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Original Research

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Remineralization Potential of Calcium Sucrose Phosphate on Demineralized Enamel: Results of an *In Vitro* Study

T N Raghu[1], S Ananthakrishna[2]

Contributor:

[1]Professor, Department of Conservative Dentistry and Endodontics, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India; [2]Reader, Department of Conservative and Endodontics, M.R. Ambedkar Dental College, Bangalore, Karnataka, India.

Correspondence:

Dr. Raghu TN. Department of Conservative Dentistry and Endodontics, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India. Email: drraghutn@gmail.com

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Abstract:

Background: To evaluate the effect of calcium sucrose phosphate (CaSP) application on demineralized enamel using energy dispersive X-ray spectroscopy (EDX) and scanning electron microscope (SEM).

Materials and Methods: About 20 non-carious pre-molars were studied before and after application of CaSP. About 15 samples were subjected for EDX spectroscopy analysis while five were subjected to SEM examination. After baseline examination of microhardness, all samples were demineralized with artificially prepared solution to produce carious lesions and microhardness was rechecked to determine the efficiency of the demineralization procedure before starting treatment with the Toothmin tooth cream. All samples were brushed with Toothmin CaSP cream twice a day till 21 days and all examinations were repeated at 7, 14, and 21 days.

Results: Baseline microhardness (357.9 ± 31.2) reduced to 130.5 ± 60.4 after demineralization ($P < 0.001$) while EDX ratio increased from 2.32 ± 0.09 at baseline to 2.36 ± 0.09, 2.34 ± 0.23, and 2.44 ± 0.20 after 7, 14, and 21 days, respectively ($P < 0.001$). The SEM analysis showed significant surface smoothness in 3/5 samples after 7 days and 5/5 samples after 14 days. Further improvement in surface changes of two samples and no change in other three was noted after 21 days.

Conclusion: Enhanced surface smoothness of enamel was observed with 2 weeks of brushing with Toothmin. The elemental analysis using EDX can be a suitable parameter to assess the remineralizing ability of anticay.

Key Words: Calcium sucrose phosphate, demineralization, Toothmin

Introduction

Dental caries is an important health problem.[1] It is more than just dental erosion because of damage on both the surface and subsurface region. There might be dissolution of mineral below the surface[2] as a consequence of demineralization.[3] Dental caries may have multifactorial etiology with involvement of several intrinsic and extrinsic causes in its pathology. Acid exposure due to vomiting, regurgitation, environmental factors, diet, and drugs are known to play a role in the causation of dental erosion. The acidic conditions can cause demineralization of the tooth and tooth decay.[4] The organic acids cause demineralization of tooth enamel. Bacteria produce organic acid from fermentable carbohydrates when surfaced to the plaque. The organic acids can diffuse the enamel surface with reduction in pH which initiates the demineralization procedure. Dental caries can be repaired or arrested at early stages by enhancing teeth mineralization.

There is an inclination towards minimal intervention procedures causing less or no destruction of tooth substance.[5] One of the approaches is application of therapeutic agents for remineralization.[3] Remineralization can start with increase in pH above acidic level, and encompass getting the calcium and phosphate into the enamel with the help of saliva, fluorides, or other agents. Remineralization occurs through formation of rebuilt crystalline structures of fluoridated hydroxyapatite and fluorapatite. Fluoride is long known to be the effective method of remineralization. The technology containing milk protein casein phosphopeptide (CPP) along with amorphous calcium phosphate-CPP (ACP-CPP) is compared for the enamel remineralization ability with fluoride.[6] ACP-CPP also decreases tooth enamel demineralization and promotes enamel remineralization. It also inhibits accumulation of plaque.[7] Its effective remineralizing action is because of its solubility in water providing high concentrations of free calcium and phosphate ions several times higher than normally present in saliva. The ACP-CPP cream has shown to be less effective that fluoride in remineralizing early enamel caries.[6]

In addition, organic phosphates such as glycerophosphate, phytate, sodium dihydrogen phosphate salts,[8] and calcium sucrose phosphate (CaSP) –calcium orthophosphate complex[9] have been studied for their potential anticaries effect.[8] Saliva calcium concentration is inversely related to solubility of enamel and therefore the incidence of caries.[10] Due to significantly higher efficiency of calcium than phosphate in restricting the demineralization, calcium phosphate ratio of

1:6 has been suggested for enamel remineralization.[11] Calcium glycerophosphate has also shown anticaries potential.[12]

Other method based on Anticay® technology, i.e., mixture of CaSPs and inorganic calcium phosphates is also available. The technology works by creation of an aqueous solution containing high concentration of calcium (10-12%) and phosphate (8-10%) by weight without precipitation.[13] Toothmin tooth cream (Abbott Healthcare, Mumbai, India) based on Anticay Technology having potential of re-mineralization is available in India, whose effectiveness has been shown in some *in vitro* studies.[14,15] However, detailed studies on morpho-physiological characteristics of tooth after its application on demineralized tooth are limited.

Objective

To evaluate the effect of CaSP application on surface hardness of demineralized enamel using energy dispersive X-ray spectroscopy (EDX) and scanning electron microscope (SEM).

Materials and Methods

Specimen preparation

About 20 non-carious pre-molars extracted for orthodontic purpose in patient between 18 and 25 years of age were collected, sterilized with autoclave and stored in distilled water until specimen preparation. The teeth were sectioned using water coolant micro motor hand piece using diamond disc in mesiodistal direction with aseptic conditions. The specimens were treated with ultrasonic radiations for 30 s and mounted on acrylic resin. The exposed surface of the enamel was polished using different polishing disc, starting from coarse, medium and fine (3M: Sof-Flex Disc™).

Baseline examination

About 15 randomly selected samples were subjected to baseline EDX spectroscopy and elemental analysis over the surface of the tooth was noted at baseline. Other five samples were subjected to SEM examination (Figure 1) at baseline. Images were taken at different resolution ×1000, ×5000, and ×10,000.

At the baseline, microhardness of all the samples was measured.

Demineralization

All samples were dematerialized in solution containing 20 ml acid buffer with 2 mmol/L $Ca2^{+}$, 2 mmol/L $PO4^{3-}$, and 0.0075 mol/L acetate at pH 4.3 at 37°C to produce artificial carious lesions. After 3 days, the specimens were transferred to distilled water and stored in it for next 15 h as wash out period. The microhardness was again checked to determine the efficiency of the demineralization procedure before starting treatment with the Toothmin tooth cream.

Post demineralization study

EDX and SEM examination were repeated after 72 h.

Remineralization

Fresh slurry was prepared for every day brushing with Toothmin paste with dilution ratio of 1:3. About 1 ml of slurry was maintained for each time brushing for each sample. About 15,000 strokes were performed on each specimen at the rate of 200 strokes/min using Colgate motorized brush (Colgate 360 Energiser Powered) with a load of 225 g (Figure 2). The procedure was continued twice a day till 21 days.

The specimen was thoroughly rinsed with distilled water without touching the enamel surface. The specimen was transferred to distilled water and kept there until next procedure. At day 7, 14, and 21, the specimen were subjected to EDX for elemental analysis and morphological assessment. During SEM examination, the images were taken at resolution of ×1000, ×5000, and ×10,000.

Statistical analysis

The changes in EDX ratio progress were compared using one-way ANOVA and Student's t-test. ANOVA test was used to examine the statistical significance at different time points compared to baseline, whereas Student's *t*-test was used to evaluate the statistical significance at specific time period.

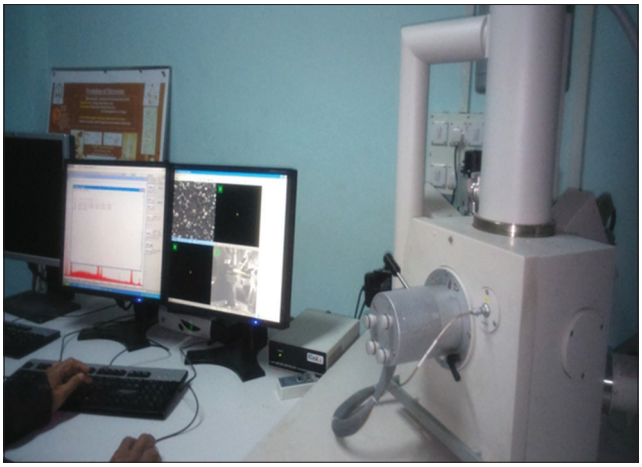


Figure 1: Scanning electron microscope (E-SEM – Quanta200, The Netherlands).

Figure 2: Brushing.

Results

The baseline microhardness significantly reduced from 357.9 ± 31.2 to 130.5 ± 60.4 after demineralization procedure (Graph 1; $t = 12.951$; $P = 0.000$).

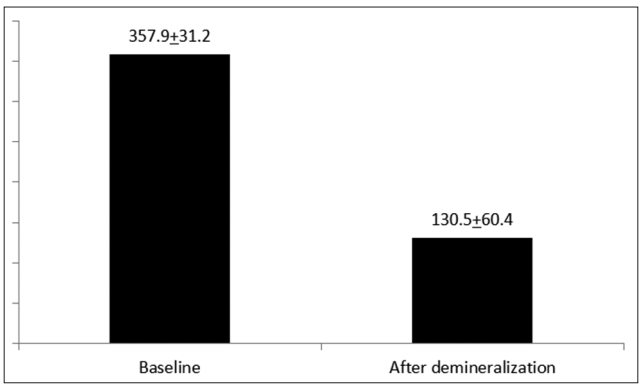
The calcium and phosphate ratios were estimated on sound, demineralized, remineralized enamel samples using one-way ANOVA analysis. A significant increase in Ca/P ratio was found after remineralization (Graph 2; $F = 8.208$; $P < 0.001$).

At baseline, all the five samples showed regular smooth enamel surface with scanty microporosity at all magnification levels (Figure 3).

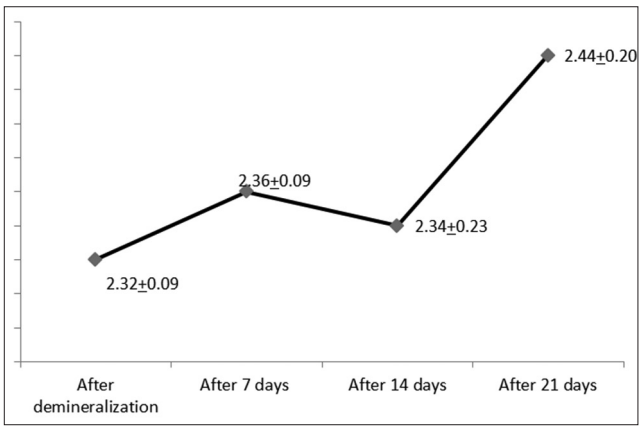
After demineralization, four samples showed enhanced surface roughness and surface irregularity at ×1000 and ×5000, whereas one sample exhibited exaggerated surface roughness at same magnification. All samples showed enhanced micro porosity, surface roughening, cracks, fish scale appearance, which were indicative of the typical demineralized surface at ×10,000.

After 7 days of brushing, significant surface smoothness was observed in three samples (Figure 3) out of five. Two samples exhibited no significant changes on the enamel surface. After 14 days of brushing with Toothmin, all the samples including those which did not show any significant changes on day 7 exhibited significant changes on the enamel surface smoothness at ×1000, ×5000 and at ×10,000 magnification.

Brushing for 21 days showed further improvement in surface changes of two samples and no further change in other three.



Graph 1: Microhardness after demineralization compared to baseline.



Graph 2: Energy dispersive X-ray ratio progress from demineralized state to 21 days of Toothmin application.

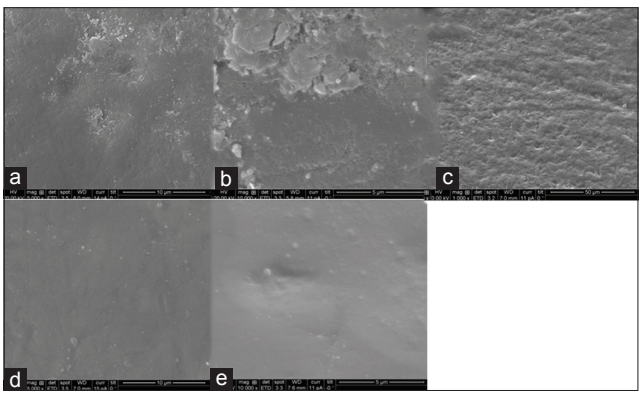


Figure 3: Scanning electron microscope results: (a) Baseline, (b) demineralized, (c) after 7 days brushing, (d) after 14 days brushing, (e) after 21 days brushing.

Discussion

In this *in vitro* study, we assessed the remineralization potential of Toothmin tooth cream on artificially demineralized tooth samples. The remineralization was determined by quantifying Ca/P ratio using EDX spectroscopy and visualization of morphology using SEM. SEM has been proved to be a useful method for testing remineralization potential of agents.[16] CPP-ACP has proven effect of significant remineralization on the artificial enamel subsurface lesions as evaluated by SEM-EDX.[17]

We performed the analysis of baseline microhardness of enamel to determine the efficiency of the demineralization procedure before starting treatment with the Toothmin tooth cream. The VHN method of verification showed significant reduction in the microhardness of the enamel confirming considerable demineralization.

Determination of Ca/P ratio using EDX spectroscopy was done to assess the extent of remineralization. The standard remineralization solution having a calcium/phosphate ratio of 1:0 has shown to be effective for remineralization.[18] In our study, Toothmin tooth cream application resulted continuous increase in the Ca/P ratio till 21 days demonstrating its efficacy. There was slight decrease in the Ca/P ratio after 14 days; however, it increased with further brushing till 21 days. The final increase in Ca/P value from the demineralized state to day 21 of Toothmin tooth cream was significant.

The elemental analysis using EDX method showed significant mineral recovery at the end of 7, 14, and 21 days suggesting elemental analysis using EDX as useful parameter to assess the remineralizing ability of Toothmin.

Demineralization in our study was also confirmed by the observation of enhanced surface roughness in the initially demineralized samples, which was contrasted by the smooth enamel surface at baseline. In addition, the baseline samples contained scanty microporosity, which can be taken as the general structure of the natural no-carious tooth samples which were selected for this study. Increased level of microporosity along with cracks, fish scale appearance after the demineralization procedure was also the evidence for adequate demineralization. The surface changes from demineralized enamel to 14 days showed complete smoothening of surface at all evaluated magnification which remained same at the end of 21st day. The SEM analysis confirmed that brushing with the Toothmin tooth cream for 14 days can result in significant remineralization in the tooth samples with no further improvement if the brushing is extended till 21 days. From the general dental practitioner's perspective, the visual changes provide simple explanation of mode of action of Toothmin. The SEM images clearly indicated the mineral recovery and enhanced surface smoothness compared to baseline non demineralized and demineralized enamel surface.

Overall, we feel that adsorption of calcium and phosphate ions on the enamel surface may result in reduced rate of acid solubility of enamel with increase in remineralization. Moreover, sucrose phosphate ions also adsorb onto the enamel surface and cause decrease in the rate of acid dissolution. Our observation support that Toothmin tooth cream, a prototype containing CaSP, when used for brushing of teeth twice daily for 14 days can efficiently increase the remineralization *in vitro*. Our results indirectly support the published reports on remineralization potential of Toothmin tooth cream.[14,15]

This study has some limitations. Being a pilot study with limited number of specimens, it is difficult to make definite conclusions. There are no demineralization standards for *in vitro* studies and no specific formulations for demineralization are designed as on today. Remineralization in the oral cavity is a complex dynamic process; hence observations of our study should be carefully extrapolated in clinical settings. We recommend further studies with specific assessment parameters such as EDX and SEM analysis with higher number of specimens before extrapolating results to the clinical conditions.

Conclusion

Our study results indicate that Toothmin can increase the surface microhardness of enamel after 14 days of brushing. SEM images clearly indicated mineral recovery and enhanced surface smoothness with 2 weeks of brushing with Toothmin. Elemental analysis using EDX can be a suitable parameter to assess the remineralizing ability.

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