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Effect of Calcium Sucrose Phosphate and Calcium Casein Phosphopeptide Containing Pastes on Mineralization of Artificially Demineralized Human Enamel an In Vitro Study

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Abstract-*Aim of the study was to assess and compare the effect of calcium sucrose phosphate and calcium casein phosphopeptide containing pastes on mineralization of artificially demineralized human enamel. The remineralizing ability of calcium sucrose phosphate and calcium casein phosphopeptide is under research.75 extracted premolar teeth were collected and a buccal window was exposed on the tooth sample by coating the rest of the teeth with nail varnish. The samples were artificially demineralized using Featherstone pH cycling technique. The medicaments were applied to the demineralized samples for 5 minutes, rinsed and then stored in de-ionized water for the next day. This was repeated for15 days. The samples were excited using diode laser and the fluorescence spectra were measured using laser induced fluorescence spectroscopy (LIFS).Both groups showedremineralization. Calcium sucrose phosphate induced the highest remineralization in the tooth samples. Calcium casein phosphopeptide showed less remineralization than calcium sucrose phosphate. The difference in remineralization showed by the pastes was statistically significant. So they may have a potential role as a remineralizing agent for daily use*

Key Words - *Remineralization, Calcium sucrose phosphate, Calcium caseinphosphopeptide,*

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I. Introduction

Dental caries has been associated with mankind since time immemorial, and its eradication is considered to be one of the holy grails of dental profession. Numerous techniques and approaches have been endeavored to deal with dental caries throughout these years. The two main modalities are surgical approach and preventive approach. The surgical approach, in clinical terms simply "drill and fill"[1]. In recent years, there is a paradigm shift in the approach to combat caries, with more emphasis being given to prevention rather than treatment, conforming to the concept "prevention is better than cure".

Arresting of caries involves shifting the equilibrium in favorofremineralization. Sodium Fluoride is the most widely accepted anticaries agent at present. It is considered as the gold standard against newly introduced remineralizing agents. Sodium fluoride is administered topically as gels or varnishes and systemically as tablets. To maintain sufficient amount of fluoride in the oral cavity for its preventive action, multiple application is required which necessitates numerous visits to the dentist. This is impractical and time consuming. Because of these disadvantages an alternative has been sought over the years[2]

This study has been designed to evaluate the remineralizing potential of calcium sucrose phosphate and calcium casein phosphopeptide containing pastes on mineralization

II. Material And Methods

75 premolars extracted for orthodontic purpose were collected. Universal precautions for handling extracted teeth according to Centre for Disease Control (CDC) were undertaken. On each tooth sample a buccal window of 6mm diameter was exposed by coating the rest of the area with layer of nail varnish. Root apices were sealed with acrylic and the teeth samples were then stored in deionized water. Initial reading was taken using Laser Induced Fluorescence

Materials used

• Tooth Mousse containing calcium casein phosphopeptide
• Enafix containing calcium sucrose phosphate
• De ionized water will be taken as control.

Study groups

The teeth samples were divided into 3 groups. Each group contained 25 samples.
The samples in each group were treated with the respective pastes
Group A - Teeth samples treated with calcium casein phosphopeptide
Group B - Teeth samples treated with calcium sucrose phosphate
Group C - Teeth samples treated with De ionized water

Artificial Demineralization

Demineralization of samples was artificially induced by Featherstone pH cycling technique. All the teeth specimen were immersed in demineralization solution for 6 hours, rinsed with deionized water and then kept in remineralization solution for 17hrs. This was repeated for ten days to induce artificial demineralization. The demineralization was then quantitatively assessed using laser induced fluorescence spectroscopy. This was done to clinically simulate the demineralization in initial enamel caries.

Remineralization

The medicaments were applied to the demineralized samples for 5 minutes, then rinsed and will be stored in de-ionized water. This was repeated for15 days. Change in mineralization was then quantitatively assessed using laser induced fluorescence spectroscopy

Measurement of Mineralization

The samples of each group were mounted in a wax moldfor ease of handling, with buccal window aspect upside. The samples were excited using diode laser at 404nm by placing the optical fiber light in the buccal window. The light emitted from the sample was recorded in the spectrometer in nanometers. A set of at least 8 measurements were taken from each sample. The fluorescence spectra were collected from the samples. The fluorescence intensity is considered to be inversely related to demineralization. The decrease in fluorescence intensity indicates either an increase in demineralization or decrease in remineralization and vice versa.

Statistical analysis

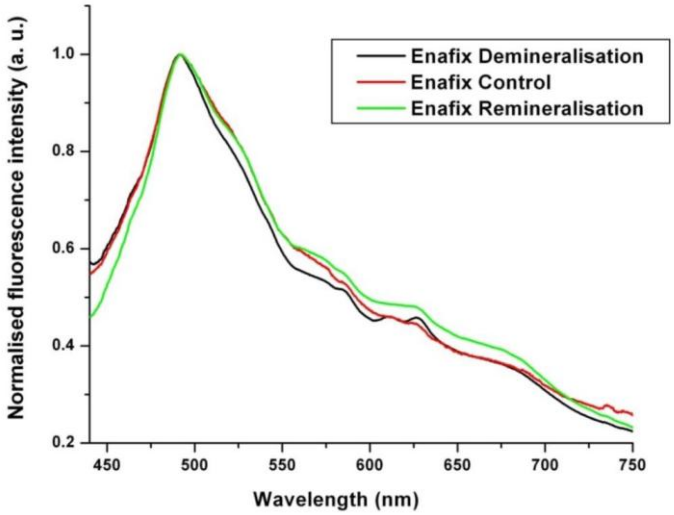
The mean changes in remineralization among the three groups were compared, using one way Anova. Significant changes found among the groups were analyzed using, Scheffe Multiple comparison (posthoc test).

III. Results

Remineralization potential of calcium sucrose phosphate and calcium casein phosphopeptide containing pastes was evaluated. The remineralization induced by these pastes were compared each other and with control group. The tooth remineralization induced by the study solutions were measured using laser induced fluorescence spectroscopy

Calcium sucrose phosphate (Enafix) shows the demineralization and effective remineralization within the stipulated time. The graph1 shows that during demineralization the fluorescence spectral intensity is decreased than control and during remineralization the fluorescence intensity is increased than control

Graph 1



Calcium casein phosphopeptide (tooth mousse) did not show significant remineralization compared to calcium sucrose phosphate within the stipulated time. Graph2 shows demineralization compared to control but remineralization is less

Graph 2

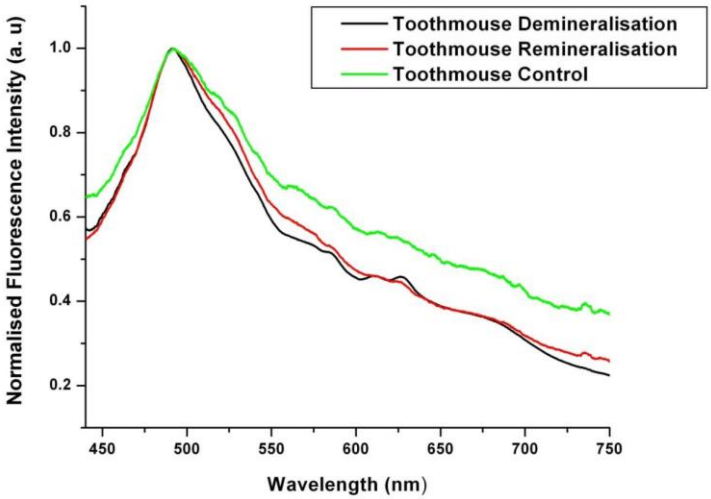


Table 1: Descriptive statistics according to study groups

Groups	Mean	SD	Min	Max
Demineralisation	112.897	46.371	48.07775	215.66888
Control	23.093	9.280	11.029500	43.016125
Calcium sucrose phosphate Remineralisation	104.200	40.396	44.467500	193.221875
Calcium casein phosphopeptide Remineralisation	12.429	3.679	7.368500	20.143125

Table 1 shows mean intensity values of demineralization and remineralization. Distilled water showed the least mean intensity. Demineralization is uniform for both groups. Calcium sucrose phosphate showed higher mean intensity compared to Calcium casein phosphopeptide. One way ANOVA test was used to compare the means of three groups for establishing whether a statistically significant difference exists in terms of fluorescence intensity.

Table 2: Comparison of the three groups

Groups	Mean	SD	f-value	p-value
Control	23.093	9.280	1682.089	.001*
Calcium sucrose phosphate Remineralisation	104.200	40.396		
Calcium casein phosphopeptide Remineralisation	12.429	3.679		

*****P-value obtained using *one-way analysis of variance and* significant at the 0.05

One way ANOVA test was used (Table 2) to compare the mean intensity among the groups. The F test value (1682.089) and the p value (< 0.001) shows that the difference in intensity among the groups is statistically significant at 0.01level. On the basis of the above results it was concluded that there was a statistically significant difference between the remineralizing ability of the calcium sucrose phosphate and calcium casein phosphopeptide

Scheffe multiple comparison (Post Hoc Test)

There was a significant difference in the mean intensity among the groups (*P* <0.001). Hence Scheffe multiple comparison test was done to compare the mean values of two groups at a time (pair -wise comparison) assess whether any significant difference existed between the two groups. In the present study pair – wise comparison was done among calcium sucrose phosphate and calcium casein phosphopeptide

IV. Discussion

The medical model of caries management involves comprehensive diagnosis of dental caries which include the detection of cariogenic bacteria, the survey of plaque acidogenicity and the recognition of tooth demineralization sites. This is followed by a comprehensive treatment plan for dental caries which encompass the elimination of cariogenic bacteria, the reduction of plaque acidogenicity, the enhancement of tooth remineralization and the repair of damaged teeth. With this new approach moving more towards the preventive aspects, remineralization has started to play a major role for eradicating caries.

The enamel contains 96% inorganic and 4% organic content. The inorganic content of the enamel is a crystalline calcium phosphate hydroxyapatite. The susceptibility of these crystals to dissolution by acid provides the chemical basis of dental caries

Research shows that there is a critical pH (5.5) of saliva, below which the equilibrium becomes deranged and the solution becomes unsaturated. So when the pH of saliva falls below 5.5, it becomes unsaturated with respect to hydroxyapatite and enamelis at risk of dissolution[6]. One of the methods to prevent demineralization of tooth is to ensure that the pH of saliva does not fall below the critical pH. Another method is to increase the levels of calcium and phosphates in the saliva which effectively lower the critical pH, so that more acid is required to make saliva unsaturated.

In remineralization, the partially demineralized apatite crystals recrystallize by absorbing the mineral ions. The sources of these ions are saliva and other exogenous sources like topical fluoride, diet etc. Remineralization of dental lesions requires the presence of partially demineralized apatite crystals that can grow to their original size as a result of exposure to solutions supersaturated with respect to apatite

Thecalcium casein phosphopeptide has a remarkable ability to stabilize calcium phosphate in the solution as amorphous calcium phosphate nano complexes. The proposed anticariogenic mechanism for CCP-ACP is by localization of amorphous calcium phosphate on the tooth surface which buffers free calcium and phosphate ions there by maintain a super saturation with respect to tooth enamel[3].

Calcium sucrose phosphate is another remineralizing agent which releases calcium, phosphate and sucrose phosphate ions. Sucrose phosphate absorbs on to enamel and decreases the acid solubility of hydroxyapatite and provides high concentrations of calcium & phosphate ions[4].

In this study, the remineralization potential of medicaments was assessed in a demineralized sample rather than in a sound intact tooth. The remineralization potential in partially demineralized enamel is more than in a sound teeth[7]and hence can be easily assessed in the study. So, in this study, the sound teeth were artificially demineralized by Featherstone pH cycling technique. There are other methods to demineralize the tooth sample like the use of citric acids and other acids, but the Featherstone technique was used in the present study as it clinically simulate the demineralization in initial enamel caries.

Numerous studies are being conducted to develop effective remineralizing agents. **Llena *et al*[8]**evaluated the remineralization potential of casein phosphopeptide with amorphous calcium phosphate. **Souza *et al*[9]**tried to ascertain whether an experimental paste with hydroxyapatite nanoparticles has an improved remineralization ability.In the present study, the remineralization potential of two possible remineralizing agents calcium sucrose phosphate and calcium casein phosphopeptide containing pastes, assessed and compared

There are various methods to measure mineralization. Commonly used method is to determine the hardness of the sample. Limitation of this method is that, hardness is not a direct measurement of the mineralization of the sample

Atomic force microscopy (AFM) and profilometry is another method to measure mineralization. Disadvantage of contacting profilometry is that the contacting stylus may damage the delicate mineralized surface layer and influence the measurement results by affecting the stylus force, also the measurement of scratch depths does not represent the actual demineralization and erosion depth[10].

Polarized microscope was used by **Hasan *etal*[11]**to determine the remineralization asit is very accurate and effective method of studying mineralization.. The main limitation of this method was that it was highly subjective and was not able to correctly delineate small changes in mineralization.

The present study employed LIFS system to assess the remineralization as it is accurate and effective method in studying mineralization. The advantage of this method is that it directly quantifies the demineralization of enamel precisely, even after a short period of exposure. Previous studies have proved that detection of very early mineral loss and its subsequent monitoring is possible in primary teeth using quantitative laser fluorescence. **Borisova*et al*** has reported that the differentiation between initial caries lesion and demineralized tooth can be done using laser induced fluorescence spectroscopy.

The pastes used in the study showed increase in intensity of fluorescence of enamel samples indicating remineralization in the demineralized tooth samples. Calcium sucrose phosphate showed the highest fluorescence intensity, followed by calcium casein phosphopeptide. Least fluorescence intensity was shown as expected by the control i.e. distilled water. There was a substantial difference in the fluorescence intensity between calcium sucrose phosphate and calcium casein phosphopeptide which was statistically significant.

V. Conclusion

Within the limitations of the present study it can be concluded that calcium sucrose phosphate and calcium casein phosphopeptide showed remineralization. Calcium sucrose phosphate induced the highest remineralization in the tooth samples. Remineralization produced by calcium casein phosphopeptide is less when compared to calcium sucrose phosphate and is significant. With high remineralizing ability of calcium sucrose phosphate it can become the primary remineralizing agent in the treatment of early enamel caries.

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