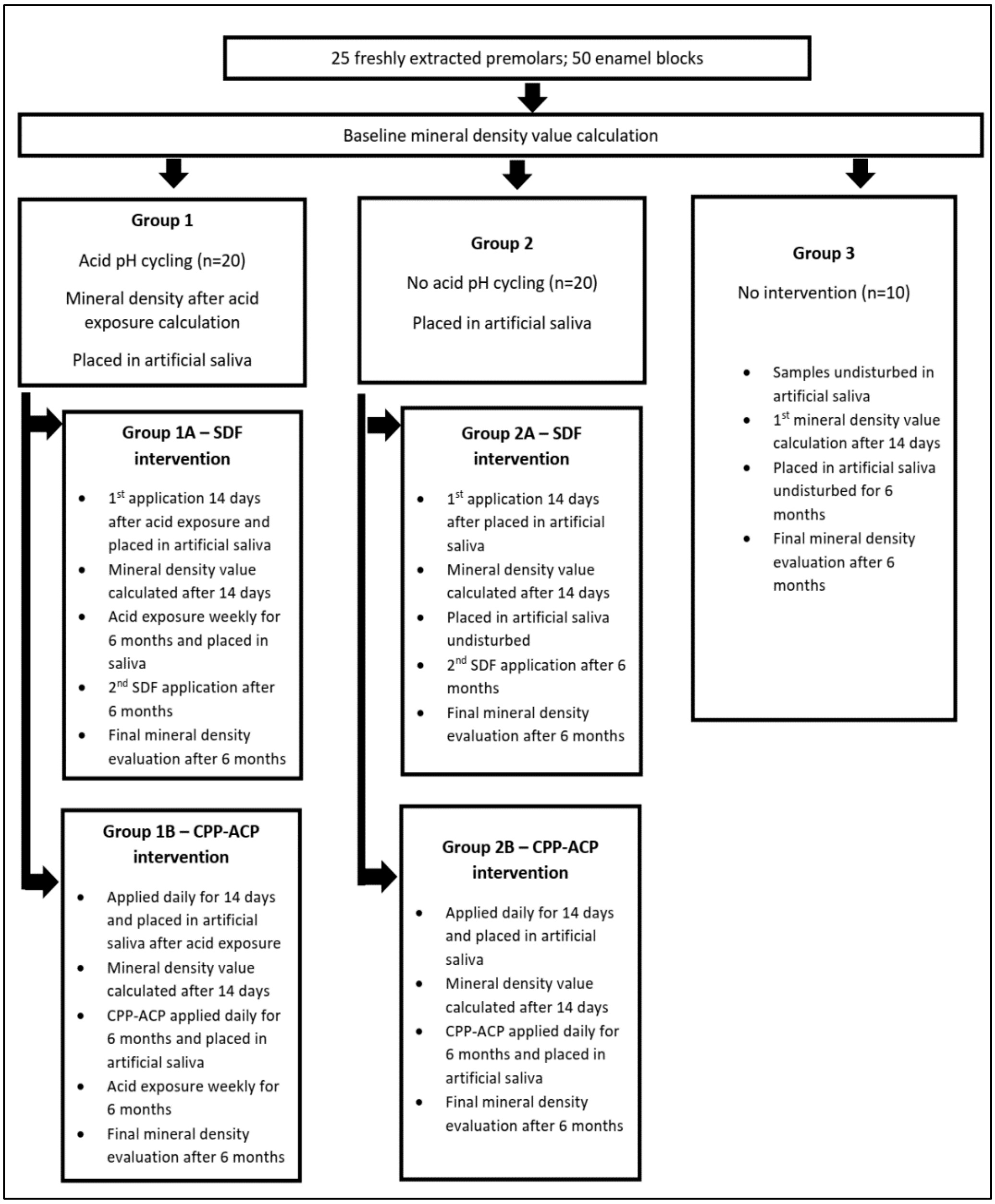

Figure 2: Experimental flowchart

SDF intervention (Group A)

Group A (Group 1A and Group 2A) samples were treated with 1–2 drops of 38% SDF according to the protocol by Horst *et al*.[18] The first post-treatment mineral density value was assessed at the end of 14 days. The second application of SDF was done at the end of 6 months to simulate the semi-annual application of SDF which is considered to be a more appropriate and effective method of SDF application.[18]

CPP-ACP intervention (Group B)

Group B (Group 1B and Group 2B) samples were treated with CPP-ACP daily for 14 days. This was done considering that 14 days is the mean time required for CPP-ACP to remineralize following acid exposure.[19,20] CPP-ACP application was similar to a study by Pai *et al*.[21] The paste was applied onto the enamel surface window with a micro-brush and left undisturbed for three minutes. Application of CPP-ACP continued daily for 6 months.

Micro-CT analysis

A Micro-CT system (MI Labs, BV Heidelberglaan 100, Netherlands) was used to evaluate the mineral density values of each specimen. The specimens were scanned at 50kVp with a current of 0.24Ma and analyzed under a resolution of 20-micron voxels. Volumetric measurements were used to establish the mineral density of each specimen. The grey scale value was computed from the Micro-CT scanned images and the mineral density was calculated in houndsfield units (h.u.). (Figure 3).

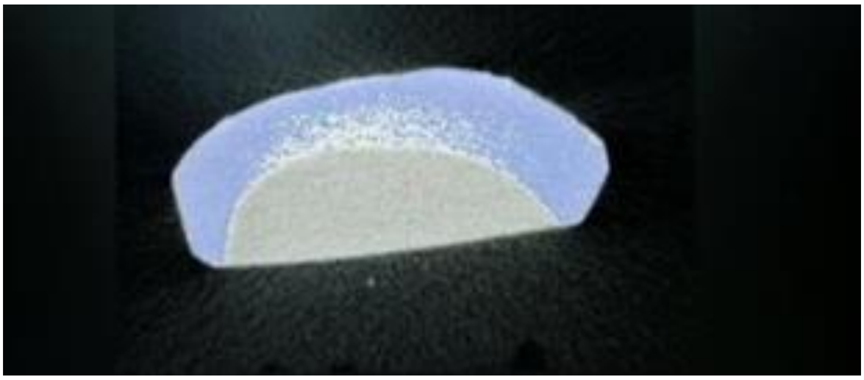

Figure 3: Micro-CT reconstructed image showing enamel (blue) and dentin (gray) layers of sectioned tooth sample

Statistical analysis

Statistical analysis was done using the IBM SPSS Statistics 20 Windows (SPSS Inc., Chicago, USA). To test the statistical differences in the average parameters among five groups, ANOVA (non-parametric Kruskal-Wallis) test followed by the Dunn Bonferroni test was applied for non-normality data. To test the statistical difference in the mean micro-CT before and after the exposure of acid, paired sample t-test was applied. A p-value of ≤ 0.05 was considered statistically significant.

3. Results

The mineral density values following acid exposure of samples showed a statistically significant decrease from the baseline mineral density values (Table 1). Inter- and Intra-group comparisons after 14 days revealed a statistically significant difference in mineral density values ($p = 0.045$). However, the mineral density value at the end of 6 months was statistically non-significant ($p = 0.615$). (Table 2)

There was a minimal difference in mineral density values between Groups 1A (SDF), Group 1B (CPP-ACP), and control, which was statistically significant (Table 2). All the groups showed an increase in the mineral density value from the baseline mineral density value. At the 14-day assessment, Group 1B (CPP-ACP) showed the highest mineral density value, while at the end of 6-months, the mineral density of Group 1A (SDF) was found to be higher than both Groups 1B (CPP-ACP) and control. (Table 3)

There was a minimal difference in mineral density values between Groups 2A (SDF), Group 2B (CPP-ACP), and control which was statistically significant (Table 3). All the groups had an increase in the mineral density value from the baseline mineral density value. At the 14-day assessment, Group 2B (CPP-ACP) had a slightly higher mineral density value when compared to the other groups whereas, at the end of 6-months, the mineral density of Group 2A (SDF) increased and was found to be higher than both Groups 2B (CPP-ACP) and control. (Table 4)

Table 1: Difference between mean mineral density values at baseline and mineral density after acid exposure

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Baseline - Acid.Exposure	**641.4200000**	**454.2299220**	**203.1377966**	**77.4190590**	**1205.4209410**	**3.158**	**4**	**.034**

Table 2: Intergroup and intragroup comparison at 14-days and 6 months using ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Micro_CT_14days	Between Groups	873436.414	2	436718.207	3.304	.045
	Within Groups	6212971.443	47	132190.882		
	Total	7086407.857	49			
Micro_CT_6months	Between Groups	9625113.556	2	4812556.778	1.931	.615
	Within Groups	117165434.003	47	2492881.575		
	Total	126790547.559	49			

Table 3: Mineral density for Group 1A (SDF), Vs Group 1B (CPP-ACP) Vs Control

	Group 1A - SDF with acid	Group 1B - CPP-ACP with acid	Control	p-value
Baseline	11027.5000	11025.5000	11027.5000	0.043
Micro - CT 14 days	11393.200	11421.100	11150.400	
Micro - CT 6 months	11791.400	11339.100	11201.100	

Table 4: Mineral density of Group 2A (SDF) vs Group 2B (CPP-ACP) vs Control

	Group 2A - SDF without acid	Group 2B - CPP-ACP without acid	Control	p-value
Baseline	11023.5000	11030.5000	11027.5000	0.043
Micro - CT 14 days	11053.100	11324.500	11150.400	
Micro - CT 6 months	11540.100	11676.500	11201.100	

4. Discussion

Dental erosion is the chemical dissolution of dental hard tissues by acids which has been widely recognized in recent years as the cause of rapid tooth structure loss in children and adolescents.[22] Coca-Cola contains phosphoric acid which results in a superficial etched zone on the tooth surface that may be permanently lost.[23] The erosivity of Coca-Cola has been established in numerous studies which were similar to the findings of this study.[23-25] In the present study, Coca-Cola was used as the demineralizing agent.

Artificial saliva is commonly utilized as a basic material for in-vitro tests to replicate the chemical environment of the oral cavity. The present study showed an increase in mineral density values from baseline in the control group over 6 months. This finding may be due to the composition of artificial saliva which includes calcium and phosphate and was in accordance with existing literature that has established the potential of artificial saliva to remineralize softened or etched enamel.[17,26,27] The formula of the artificial saliva used in the present study has been reported to be comparable to natural saliva in composition and pH.[17] The artificial saliva as a supersaturated calcium and phosphate solution may have exhibited spontaneous precipitation of calcium phosphate on the enamel surface during the long storage of the specimens, with consequent remineralization, and rehardening by deposition of calcium and phosphate minerals which may explain the increase in mineral density over time.[17]

CPP–ACP has also been introduced based on the calcium–phosphate remineralization technology, where it is claimed that the CPP stabilizes high concentrations of calcium and phosphate ions, together with fluoride ions, at the tooth surface.[28] Tantbirojn *et al*[29] found an increase in enamel microhardness and reduced erosion by cola drinks following the use of CPP-ACP, implying that CPP–ACP is capable of remineralizing eroded lesions. In the present study, a substantial increase in mineral density that followed the application of CPP-ACP confirmed the remineralization potential of CPP-ACP and is consistent with previous studies by Yu *et al*[30] and Hamba *et al.*[31] The results showed that CPP-ACP had the highest remineralizing potential after 14-days, which showed a slight decrease over 6 months. The variation over 6 months was not however statistically significant. The application of CPP-ACP was thus found to be more effective as a remineralizing agent within 14 days of application than SDF. The mechanism of remineralization of eroded lesions by CPP-ACP may be attributed to its ability to localize ACP on the tooth surface, which buffers the free calcium and phosphate ion activities, and thereby aids to maintain a state of supersaturation by suppressing demineralization and enhancing remineralization.[32] Thus, CPP-ACP can promote an increase in the surface hardness, reduce tooth wear and erosion depth and increase the remineralization of the eroded enamel. **The consistency in increased mineral density over 6 months indicates the effectiveness of CPP-ACP over a longer period of time.** However, most studies conducted are *in-vitro*, and randomized clinical trials evaluating the effectiveness of these remineralizing agents in the prevention of enamel erosion are scarce.[33]

Numerous clinical trials show that SDF has a higher level of prevention with less frequent applications than other topical therapies such as fluoride varnish.[34] The different mechanisms of action of SDF have been reported by Zhao *et al*.[35] In the 1970s, Yamaga *et al*[36] suggested that the principal chemical reactions between SDF and the tooth component hydroxyapatite involved the formation of an impermeable layer of silver phosphate (Ag_3PO_4) and calcium fluoride (CaF_2) on the treated tooth surfaces.[36] The present study showed significant remineralization following the use of SDF. The remineralization was seen with a significant increase from the baseline values within 14 days after the first SDF application. This was found to be less than the remineralization value obtained by applying CPP-ACP. The semi-annual application of SDF however, showed a greater increase in mineral density value after 6 months which was more than the remineralization value by CPP-ACP. The possible mechanism for this outcome could be explained by the study by Li *et al,*[37] where they found silver deposition in demineralized enamel, and the intensity was related to the degree of enamel demineralization. Close investigation of the zones revealed that extremely concentrated silver particles were precipitated in the innermost demineralized lesions and the dentinal tubules were partially sealed by silver particles and other minerals.[37]

Ex-vivo models are extremely useful for evaluating dental erosion as it helps in experimenting over a short period of time, requires low operating cost, and do not depend on patient consent. However, they are not able to replicate the oral cavity with all the biological characteristics which are known to interfere with erosion development, such as saliva. Our study has the limitations of being an *ex-vivo* study, and the effects were observed for a short period

Overall, the present study deduced a possible role of SDF in the remineralization of eroded dentin surfaces. The findings however can be utilized for further research to assess the potential of SDF in the management of erosive lesions with an effectively larger sample size for reproducible results in clinical practice.

5. Conclusion

Within the limitations of this study evaluating the potential of SDF in the remineralization of dental erosion, SDF may be suggested to be an effective alternative agent to CPP-ACP in the management of dental erosion. However, more studies are warranted on a larger sample before evaluating its potential in clinical trials.

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