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Comparative evaluation of surface microhardness and morphology of primary teeth using three different remineralizing agents - An Invitro study

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Abstract

Background: In modern dentistry, one of the goals is to prevent disease progression and improve strength, esthetics, and function of teeth by remineralizing non-cavitated carious lesions. The study aimed to evaluate the enamel remineralization potential of CPP-ACP, CPP-ACPF, NaF using the Vickers hardness test to compare the surface microhardness, and SEM analysis for surface morphology of primary teeth.

Materials and methods: Sample size consists of fifty human primary molar teeth divided into five groups of 10 samples each, Group I (Sound enamel, no treatment), Group II (10% CPP-ACP), Group III (10% CPP-ACPF), Group IV (NaF), Group V (Demineralized and not subjected to remineralization). Enamel samples were prepared, windows were created and the sample was made completely resistant to acid attack by coating nail varnish. To induce artificial caries formation, the enamel samples were immersed in the demineralizing solution. The slurry of CPP-ACP and CPP-ACPF along with sodium fluoride was prepared. The pH cycling model was adopted and after the completion of pH cycling for a period of 28 days, all the samples were subjected to surface microhardness and SEM analysis.

Results: CPP-ACPF has the highest remineralizing potential followed by NaF and CPP-ACP.

Conclusion: CPP-ACPF agents are promising remineralizing agents in the remineralization of artificial carious lesions in primary teeth.

Keywords: Enamel, Microhardness, Primary teeth Demineralization, Remineralization,

Introduction

An improved understanding of the dental caries process has enabled the development of a new class of remineralizing therapeutics as a result of more diverse methods of assessing early demineralization. Therefore,

one of the emerging goals of modern dentistry is to manage non-cavitated carious lesions non-invasively through remineralization in order to prevent disease progression and to improve strength, esthetics, and function.[1]

Researchers have tried out a number of remineralization techniques, among which milk products appeared to be protective against dental caries. Various newer systems were developed which are complex of casein phosphopeptide and amorphous calcium phosphate, sodium calcium phosphosilicate (bioactive glass), calcium carbonate carrier - Sensi Stat, xylitol carrier, nano-hydroxyapatite, the TriMet phosphate ion, alpha-tricalcium phosphate, dicalcium phosphate dihydrate, nova min, enamel on, and ion exchange resins. [2]

Casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACPF) contains nanocomplexes of milk protein. The fluoride ion incorporated into the ACP phase was responsible for the anti-cariogenic effect which is stabilized by the CPP to produce a novel ACPF. When CPP-ACPF is applied to the oral environment, a supersaturated state of essential minerals is maintained by the sticky CPP, as it binds readily to the enamel, biofilm, and soft tissues, delivering the calcium phosphate ions. Fluoride ions help in remineralization by forming fluorapatite in the presence of calcium and phosphate ions over the enamel surface.[3]

Recent literature showed CPP-ACP and CPP-ACPF, have equal remineralizing potential with that of fluoride. The use of fluorides in endemic fluorosed areas is its limitation and needs search for the materials with equal or superior remineralization potential. With the above background, the purpose of the present invitro study was to evaluate enamel remineralizing potential using surface microhardness test and morphology using SEM.

Materials and methods

The present study was conducted in the Department of Pedodontics and Preventive Dentistry, Mamata Dental College and Hospital, Khammam. Ethical clearance (MDC-KT-19205103001D) was obtained from the Ethical committee of Mamata Dental College, Khammam, Telangana state.

Fifty extracted human primary molar teeth were used in this study. Enamel samples (2 mm thickness) were prepared from the buccal and lingual surfaces of the teeth selected, using a double-faced diamond disc (Deccan Pvt Ltd, Hyderabad) mounted on a contra-angle handpiece (Marathon, Mumbai). Following sample preparation windows were created dimension of (5×5 mm) using adhesive tape and the sample was made completely resistant to acid attack by coating nail varnish (Colo Rama nail varnish, Maybelline). The adhesive tape was then removed from the enamel after drying. A total of 50 enamel slabs were randomly divided into five groups of 10 samples each based on the remineralizing agent used. (Table 1)

The enamel samples were then immersed into 40 ml demineralizing solution (acetate 0.1 Mol/L, calcium 0.1 Mol/L, phosphate 0.1 Mol/L, fluoride 0.1 mg/L, pH 5.0) for a period of 4 days at a constant temperature of 37°C, in an incubator to induce artificial caries lesion formation, simulating an active area of demineralization. [3]

The dynamic process of demineralization and remineralization was simulated by a pH cycling model and was carried out for a period of 28 days. The enamel samples were treated with the respective remineralizing agents for a period of 2 min, the samples were then immersed in a 20 ml demineralizing solution (calcium 2.0 mMol/L, phosphate 2.0 mMol/L, acetic acid 75.0 mMol/L, pH 4.4) for a period of 3 hrs. Followed by treatment with slurries of the respective remineralizing

agents for 2 min followed by final immersion into a 30 ml remineralizing solution (1.5 mM calcium, 0.9 mM phosphate, 0.15 M KCl in 0.1 m Tris buffer, pH 7) for a period of 17 hrs. The remineralizing solution was replaced every 48 hrs. and the demineralizing agent was replaced every 5 days.

After the completion of the process of pH cycling, all the enamel slabs were assessed for surface microhardness using the Vickers hardness test followed by SEM analysis. The results were subjected to statistical analysis.

Results

One-way ANOVA and posthoc Tukey test were performed using SPSS software, version 22.0. Table 2, Graph 1: represents the comparison of mean surface microhardness values of different groups. The surface microhardness values of enamel samples in Group I (338±7.08), Group II (311±15.23), Group III (325±10.82), Group IV (315±13.08), and Group V (235±21.66). Enamel samples in Group III (325±10.82), showed increased remineralization of enamel followed by Group IV (315±13.08), which showed a marginal increase in remineralization compared with Group II (311±15.23). Where Group V showed the least mean value. Table 3: Posthoc Tukey's test, the intergroup comparison of surface microhardness observed between all groups. There was a modest statistically significant difference observed in Group V compared with other groups. No other group showed statistical significance upon intergroup comparison.

SEM analysis

The sound enamel had an orderly rod appearance and enamel crystals were homogenously arranged. In contrast, demineralized enamel was disorganized with variable rod widths and the small number of enamel rods and the enamel crystals were irregularly arranged. In the CPP-ACPF group and the NaF group, numerous particles and amorphous crystals were arranged on the surface, but in the CPP-ACP group, those crystals seemed to be more homogeneous than those in the NaF group, and there was no obvious intercrystalline space. (Fig 1)

Discussion

The better understanding of the caries process, and the ability to detect enamel demineralization in its early stage, provides an opportunity to promote preventive therapies that can be used to arrest the early caries lesion progression. Subsequently, these therapies can reverse the early lesions leading to the preservation of tooth structure, function, and esthetics. Currently, a range of therapeutic procedures and a variety of new agents have been developed to conservatively manage early caries lesions. These include intensive use of topical fluoride treatments and other preventive measures consisting of calcium, phosphate, and fluoride ions, improved oral hygiene, and reducing the number of daily exposures to fermentable carbohydrates.[4]

Fluoride is a widely accepted and also the most effective tool in terms of caries prevention. Many studies have demonstrated the profound effect of fluoride on enamel remineralization that resulted from regular use of toothpaste, even with low levels of fluoride.[5] Fluoride action is limited by the bio-availability of calcium and phosphate ions. The salivary reservoirs of calcium and phosphate are rapidly depleted under acid challenges leading to a net loss of enamel minerals. Therefore, the use of a delivery system for bio-available calcium and phosphate ions is an important adjunct to fluoride treatment for the non-invasive management of early caries lesions.[6]

Molecular interaction of topically applied fluoride with enamel results in the formation of calcium fluoride

which further reacts with enamel leading to the formation of fluoridated hydroxyapatite protecting the enamel from demineralization. Reynolds [1997] found a trend for increasing rates and percentages of remineralization as the pH decreased from 9.0 to 7.0. This study found that this trend of increasing remineralization continued to a maximum value at pH 5.5 and then decreased to a lower value at pH 4.5. The levels of remineralization produced by the CPP-ACPF and CPP-ACP solutions were similar from pH 7.0 to 6.0. However, the CPP-ACPF solutions exhibited significantly greater remineralization than the CPP-ACP solutions at pH 5.5, 5.0, and 4.5. The difference in remineralization between CPP-ACP and CPP-ACPF at the lower pH values was attributed to the presence of fluoride. Only the ion activity gradient of the neutral ion pair $CaHPO_4$ could be correlated with the rate of remineralization for both the CPP-ACP and CPP-ACPF solutions. Both the activity gradient of $CaHPO_4$ and the rate of remineralization reached a maximum value in the acidic pH range at around 5.5. Therefore, it was concluded that the neutral ion species $CaHPO_4$ and HF had significance in the remineralization of enamel subsurface lesions. Both of these species have no charge and therefore their diffusion into the lesion would not be impeded by the charged enamel surface. Remineralization of enamel subsurface lesions involves diffusion of ions through the lesion's surface layer and then deposition of the ions into crystal voids of the demineralized enamel of the lesion. The correlation of the neutral ion $CaHPO_4$ activity gradient with the rate of remineralization suggests that it is the diffusion of ions into the subsurface lesion that is the rate-limiting step in subsurface lesion remineralization as the diffusion of charged ions through a charged surface layer would be impeded relative to the neutral ion.[7]

The synergistic effect of CPP-ACP and fluoride has been attributed to the formation of a stabilized ACFP phase.CPP-ACPF product increased the concentration of calcium, phosphate, and fluoride ions in saliva which prevents spontaneous precipitation and allows penetration of the ions deep into subsurface lesions. The low solubility of calcium and phosphate ions, especially in the presence of fluoride ions, made it unsuccessful for remineralization in the past. Acid is required to produce the ions which are then able to diffuse into the enamel subsurface lesion. In addition, due to the intrinsic insolubility of calcium fluorophosphate, only very low concentrations of soluble calcium and phosphate can be used. These soluble calcium and phosphate ions neither incorporate into dental plaque nor localize at the tooth surface to produce effective concentration gradients to drive diffusion into the subsurface enamel.[8,9]

CPP-ACP is the acronym for a complex of casein phosphopeptide (CPPs) and amorphous calcium phosphate (ACP). The ability to bind multiple ions of calcium, phosphate, and fluoride serves as the basis to combine CPP with ACP in the form of CPP-ACP, and optionally fluoride, CPP-ACPF. The precipitation of CPP with calcium and phosphate ions is controlled by ACP. The clusters of ACP in the metastable solution formed through their multiple phosphoryl residues (terminal phosphate ion groups), the CPPs can bind to amorphous calcium in ACP to form these clusters thus preventing their growth to the critical size required for nucleation and precipitation. This mechanism prevents the premature loss of solubility of the calcium-phosphate complex, which allows diffusion of calcium and phosphate within the surface layers of tooth structure. The state of supersaturation is maintained by the proposed mechanism of anti-cariogenicity is that it localizes ACP in dental plaque which further buffers the

free calcium and phosphate ions, thereby suppressing demineralization and initiating remineralization. The main function of casein phosphopeptide appears to regulate the bioavailability of calcium phosphate levels by maintaining ionic phosphate and calcium supersaturation to increase remineralization.[1]

The casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a multi-phosphorylated peptide that stabilizes calcium and phosphate ions in solution form and localized onto the tooth surfaces during acidogenic challenges.

This maintains the supersaturation of calcium and phosphate ions and ion pairs into the subsurface lesion thereby decreasing enamel demineralization and regulating remineralization. [10]

Stabilized ACFP phase formed by the synergistic effect of CPP-ACP and fluoride.[8] CPP-ACPF increased the concentration of calcium, phosphate, and fluoride ions in saliva which prevents spontaneous precipitation and allows penetration of the ions deep into subsurface lesions.[11] The observations of the present study showed that CPP-ACPF has the highest remineralizing potential followed by NaF which acquires just a marginal difference compared with CPP-ACP. It has been reported that enamel subsurface lesions which were remineralised by CPP-ACPF were more resistant to demineralization.

Research has proven, the effect of ion composition of CPP-ACP and CPP-ACPF solutions on enamel subsurface lesion remineralization. The CPP-ACPF solutions produced greater remineralization than the CPP-ACP solutions. The mineral formed in the subsurface lesions was consistent with hydroxyapatite and fluorapatite for remineralization with CPP-ACP and CPP-ACPF (N J Cochrane et al). In the present study the NaF group showed higher remineralization potential compared to the CPP-ACP group. A study by Lata et al, concluded that CPP-ACP cream is effective, but to a lesser extent than fluoride in remineralizing early enamel caries at surface level. When fluoride ppm factor was considered as a variable, Tantbirojn et al concluded that probably due to the lower level (1ppm) of fluoride agent used on early stages of erosion on the enamel subsurface showed no synergy between CPP-ACP and fluoride. [12,13,14]

Conclusion

Within the limitations, the observations of the present study inferred that among the groups tested, the CPP-ACPF agent is the most effective means of enhancing remineralization than NaF and CPP-ACP in primary teeth. Before deducing the results of our study into clinical practice it must be restated that there is a lot of research studying demineralization/ remineralization of enamel lesions in permanent teeth using CPP-ACP, CPP-ACPF, and NaF and very sparse literature comparing demineralization/remineralization of enamel lesions in primary teeth. Our results were obtained in an in-vitro ideal environment, independent of any antimicrobial effect, saliva, plaque, and many other confounding factors that may affect the release of remineralizing agents through such systems.

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Legend Figures and Tables

Fig 1: SEM Analysis

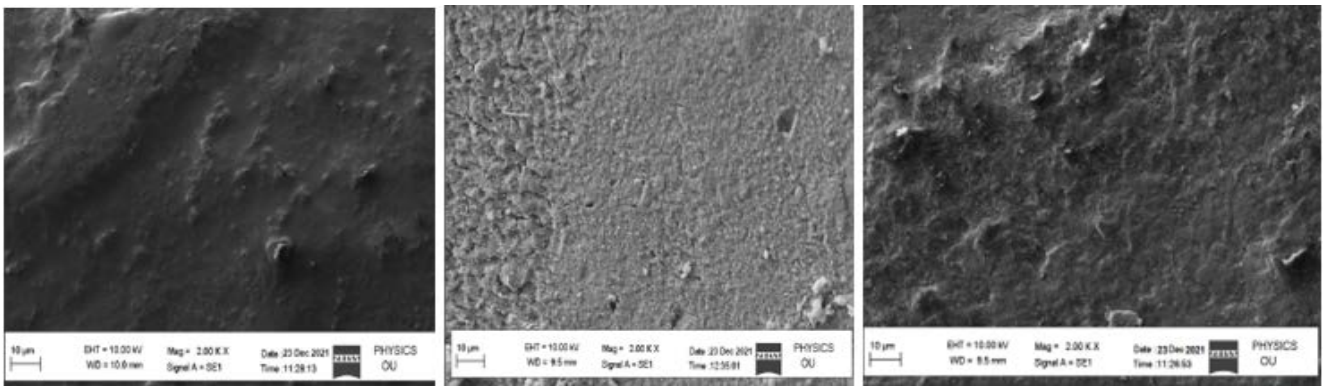

Table 1: Group allocation based on remineralizing agents

Groups	n	Remineralizing agents
Group I	10	Sound enamel
Group II	10	Demineralized and treated with CPP-ACP
Group III	10	Demineralized and treated with CPP-ACPF
Group IV	10	Demineralized and treated with sodium fluoride solution
Group V	10	Demineralized and not subjected to remineralization

Table 2: Comparison of mean surface microhardness values of all groups.

Surface microhardness	n	Mean	SD	F-value	p-value
Group I	10	338.64440	29.1103	9.075	<0.001
Group II	10	311.7467	41.5397		
Group III	10	325.6667	72.3852		
Group IV	10	315.8478	32.8696		
Group V	10	235.0656	19.8188		

SD = Standard deviation, F = Degree of freedom, P = Probability, N = Number

Table 3: Posthoc Tukey's test

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p-value
Surface microhardness	Group I	Group V	111.88234	18.77142	<0.001
		Group II	24.8	18.77142	0.644
		Group III	28.68842	18.77142	0.789
		Group IV	15.97778	18.77142	0.975
	Group V	Group II	-79.68111	18.77142	0.001
		Group III	-82.78222	18.77142	0.001
		Group IV	-94.60111	18.77142	<0.001
	Group II	Group III	-3.10111	18.77142	1.000
		Group IV	-14.92000	18.77142	0.941
	Group III	Group IV	-11.81889	18.77142	0.975

P = Probability

Graph 1: Comparison of mean surface microhardness values of all groups.