Incipient Enamel Lesions Remineralization using Casein Phosphopeptide Amorphous Calcium Phosphate Cream with and without Fluoride: A Laser Fluorescence Study

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*The **aim** of this in vitro investigation was to evaluate and compare the incipient enamel lesions remineralized by topical application of casein phospho peptide amorphous calcium phosphate (CPP-ACP) cream with and without fluoride. **Method:** Sixty caries free teeth were used in the study. They were divided into four groups as positive control, negative control and two experimental groups.The samples were demineralized and then remineralized using a CPP-ACP Cream with and without fluoride. The remineralization was evaluated at 7, 14 and 21 days using laser fluorescence. **Results** of this study showed that the laser fluorescence readings of test samples for remineralization were very highly significant at 14 and 21 days ($p < 0.001$). **Conclusions:** The degree of remineralization achieved between CPP-ACP and CPP-ACP with fluoride was statistically significant ($p = 0.040$) at 21 days.*

Keywords: *Casein PhosphoPeptide – Amorphous Calcium Phosphate(CPP-ACP), remineralization, laser fluorescence.*

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INTRODUCTION

Dental caries is not an irreversible process. The tooth is subjected to phases of demineralization and remineralization depending on the oral environment. Clinically detectable white spot lesions " involute" or remineralize over time.[1,2] Reports on remineralization of enamel and dentin *in vivo* can be found in early literature in the form of clinical observations of naturally occurring arrestment of carious lesion. The first systematic clinical study on caries reversal was reported in 1966[1] in which about half of the 72 white spot lesions observed on the buccal surfaces of first molars at age 8 were found to have remineralized at age 15. Other *in vitro* studies have shown that artificially formed lesions in enamel can be partially remineralized as evidenced by increasing hardness[3] or mineral content.[4] Consequently, remineralization is accepted as a viable non-invasive approach for restoring carious teeth at least at the earlier stages of the disease.

Since enamel lacks cellular repair mechanisms, the events surrounding the development and reversal of caries is dependent upon physicochemical events at the tooth-pellicle/plaque interface.[5] The enamel of teeth is a porous structure that allows the access of ions in its deeper layers.[6]

The influence of milk and its derivatives on caries is known since the 1950s when cheese was considered to have a substantial cariostatic effect. This action was attributed to the physical nature of cheese and the presence of casein, calcium and phosphate contents.[7] Later investigations on role of casein phosphate concentrates derived from milk on remineralization concluded that casein phosphate stabilized the calcium phosphate and remineralized the incipient lesion.[8,9] Since then efforts to halt the progression of caries or revert the lesion in its early stages with the use of CPP-ACP have been phenomenal. CPP-ACP is now made available in chewing gums, topical cream and lozenges that are used clinically for cariostasis.[6]

Fluoride ions can also promote the remineralization of previously demineralized enamel if salivary or plaque calcium and phosphate ions are available in adequate amounts when the fluoride is applied. For every 2 fluoride ion, 10 calcium and 6 phosphate ions are required to form one unit cell of fluorapatite. Hence on topical application of fluoride ions, the availability of calcium and fluoride ions can be a limiting factor for enamel remineralization to occur.[10] G C Tooth

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Mousse Plus (CPP-ACP with Fluoride) provides the fluoride ions with calcium and phosphate ions in the right ratio for formation of fluorapatite.

Hence this *in vitro* study was carried out with the objective to compare the remineralizing potential of CPP-ACP and CPP-ACP with fluoride using laser fluorescence.

MATERIALS AND METHOD

Sixty caries free premolar teeth extracted for orthodontic reasons without any detectable caries, hypoplastic lesions, and stains (intrinsic or extrinsic and white spot lesions) were included in the study.

The teeth were divided into four groups of fifteen each.

The groups being: a) positive control b) negative control, c) Experimental group 1 (Remineralized using GC Tooth Mousse i.e CPP-ACP Cream); d. Experimental group 2 (Remineralized using GC Tooth Mousse Plus i.e CPP-ACP Cream with Fluoride)

All the teeth were painted with an acid resistant nail varnish exposing a window of 2x2 mm on the centre of the buccal/lingual surfaces. The baseline laser fluorescence readings within the exposed window of all the samples were recorded with Kavo DIAGNOdent 2095(Best Nr 5740500 Kaltenbach and Voigt Gmb and Co, Biberach) using the tip B probe used for smooth surfaces. The device was calibrated against a porcelain reference object prior to examination and recalibrated after reading ten teeth. The peak values of the readings were recorded.

The teeth were then demineralized by immersion in the demineralizing solution at 37ºCelcius,in an incubator for a period of 16 hours. The demineralizing solution was prepared by adding 1% of sodium carboxymethylcellulose to 0.1M Lactic acid containing 3mM of calcium and 1.8mM of phosphate. The pH of the solution was adjusted to 4. The laser fluorescent device was used to evaluate the presence of demineralization using the cut off point recommended by the manufacturer.[11]

The test samples were then remineralized by the once daily application of GC Tooth Mousse (CPP-ACP cream) for the experimental group 1 and GC Tooth Mousse plus (CPP-ACP Cream with fluoride) for experimental group 2 (GC Corporation JAPAN). The paste was applied onto the tooth surface window with a brush and left in place for three minutes. The samples were then immersed in artificial saliva[12] and incubated at 37ºCelcius. The artificial saliva was changed everyday.

The positive control samples were left in artificial saliva and negative control samples were left in normal saline during the test period.

All the samples were evaluated for remineralization at 7, 14 and 21 days. Peak readings were recorded and the data was subjected to statistical analysis.

The digital readings of the changes in fluorescence from baseline to demineralization to remineralization of all the groups were analyzed using Kruskal Wallis test.

The digital readings of the changes in fluorescence from baseline to demineralization to remineralization between the 2 experimental groups were analyzed using the Mann Whitney U Test at 7, 14 and 21 days.

RESULTS

A change in fluorescence of experimental groups from demineralization to remineralization indicated that significant amount of remineralization had occurred in both the experimental groups at 14 and 21 days.

No significant change from demineralization to remineralization was seen in both the control groups.

The mean digital readings of the changes in fluorescence from demineralization to remineralization of all the groups are shown in Table 1

The digital readings of the changes in fluorescence from baseline to demineralization to remineralization between the 2 experimental groups are shown in Table 2.

Table 1. The mean digital readings of the changes in fluorescence from demineralization to remineralization of all the groups at 7,14 and 21 days using Kruskal Wallis test (ns-Not significant, vhs- Very Highly Significant)

	N	MEAN	STANDARD DEVIATION	p
Baseline readings				
Positive control	15	2.4667	. 99043	
Negative control	15	2.0667	1.09978	
Experimental group 1	15	1.8000	1.01419	
Experimental group 2	15	1.8667	1.06010	.29 ns
Demineralization				
Positive control	15	11.3333	1.23443	
Negative control	15	11.2667	.88372	
Experimental group 1	15	11.3333	1.17514	
Experimental group 2	15	11.0667	1.03280	.962 ns
Remineralization 7 days				
Positive control	15	11.1333	1.35576	
Negative control	15	11.2667	.88372	
Experimental group 1	15	11.0667	1.03280	
Experimental group 2	15	10.3333	.81650	.078 ns
Remineralization 14days				
Positive control	15	11.0667	1.27988	
Negative control	15	11.2000	.94112	
Experimental group 1	15	9.0000	1.25357	
Experimental group 2	15	8.6667	.81650	0.001 vhs
Remineralization 21days				
Positive control	15	10.9333	1.43759	
Negative control	15	10.8000	1.08233	
Experimental group 1	15	8.6000	.82808	
Experimental group 2	15	7.8667	1.06010	0.001 vhs

Table 2. Comparision of the DIAGNOdent readings between experimental group 1 and 2 using Mann Whitney Test.

	Baseline readings	Demineralization	Remineralization 7 days	Remineralization 14 days	Remineralization 21 days
Z	-.174	-.433	-1.866	-.803	-1.973
p	.862	-.665	.062	.422	0.040 significant

DISCUSSION

Changing patterns of dental caries can be attributed to not only preventive approaches but also to early interceptive strategies employed. This includes early detection and remineralization of incipient lesions. This is made possible by use of non invasive techniques like laser fluorescence and use of CPP-ACP, which is derived from milk constituents.[6]

Casein-a milk protein, forms a protective coat on the enamel surface and prevents demineralization. The calcium and phosphate complexes bring about the remineralization. The caseinate protein resists the proteolytic action of the enzymes and continues to stabilize calcium phosphate complexes, favoring remineralization.[13]

The casein phosphopeptide (CPP) contains a cluster of phosphoseryl residues which markedly increases the apparent solubility of calcium phosphate by stabilizing amorphous calcium phosphate (ACP) under both neutral and alkaline conditions forming metastable solutions that are supersaturated with respect to calcium phosphates in plaque. CPP-ACP acts as a calcium reserviour, buffering the activities of free calcium phosphates ions in plaque fluid helping to maintain a state of super saturation with respect to enamel mineral, thereby depressing demineralization and enhancing remineralization.[6]

Remineralization as well as post eruption maturation is exemplified by increase in the fluoride content in the superficial aspect of enamel caries lesion. Intra oral models have also found that low concentrations of fluoride in demineralizing and remineralizing solutions are more critical than fluoride within the enamel during demineralization and remineralization. This is because fluoride in solution limits demineralization and enhances remineralization. CPPs can bind up to 25 calcium, 15 phosphate and 5 fluoride ions per molecule.[5]

In this *in vitro* investigation, both CPP-ACP Cream and CPP-ACP cream with fluoride produced significant degree of remineralization as evaluated with laser fluorescence. The difference in the degree of remineralization between CPP-ACP and CPP-ACP with Fluoride was statistically significant at 21 days with the latter being more effective.

Since any clinical evaluation of the remineralizing agents used has to be non invasive, laser fluorescence was used for evaluation in this study.

CPP-ACP has been shown to localize on tooth surfaces and during acidogenic challenge may release calcium, phosphate and Fluoride and maintain the saturation of calcium and phosphate ions in the vicinity of the tooth surface. Using solutions containing small quantities of CPP-ACP has shown to increase mineral deposits by 44-64%. Low levels of fluoride are necessary to markedly increase mineral deposits in carious lesions as fluoride acts as a catalyst in the remineralization process.[5]

This study was conducted simulating a few intra oral conditions under an *in vitro* model. The replication of the dynamics of caries process and complexity of the oral environment is limited in such *in vitro* models.

Given these limitations, further *in vivo* studies and studies to evaluate the quality of remineralization achieved using CPP-ACP and CPP-ACP with fluoride must be planned to validate their remineralizing potential in clinical situations.

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