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Comparative Evaluation of the Surface Microhardness of Demineralized Enamel After the Application of Three Remineralizing Dentrifices - an in-Vitro Study

KEYWORDS: Early carious lesions, Remineralizing dentrifices, Calcium Sucrose Phosphate

Reaba Rebecca Thomas
: Department of Conservative Dentistry & Endodontics,Manipal College of Dental Sciences, Manipal, Karnataka

Shashi Rashmi Acharya
Professor,Department of Conservative Dentistry & Endodontics, Manipal College of Dental Sciences, Manipal, Karnataka

ABSTRACT *AIM: To evaluate the surface microhardness of enamel after the use of three different remineralizing dentrifices containing Casein Phosphopeptide-Amorphous Calcium Phosphate(CPP-ACP), Calcium Sucrose Phosphate(CSP) & Calcium Sodium Phosphosilicate(CSPS).*

MATERIALS & METHOD: Enamel specimens prepared from thirty freshly extracted anterior teeth were assigned to three groups of ten specimens each. After assessment of baseline microhardness values, demineralization was carried out with McInne's solution and microhardness was reassessed. Remineralization for fifteen days was then carried out using CCP-ACP (I), CSP (II), CSPS(III) dentrifices and final readings were recorded.

RESULTS: Data was analyzed using ANOVA and multiple comparisons done using Bonferroni method &Kruskal Wallis test showed that although all remineralization dentrifices produced increased microhardness values on demineralized enamel surfaces, CSP containing dentrifices gave higher microhardness values compared to CCP-ACP and CSPS

CONCLUSION: The use of remineralizing agents increases microhardness of enamel in early carious lesions.

INTRODUCTION

As the age old saying goes, 'Prevention is better than cure', thus minimal intervention has become the key phrase in the world of dentistry today. Minimal intervention dentistry focusses on the least invasive treatment possible to minimize tissue loss & patient discomfort.

Our oral cavity is constantly subjected to activities of demineralization & remineralization. **Featherstone (1999)** stated that the ratio of these processes is crucial in determining the hardness & strength of the tooth structure.

Early enamel carious lesions appear white due to the loss of translucency of the enamel. Though initial enamel lesions have intact surfaces, it has a low mineral content and shows a lower hardness value than sound enamel tissue (**Koulourides T Ann, 1965)** (**Arend J & Cate JM., 1981**)

With changing times, the focus has shifted to prevention of caries rather than its repair, thereby making the concept of remineralization the epicenter of this cause. Remineralization aids in restricting progress of caries & preventing decrease in the surface hardness. The lowered surface hardness brought about by the loss of minerals during the process of acid dissolution by the cariogenic bacteria. (**Winston AE &Bhaskar SN., 1998**)

The aim of the study was thus to evaluate the surface microhardness of enamel after the use of three different remineralizing dentrifices containing Casein Phosphopeptide-Amorphous Calcium Phosphate, Calcium Sucrose Phosphate & Calcium Sodium Phosphosilicate.

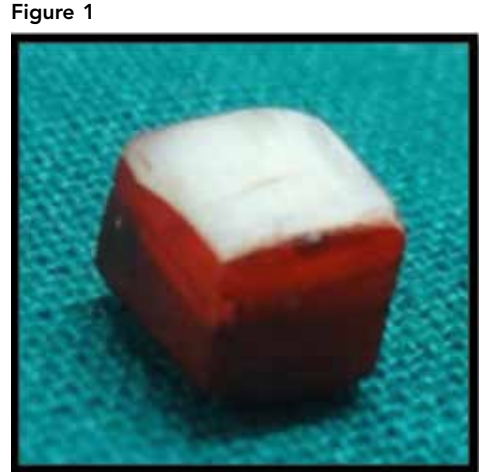
The objectives were to evaluate the surface microhardness of enamel after the use of three different remineralising dentrifices and to compare their effect on the microhardness of enamel after demineralization.

MATERIALS AND METHOD

SELECTION CRITERIA & SAMPLE PREPARATION

Thirty freshly extracted anterior teeth free from dental decay, restorations or developmental defects were selected. Using a diamond disc (Axis dental, Texas) with a slow speed straight hand piece (NSK Japan) at 15,000 rpm, all teeth were sectioned horizontally at the level of CEJ, separating the crown part of the tooth. A 4×4mm section was marked for sample preparation on the facial surface of the crowns of teeth. Incisal edge, mesial, distal, and lingual sides were cut to obtain flat surfaces. The labial side was flattened with minimum reduction and polished using 200, 400, 800, 1000, 1200 grit abrasive paper to preserve sufficient enamel thickness. A 4×4×6 mm section of the tooth was then made and nail varnish (Lakme, India Ltd) was applied on all surfaces except facial surface. Each tooth cube was then embedded into 1×1 cm cylindrical molds filled with self-cure acrylic and the blocks were then numbered**.** (Shetty S, Hegde MN, Bopanna TP. 2014)

Figure 1



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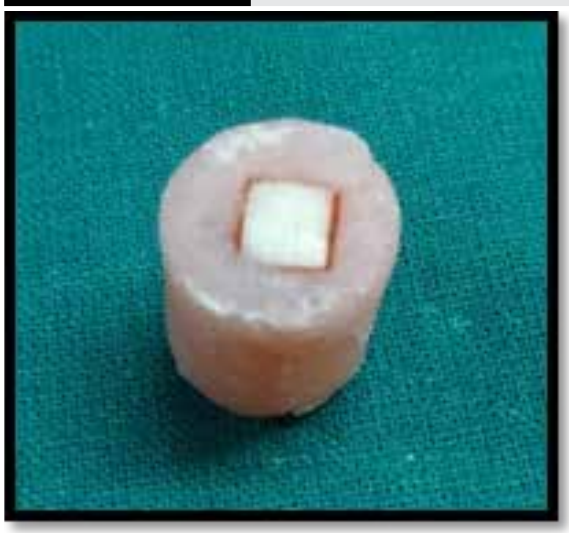


Figure 2

MICROHARDNESS TESTING

Baseline readings were measured using Vickers Microhardness Test (MatzusawaMicrovickers hardness tester) by subjecting samples to a force of 50g force for a Dwell time of 30 seconds. Readings were amplified by 30% to compensate for error of the Vickers microhardness tester and were tabulated. Dehydration of samples was prevented by storing them in 0.9% normal saline.

Demineralization of samples was carried out by Mc Innes solution (**Kamath U, Sheth H, Mullur D &Soubhagya M., 2013**) in two cycles with an application time of 5 minutes every 24 hours. Samples were then washed and damped dry and subjected to microhardness testing and the values obtained were tabulated.

The samples were then segregated into 3 groups with 10 samples each. The samples in each group were treated with three different remineralizing dentrifices- GC Tooth Mousse (CCP-ACP), Enafix(5%) (Calcium Sucrose Phosphate), Vantej (Calcium Sodium Phosphosilicate). Remineralizing pastes specific to each group were applied with applicator tips for three minutes twice daily for 15 consecutive days. The samples were subsequently washed with running tap water, dried and then subjected to microhardness testing and the readings were recorded.

STATISTICAL ANALYSIS

Using SPSS Software version,statistical analysis was perfomed and a p-value of less than 0.05 was deemed statistically significant.

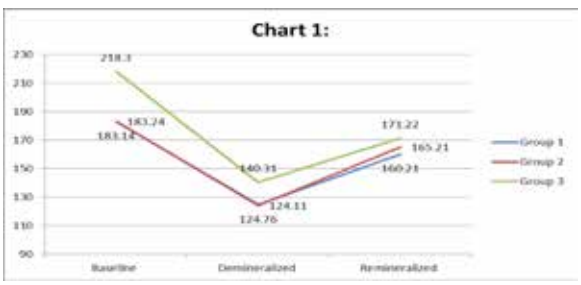
Comparison of mean values was done using ANOVA with post-hoc Bonferroni test &Kruskal Wallis test.

RESULTS

INTRAGROUP COMPARISONS

A decrease in surface microhardness after demineralization and a subsequent increase following remineralization was observed among the specimens in all the three groups. The results were consistent in all the three groups and were statistically significant.

Group		Mean	SD	p-value	Post-hoc test
1	Baseline	183.14	35.92	0.074; S	1>2
	Demineralized	124.76	24.78		
	Remineralized	160.21	68.73		
2	Baseline	183.24	32.26	0.004; S	1>2
	Demineralized	124.11	54.82		
	Remineralized	165.21	72.82		
3	Baseline	218.30	40.98	0.002; S	1>2
	Demineralized	140.31	29.68		
	Remineralized	171.22	45.29		



INTERGROUP COMPARISONS

Values were compared to assess the percentage increase in surface microhardness of demineralized enamel after application of the three different remineralizing dentrifices. Although Enafix (Group 2) showed the highest value followed by GC Tooth Mousse (Group 1) and then Vantej (Group 3), the results were not found to be statistically significant.

	Group						p-value
	1		2		3		
	Mean	SD	Mean	SD	Mean	SD	
Difference	29.07	48.68	35.20	27.58	25.55	40.61	0.418

DISCUSSION

Our teeth can repair themselves by replenishing lost minerals by those available in saliva through a natural remineralization process. The 'eureka moment' occurred with the advent of remineralizing dentrifices which were able to supersaturate the saliva with mineral ions thereby aiding in repair of initial defects. The three dentrifices used in this study function on the basis of similar mechanisms.

Casein Phosphopeptide-Amorphous Calcium Phosphate (CCP-ACP) (GC Tooth Mousse), reduces demineralization and promotes the remineralization potential of subsurface enamel(**Aimutis,2004**) .The sticky CPP binds readily to the enamel, biofilm, and soft tissues and propagates the delivery of the calcium and phosphate ions to areas of low mineral content. These free calcium and phosphate ions that are released, enter the enamel rods, and reform the apatite crystals and thereby preserve microhardness of enamel.

Calcium Sucrose Phosphate (CSP) (Enafix) was the newly introduced dentrifice tested in this study. It allows rehardening of softened enamel and super hardening of intact

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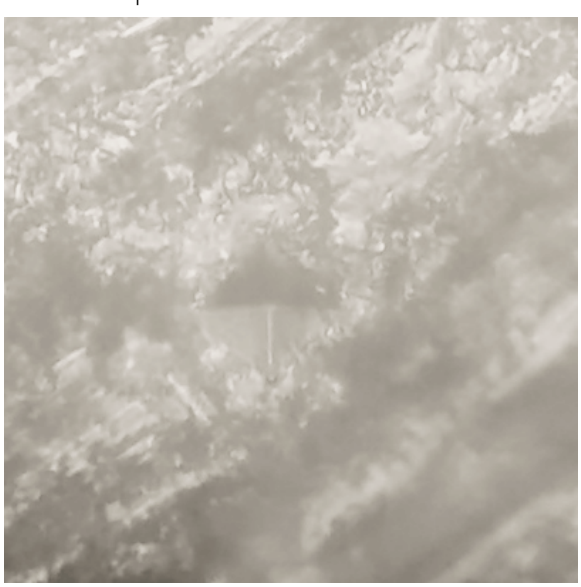
enamel by supersaturation of saliva by deposition of calcium and phosphate ions through common ion effect. It prevents acid dissolution of enamel by depositing a layer of sucrose phosphate ion layer over the exposed hydroxyapatite of tooth. The layer of deposited sucrose phosphate ions was claimed to be the cause of increased remineralization potential of CSP.

Calcium Sodium Phosphosilicate(CSPS) (Vantej) on exposure to fluid (saliva or tap water) instantly releases calcium and phosphate ions which causes the crystallization into hydroxycarbonate apatite and thereby increases surface microhardness**.(Burwell AK & Muscle D, 2009)**

In this study all the dentrifices used, showed an increase surface microhardness depicting remineralization. However Enafix (Calcium Sucrose Phosphate) showed a higher increase which could be attributed to the sucrose phosphate layer formed to protect tooth against acid dissolution.

Lata S, Varghese NO, Varughese JM (2010)stated that surface microhardness (SMH) measurement an appropriate technique for analyzing demineralization &remineralization process for a material having fine microstructure, non-homogenous or prone to cracking like enamel. SMH indentations provide a relatively simple, non-destructive and rapid method in demineralization and remineralization studies. The surface microhardness of the specimens was thus determined using digital microhardness tester (MATSUZAWA Co., Ltd. Model - MMT X7, Japan) with a Vickers elongated diamond pyramid indenter and a ×40 objective lens. A load of 50 g was applied to the surface for a dwell time 30 seconds.Precision microscopes of magnification of 400× were used to measure the indentations. Size of indentation is inversely proportional to the surface hardness of the material i.e. smaller the indentation, higher the microhardness value of the specimen.



Vickers Microhardness tester was preferred over Knoops Hardness tester for this study for the following reasons: (**Anonymous,** 2003)

- Its indentation diagonal is about 1/3 of the length of Knoop's major diagonal and penetrates about twice as deep
- Test indentations are very small and ideally suited for small rounded areas
- Less sensitive to surface conditions
- More sensitive to measurement errors

Enamel demineralization and remineralization were investigated in relation to change of surface microhardness. The softening and rehardening were shown to take place in the outer 5 μ of enamel surface. The acid resistance of the rehardened enamel was found to be similar to that of the original. (**F. Feagin, T. Koulourides& W. Pigman, 1969**)

CONCLUSION

Milton Berle said, "If opportunity doesn't knock, build a door." With the increasing awareness about dental health it is imperative to diagnose enamel subsurface lesions so as to facilitate remineralization at the earliest. Remineralizing dentrifices open a portal which affords our teeth a second chance is regaining their surface hardness with minimal intervention from a dentist.

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