ISSN Print: 2394-7489
ISSN Online: 2394-7497
IJADS 2023; 9(2): 380-386

www.oraljournal.com
Received: 08-04-2023
Accepted: 12-05-2023

Dr. Shivani PS
Department of Conservative dentistry and Endodontics, Government Dental College, Thiruvananthapuram, Kerala, India

Dr. Shiji Dinakaran
Department of Conservative dentistry and Endodontics, Government Dental College, Thiruvananthapuram, Kerala, India

Dr. Sam Joseph VG
Department of Conservative dentistry and Endodontics, Government Dental College, Thiruvananthapuram, Kerala, India

Dr. Aishwarya P
Department of Conservative dentistry and Endodontics, Government Dental College, Thiruvananthapuram, Kerala, India

Corresponding Author:
Dr. Shivani PS
Department of Conservative dentistry and Endodontics, Government Dental College, Thiruvananthapuram, Kerala, India

Evaluation of caries remineralization potential of toothpastes containing three remineralizing agents - calcium sucrose phosphate, β tricalcium phosphate and fluoro calcium phosphosilicate: An *in vitro* study

Dr. Shivani PS, Dr. Shiji Dinakaran, Dr. Sam Joseph VG and Dr. Aishwarya P

DOI: https://doi.org/10.22271/oral.2023.v9.i2e.1752

Abstract
The "Minimally invasive" approach for caries incorporates detecting carious lesions in the incipient stage and treating those lesions using remineralizing agents, thus emphasizing prevention rather than the traditional method.
Aim: To evaluate the remineralizing potential of three different tooth remineralizing agents (Calcium Sucrose Phosphate, Functionalised β Tricalcium Phosphate and Fluoro Calcium Phosphosilicate) on demineralized tooth surfaces using Vickers microhardness test and Atomic Force Microscopy.
Materials and Methods: Thirty freshly extracted mandibular premolars were collected. The prepared enamel samples were assigned to three different groups with ten specimens each. The specimens were then demineralized using McInne's demineralizing solution in two cycles. Remineralization was carried out for fifteen and thirty days in two cycles using Calcium Sucrose Phosphate (ENAFIX), Functionalised β Tricalcium Phosphate (Clinpro Tooth Crème), and Fluoro Calcium Phosphosilicate (ELSENZ) containing toothpastes for groups I, II, III respectively. The specimens were subjected to Vickers microhardness test and Atomic force microscopic evaluation post-demineralization and remineralization cycles. The results were tabulated and statistically analysed.
Results: Study results showed that the three remineralizing agents tested increased Vicker's hardness number values and decreased the surface roughness significantly in a span of 30 days. Their remineralisation potential was effective.
Conclusion: β TriCalcium Phosphate (Clinpro tooth crème) has superior remineralizing efficacy compared to the other two remineralizing toothpastes.

Keywords: Caries, Calcium sucrose phosphate, functionalised β tricalcium phosphate, fluoro calcium phosphosilicate, vickers microhardness test and atomic force microscopy

Introduction

Dental caries is a chronic, infectious microbial oral disease present worldwide it causes demineralization and cavitation of teeth, that causes pain, discomfort, limits function and compromises aesthetics [1]. Reversible and irreversible changes is of dynamic nature therefore early management is critical [2].

Streptococcus mutans is acquired from their mothers or caretakers to children and is called "discrete window of infectivity" [3, 4]. Caries is a cyclic event with periods of demineralization and remineralization. When the demineralization process predominates, it leads to cavitation [5]. When pH is low the calcium and phosphate are driven away from the tooth into the biofilm to maintain equilibrium, this process is called Demineralization. Remineralisation is when supersaturated concentration of soluble calcium and phosphate of biofilm drives minerals back into tooth structure that was previously demineralised [6].

Tooth remineralizing agents include Fluoride and Non-Fluoride preparations. Decay is prevented by fluoride as it makes tooth resistant. In the recent times non-fluoride remineralizing agents are gaining wide acceptance [7].

Calcium Sucrose Phosphate (CaSP), also known as Anticay, a recent advancement which uses 85% by weight CaSP and 15% by weight of inorganic calcium orthophosphate complex to supply both calcium and phosphate ions, that are highly soluble common ion forms of enamel and maintains alkaline pH [8].

Clinpro tooth Crème contains 0.21% sodium fluoride and tri-calcium phosphate. Damaged crystals of hydroxyapatite are repaired by Calcium and phosphate ions penetration into enamel surface. Fluoride ions accelerate the penetration of calcium and phosphate ions and permeate into hydroxyapatite crystals to form fluorapatite crystals that are more stable and resistant to acids [9].

ElsenzTM contains fluoride (530 ppm) with bioactive glass (BiominF). Fluoro calcium phosphosilicate is the main ingredient. Elsenz TM releases calcium, phosphate and fluoride ions in an 8–12-hour time period forming fluorapatite mineral which helps in remineralization of tooth structure [6].

There are limited number of studies comparing the remineralization potential of Enafix (Calcium Sucrose Phosphate), Clinpro Tooth Crème (Functionalised β Tricalcium Phosphate) and Elsenz (Fluoro Calcium Phosphosilicate). So the present study was conducted to evaluate and compare the remineralization potential of these three remineralizing agents [10].

There are various methods to detect remineralization of tooth such as Scanning Electron Microscopy, Laser Induced Fluorescence Spectroscopy, Confocal Laser Scanning Microscopy, Diagnodent, Fluorescence Lifetime Imaging (FLIM), X-ray diffraction (XRD), Knoop hardness number, Energy-dispersive X-ray spectroscopy (EDX), and Vickers microhardness test [11]. In this study for testing surface microhardness, Vickers microhardness test was used and enamel surface morphology and roughness at different stages were assessed using Atomic Force Microscopy (AFM).

Materials and Methods

Sample selection

Freshly extracted 30 mandibular premolar teeth of age group 18-30 years were included in the study.

Sample preparation and grouping

Teeth were handled according to Centre for Disease Control (CDC) precautions. They were cleaned and stored in 3% H_2O_2 for 7 days at room temperature (CDC).

The samples were further divided into three groups and each group was treated with the assigned respective paste: Group A – Calcium Sucrose Phosphate containing paste (ENAFIX), Group B –Functionalised β TriCalcium Phosphate containing paste (Clinpro Tooth CRÈME) and Group C - Fluoro Calcium Phosphosilicate containing paste (ELSENZ).

Preparation of enamel blocks

The crown of all the premolars were detached from the root at the Cementoenameljunction. Enamel blocks of 4 × 4 × 4 mm were prepared. The labial surface remained untouched and were embedded in acrylic blocks with its labial surface not covered. The teeth were sectioned using carborundum disc.

Pre-demineralization Vicker's Microhardness test and AFM examination (baseline values)

All the samples were then subjected to Vicker's microhardness test to record the baseline microhardness values as SMH1 and Atomic Force Microscopy testing to record morphological changes and surface roughness recorded as AFM1.

Demineralisation of samples

Demineralization of samples was done using freshly prepared McInnes solution. It was applied on tooth samples for 5 minutes at 24-hours interval. This was done in two cycles DML1 and DML2. Then subjected to Vicker's microhardness test (recorded as SMH2) and Atomic Force Microscopy (recorded as AFM2).

Remineralization first cycle

Remineralization was also carried out in two cycles. In the first cycle of remineralization, the remineralizing pastes were applied (0.25g) on specific groups respectively for 3 minutes and twice daily for 15 consecutive days. Later subjected to micro hardness testing and Atomic Force Microscopy observation.

Remineralization second cycle

The same procedure was continued for second cycle for another 15 consecutive days. Samples were stored in artificial saliva and at the end of 30 days were again subjected to Micro hardness testing and Atomic Force Microscopy observation.

Statistical Analysis

Data was entered in Microsoft excel and analysed using IBM SPSS Statistics for Windows (Version 20.0. Armonk, NY: IBM Corp. IBM Corp). Numeric variables were expressed using mean and standard deviation. To compare the mean values of outcome variables like surface roughness and micro hardness among the three different tooth paste groups, One-way ANOVA was used. Post hoc analysis was done using Tukey HSD. The change in surface roughness and micro hardness was calculated from post demineralization to the end of second cycle of remineralisation. This change was compared between the toothpaste groups using One-way ANOVA and post hoc analysis was performed using Tukey HSD. For all these statistical interpretations, $p<0.05$ was considered the threshold for statistical significance.

Results

Table 1: Comparison of change in micro hardness post remineralization cycles and change in surface roughness post remineralization cycles (One way Anova).

	N	Mean (Microhardness)	Std. Deviation	Std. Error	F	P Value	Mean (Surface roughness)	Std. Deviation	Std. Error	F	P Value
Enafix	10	62.4250	38.61565	12.21134	9.4	0.001*	8.4947	1.86711	0.59043	5.028	.014*
Clinpro tooth crème	10	107.2000	6.36588	2.01307			62.1702	14.26689	4.51159		
Elsenz	10	76.1070	11.86310	3.75144			32.7364	64.06471	20.25904		

*P Value < 0.05

One way ANOVA test

Inference

The microhardness and surface roughness were tested for each sample of all 3 groups at baseline, post demineralization and after each cycle of remineralization. The mean intensity and standard deviation (SD) of the three groups were calculated based on descriptive statistics.

In one way ANOVA test, for microhardness the F value (9.4) and the p value (< 0.001) shows that the difference in microhardness among the three groups is statistically significant. In one way ANOVA test, for surface roughness the F value (5.028) and the P Value (< 0.05) shows that the difference in surface roughness among the three groups is statistically significant. On the basis of the above results, it was concluded that there is a statistically significant difference between the change in microhardness and surface roughness of the three study groups.

Table 2: Post Hoc test for pairwise comparison of microhardness and surface roughness-Tukey HSD

Pairwise comparison	Mean difference (Microhardness)	P Value	Mean difference (Surface roughness)	P Value
Enafix-clinpro tooth crème	-44.77	0.001*	-53.67	0.01*
Enafix- elsenz	-13.68	0.41	-24.24	0.34
Clinpro tooth crème – elsenz	31.09	0.017*	29.43	0.01*

*P Value < 0.05

Inference

Post hoc comparison shows Clinpro has a statistically significant increase in micro hardness compared to Enafix and Elsenz, while Enafix and Elsenz have similar micro hardness.

Clinpro has significantly higher reduction in surface roughness than Enafix and Elsenz. However, the surface roughness reduction by Enafix and Elsenz is similar

Discussion

Caries management's medical model includes detailed and structured diagnosis of dental caries to include the detection of cariogenic bacteria, test the survey of plaque acidogenicity and the recognise demineralisation sites. Followed by planning the treatment for dental caries that includes enhancement of tooth remineralisation and repair of damaged teeth [12].

In this study, the remineralisation potential of three commercial remineralising agents were assessed in demineralised specimen rather than in an intact tooth. The remineralisation potential in partially demineralised enamel is more compared to a sound tooth and could be assessed better [13]. In this study, the sound tooth samples were artificially demineralised by using McInnes demineralising solution [14].

In studies done earlier change in the microhardness immediately after first cycle of demineralisation was negligible (0.3%), (p=0.034). The repeated 24 hours demineralisation procedure reduced VHN by 2.3% which was significant (p<0.001), [15]. In the present study samples were subjected to two cycles of demineralisation so that the remineralization potential of toothpastes could be better evaluated.

Numerous studies are conducted to develop effective remineralising agents. In the present study, the remineralisation potential of three remineralising agents Calcium Sucrose Phosphate, Functionalised β TriCalcium Phosphate and Fluoro Calcium Phosphosilicate were being assessed and compared.

Studies done by Karad *et al.*, Sebastian *et al.* and Thabitha rani *et al.* reported microhardness of enamel increased post Calcium Sucrose Phosphate application, along with arresting white spot lesions. It was found to be superior remineralizing agent in comparison to casein phosphopeptide-amorphous calcium phosphate with fluoride (CPP-ACPF), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and other remineralizing agents in terms of increasing enamel microhardness. In addition, Calcium Sucrose Phosphate application was also found to have a beneficial in restoring the color of white spot lesion (s) to that of normal enamel [15-17].

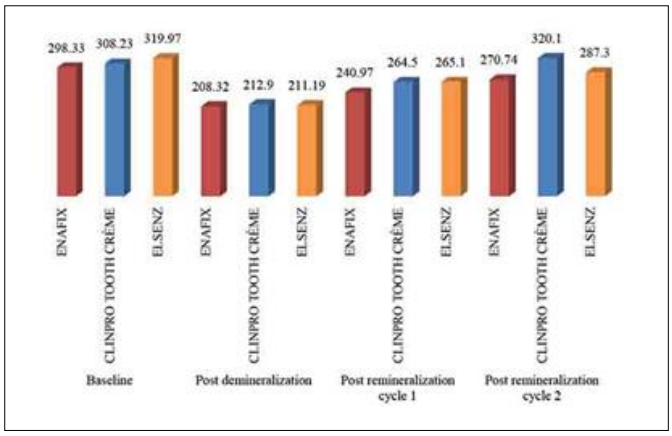
Tri Calcium Phosphate aids in better depth of penetration fluoride and remineralizes the lesion. Normally fluoride penetration into the enamel layer is constrained and declines with depth. According to studies done by Al feel *et al.*, Tulumbaci *et al.* combining TCP and fluoride could have a greater impact than just applying fluoride topically. When there is an acid challenge nearby, the anticariogenic activity of Tri Calcium Phosphate's benefit of supplying calcium and phosphate to the enamel-surrounding media. Tri Calcium Phosphate is an effective delivery method that gradually releases calcium and phosphate to the tooth surface. The three main remineralizing components of Clinpro Tooth Creme are calcium, phosphate, and fluoride ions. Thaper and Karlinsey *et al.* studied the Tri-calcium phosphate pastes (TCP) effect on remineralization of early enamel caries and noticed that products like Clinpro Tooth Creme had significant effect on remineralization process [9, 18].

Elsenz TM contains bioactive glass (BiominF) with fluoride (530ppm). So bioactive glass with fluoride when introduced into a dentifrice, forms Nano complexes at enamel surface. The particles get deposited onto the dentine surfaces and occlude the dentinal tubules mechanically. In salivary environment the sodium in calcium sodium phosphosilicate particles within a minute, starts to exchange with hydrogen ions. This rapid exchange of ions allows calcium and phosphate species to be released from particle structure and calcium phosphate layer is formed on tooth surface. Nagaveni *et al.* study reported that periodic application of Elsenz TM increases micro hardness of enamel and it can be effectively used as remineralizing agent [19].

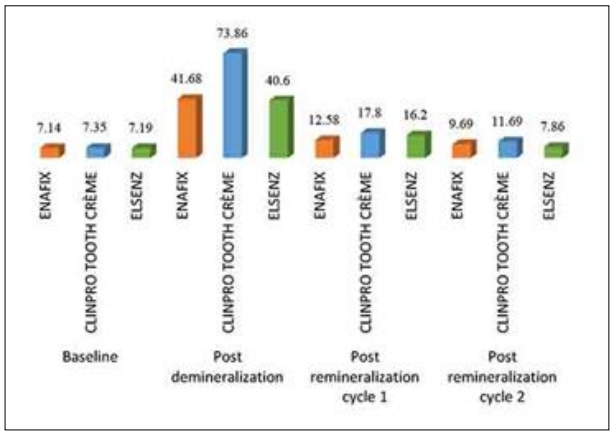
Atomic force microscopy (AFM) and Profilometry is one such way measure mineralisation. They were done on the enamel samples by Beyer *et al.* in demineralisation study. Mineralisation is determined by measuring the depth of scratches in the enamel [20]. In our study, we used teeth with natural contour, without cutting or grinding the enamel surface to estimate the changes of surface morphology in Atomic Force Microscopy, in order to simulate the clinical intraoral situation.

Vicker's hardness method is non-destructive, extremely reliable, quick, and affordable compared to other hardness tests. Hence, it was employed to examine microhardness as the same samples were studied at two time periods in the present study. Due to the long and narrow (elongated) test indent, the Knoop method is best advised for use with small, long test specimens, such as Vickers method is better for small, rounded specimens (square indentation) [20].

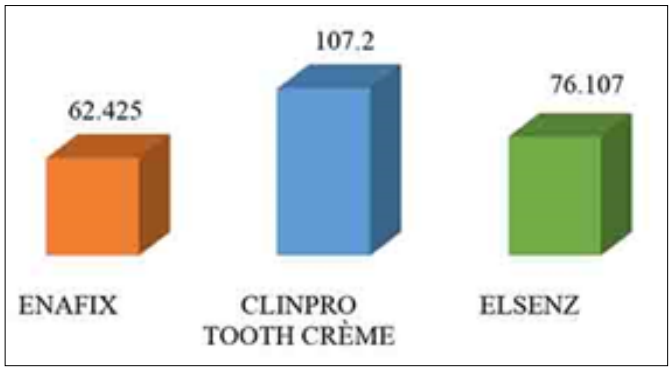
It is of vital importance to note that remineralisation in vitro may be quite different when compared to changes occurring in the oral cavity. Thus, it is necessary to apply direct extrapolations to clinical scenarios.



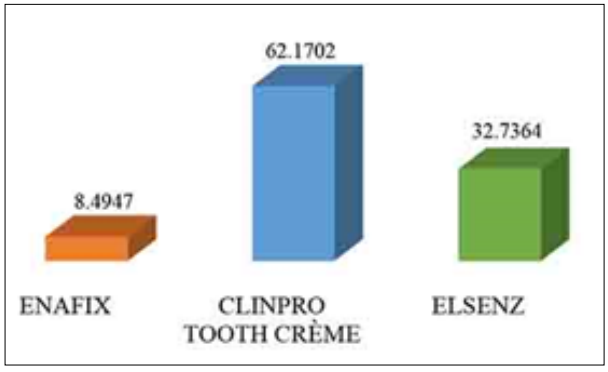
Graph 1: Mean microhardness values



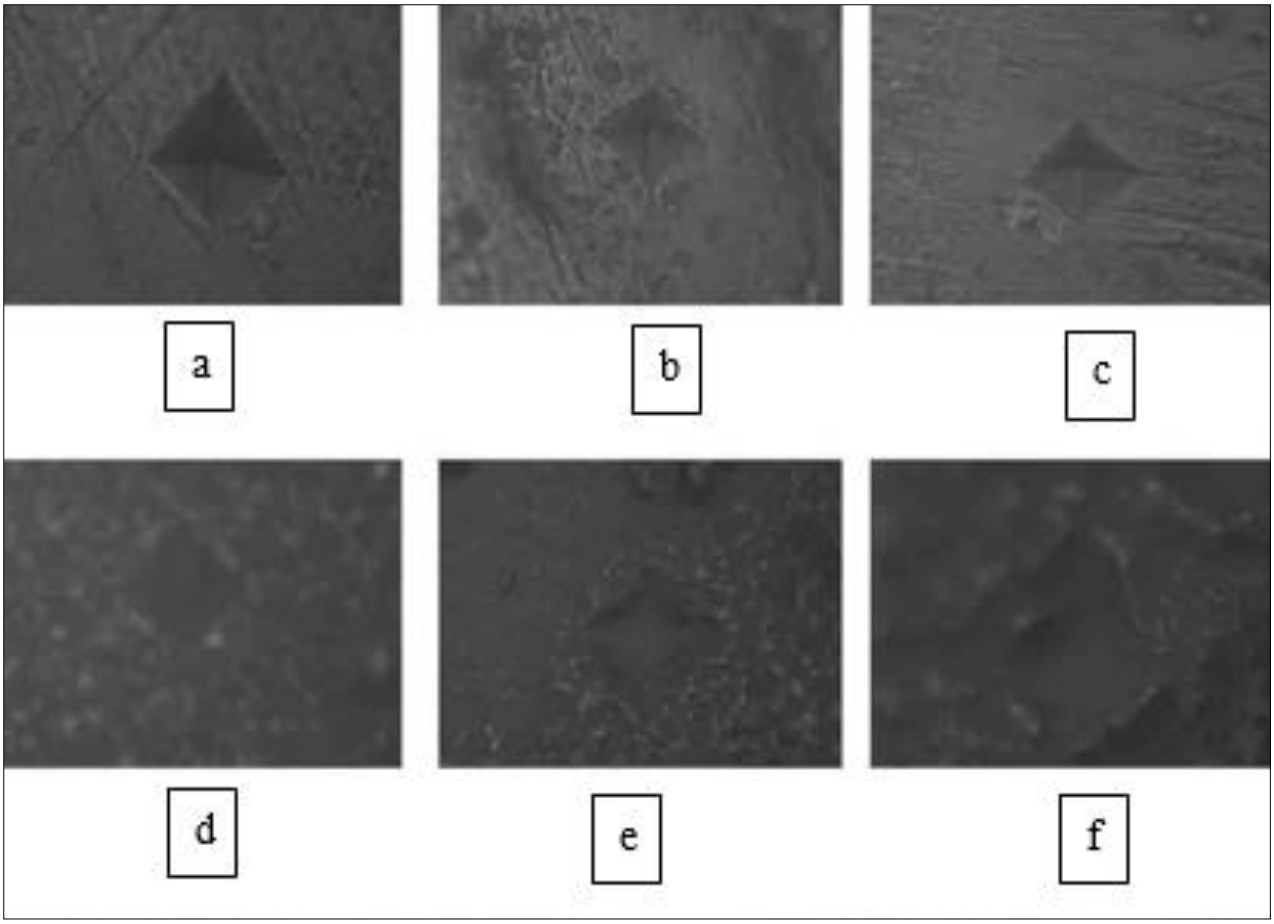
Graph 2: Depicting the mean surface roughness values

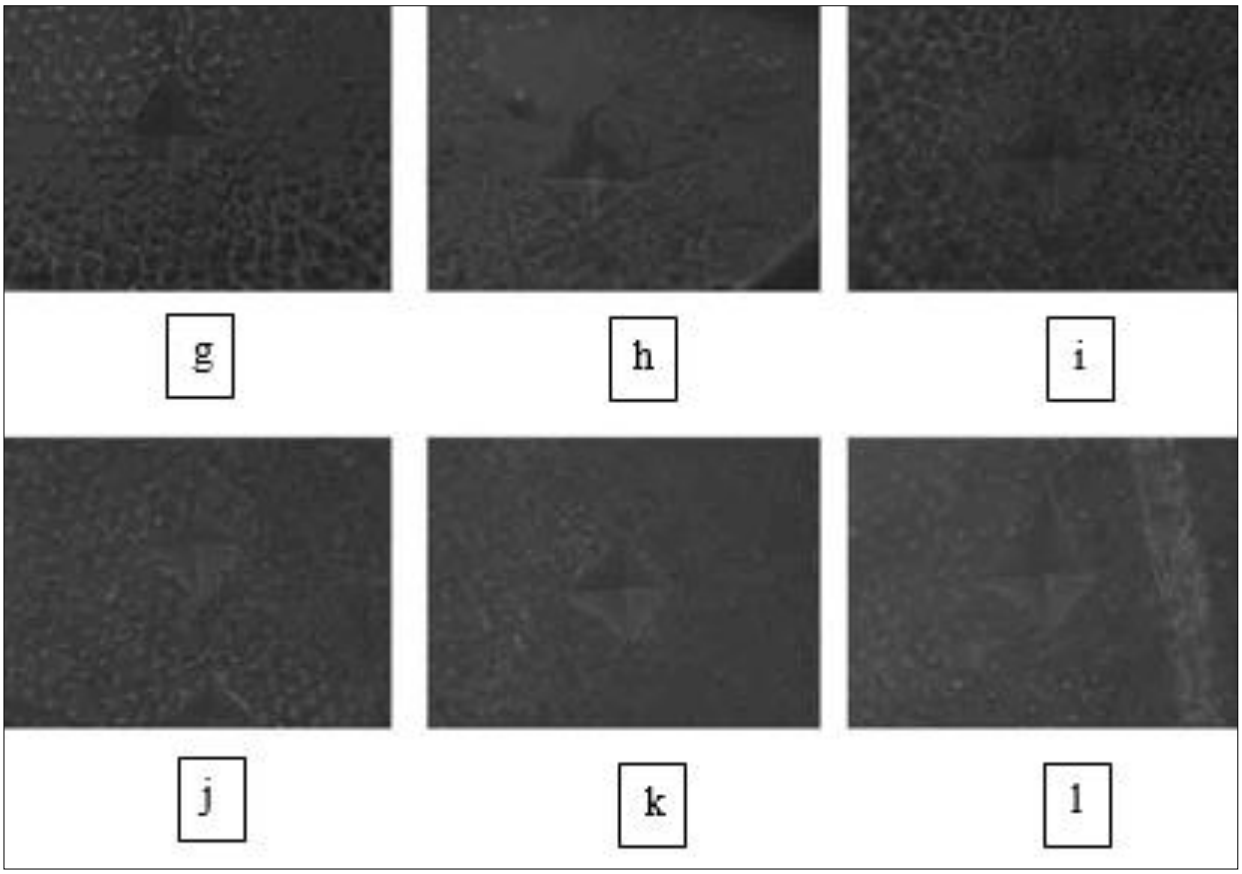


Graph 3: Depicting change in the microhardness values of three groups



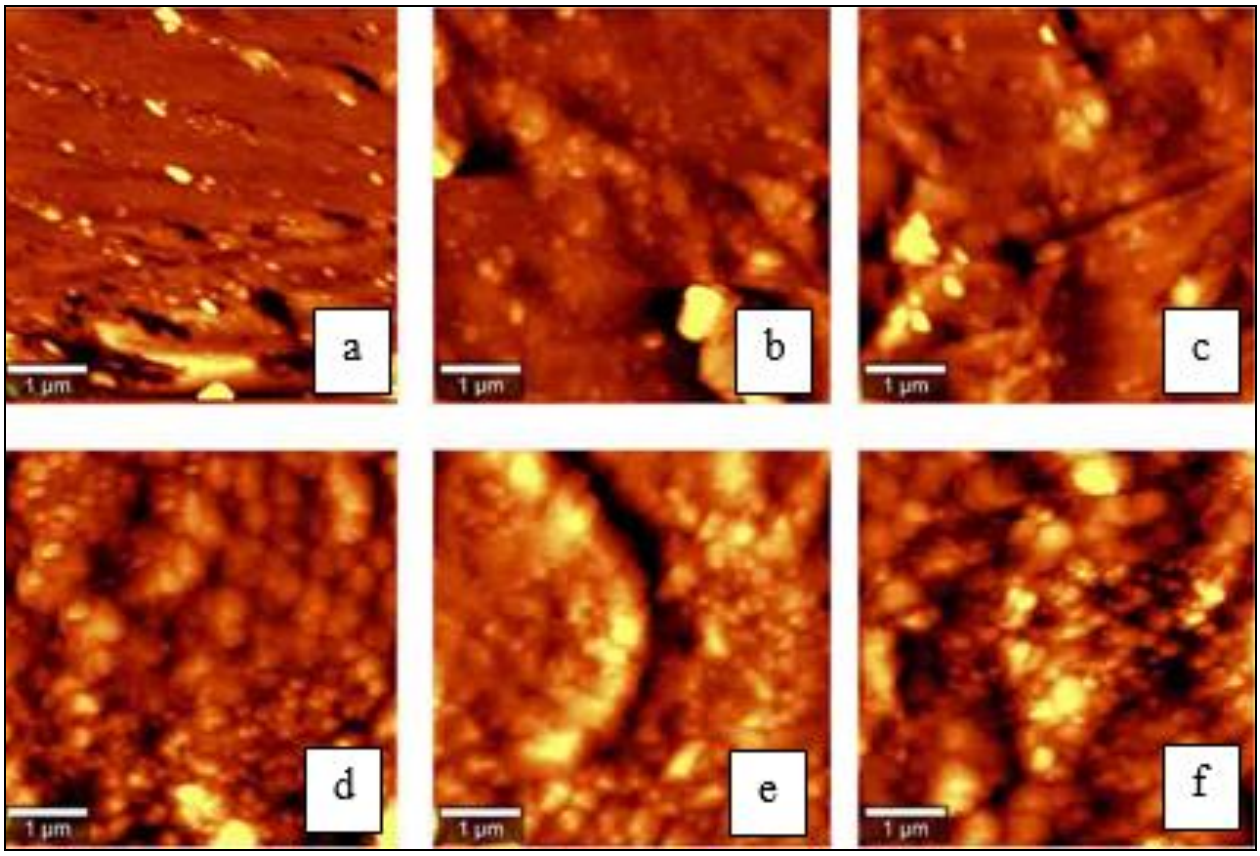
Graph 4: Depicting change in the surface roughness values of three groups

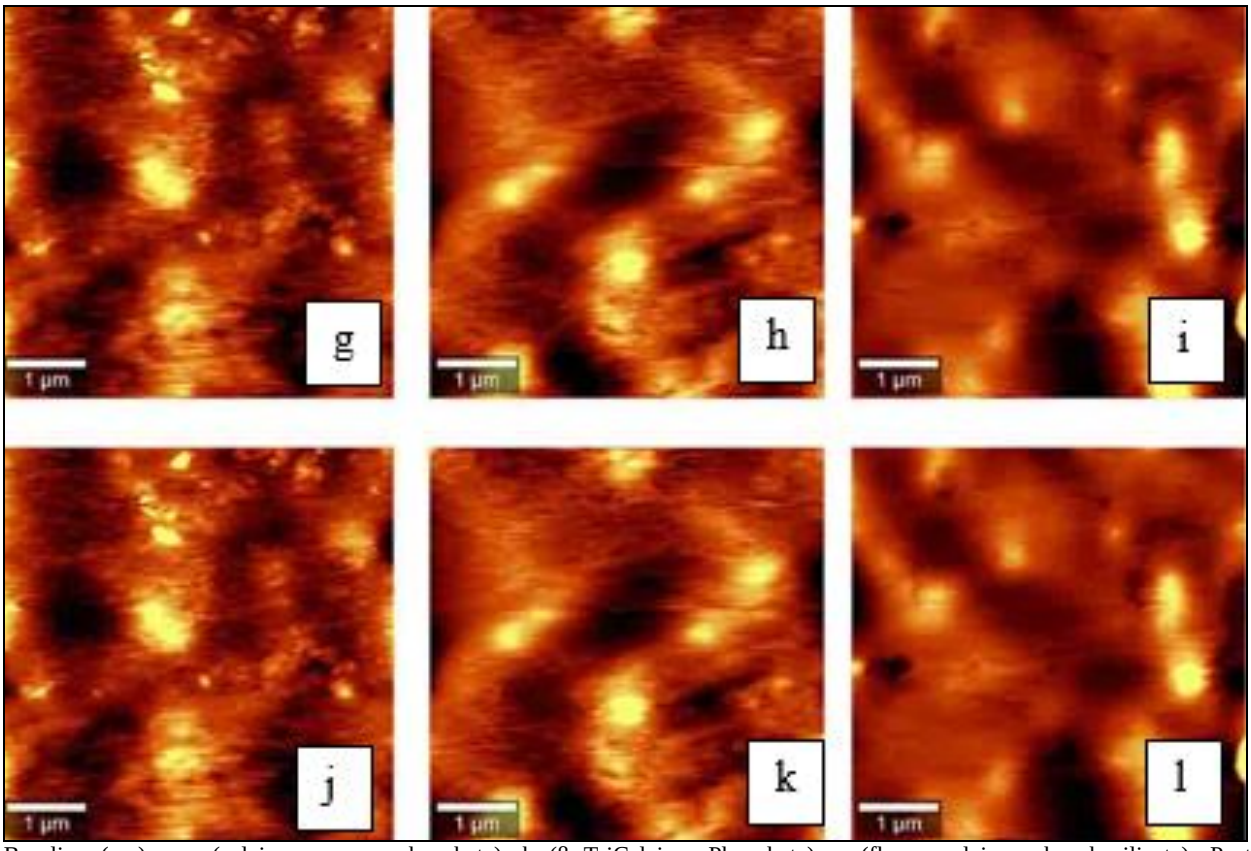




Baseline (a-c) – a (calcium sucrose phosphate), b (βTriCalcium Phosphate), c (fluoro calcium phosphosilicate), Post Demineralisation (d-f) – d (Calcium sucrose phosphate), e (β TriCalcium Phosphate), f (fluoro calcium phosphosilicate), Post first cycle of remineralisation (g-i) – g (Calcium sucrose phosphate), h (βTriCalcium Phosphate), I (fluoro calcium phosphosilicate) and Post second cycle of remineralisation (j-l) - j (calcium sucrose phosphate), k (β TriCalcium Phosphate), l(fluoro calcium phosphosilicate)

Fig 1: Image of enamel surface after indentantion using Vickers microhardness tester





Baseline (a-c) – a (calcium sucrose phosphate), b (β TriCalcium Phosphate), c (fluoro calcium phosphosilicate), Post Demineralisation (d-f) – d (calcium sucrose phosphate), e (β TriCalcium Phosphate), f (fluoro calcium phosphosilicate), Post first cycle of remineralisation (g-i) – g (calcium sucrose phosphate), h (β TriCalcium Phosphate), I (fluoro calcium phosphosilicate) and Post second cycle of remineralisation (j-l) - j (calcium sucrose phosphate), k (βTriCalcium Phosphate), l (fluoro calcium phosphosilicate)

Fig 2: Two dimensional AFM image

Conclusion

Within the limitations of the present study, it can be concluded that

- Functionalised β TriCalcium Phosphate containing paste (Clinpro Tooth Crème) has superior remineralizing efficacy compared to the other study groups.
- Clinpro tooth crème can be considered as a promising future agent for remineralization within the subsurface body of incipient carious lesions.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Arifa MK, Ephraim R, Rajamani T. Recent Advances in Dental Hard Tissue Remineralization: A Review of Literature. Int J Clin Pediatr Dent. 2019;12(2):139–44.
2. Chandru T, Yahiya M, Peedikayil F, Dhanesh N, Srikant N, Kottayi S. Comparative evaluation of three different toothpastes on remineralization potential of initial enamel lesions: A scanning electron microscopic study. Indian Journal of Dental Research. 2020 Mar 1;31(2):217-23.
3. Berkowitz RJ, Turner J, Green P. Maternal salivary levels of Streptococcus mutans and primary oral infection of infants. Arch Oral Biol. 1981;26(2):147-9.
4. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: Evidence for a discrete window of infectivity. J Dent Res. 1993 Jan;72(1):37-45.
5. Usha C, R S. Dental caries - A complete changeover (Part I). J Conserv Dent. 2009 Apr;12(2):46-54.
6. Balakrishnan A, Jonathan R, Benin P, Kuumar A. Evaluation to determine the caries remineralization potential of three dentifrices: An in vitro study. Journal of Conservative Dentistry. 2013 Jul;16(4):375-9.
7. Lenzi TL, Montagner AF, Soares FZM, de Oliveira Rocha R. Are topical fluorides effective for treating incipient carious lesions? The Journal of the American Dental Association. 2016 Feb;147(2):84-91.e1.
8. Menon LU, Varma RB, Kumaran P, Xavier AM, Govinda B, Kumar JS. Efficacy of a calcium sucrose phosphate based toothpaste in elevating the level of calcium, phosphate ions in saliva and reducing plaque: A clinical trial. Contemp Clin Dent. 2018;9(2):151–7.
9. AlFeel J, Laflouf M, AlKurdi S, Alkhouli M. Evaluating the effect of Clinpro Tooth Crème on remineralization of pre-carious White Spot Lesions in anterior primary teeth: Randomized controlled clinical trial. Pediatric Dental Journal. 2021 Aug 1;31(2):152-8.
10. Soares R. Assessment of enamel remineralisation after Treatment with Four Different Remineralising Agents: A Scanning Electron Microscopy (SEM) Study. Journal of Clinical and Diagnostic Research; c2017
11. Abdulla. Enamel slab preparation and sterilization preparation of slabs for microhardness testing. Varghese. 10.3290@j.ohpd.a30604.
12. Mei ML, Ito L, Cao Y, Li QL, Lo ECM, Chu CH. Inhibitory effect of silver diamine fluoride on dentine

demineralisation and collagen degradation. J Dent. 2013 Sep;41(9):809-17.
13. Kargul B Ömpsntswfau. Evaluation of human enamel surfaces treated with theobromine: a pilot study. Oral Health Prev Dent. 2012;10(3):275-82.
14. Duggal MS, Nikolopoulou A, Tahmassebi JF. The additional effect of ozone in combination with adjunct remineralisation products on inhibition of demineralisation of the dental hard tissues in situ. J Dent. 2012 Nov;40(11):934-40.
15. Milly H, Festy F, Watson TF, Thompson I, Banerjee A. Enamel white spot lesions can remineralise using bio-active glass and polyacrylic acid-modified bio-active glass powders. J Dent. 2014 Feb;42(2):158-66.
16. C Llena 1 AML 1, LF 1. CPP-ACP and CPP-ACFP versus fluoride varnish in remineralisation of early caries lesions. A prospective study.
17. Tulumbacı F, Oba AA. Efficacy of different remineralization agents on treating incipient enamel lesions of primary and permanent teeth. J Conserv Dent. 2019;22(3):281-6.
18. Research O, B NN, student P, B Professor NN. International Journal of Pedodontic Rehabilitation Comparative evaluation of Elsenz TM and GC tooth mousse in remineralization of enamel-a clinical study How to cite: N Nagaveni *et al.*, Comparative evaluation of Elsenz TM and GC tooth mousse in remineralization of enamel-a clinical study. Vol. 7, Int J Ped Rehab. 2022.
19. Beyer M, Reichert J, Heurich E, Jandt KD, Sigusch BW. Pectin, alginate and gum Arabic polymers reduce citric acid erosion effects on human enamel. Dent Mater. 2010 Sep;26(9):831-9.

How to Cite This Article
Shivani PS, Dinakaran S, Joseph SVG, Aishwarya P. Evaluation of caries remineralization potential of toothpastes containing three remineralizing agents - calcium sucrose phosphate, β tricalcium phosphate and fluoro calcium phosphosilicate: An *in vitro* study. International Journal of Applied Dental Sciences. 2023;9(2):380-386