

RESEARCH ARTICLE

Comparative Evaluation of Surface Microhardness of Artificially Demineralized Human Enamel with Nano Hydroxyapatite, Calcium Phosphate, and Potassium Nitrate Remineralizing Agents: An *In Vitro* Study

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ABSTRACT

Aim: To evaluate and compare the microhardness of artificially demineralized human enamel treated with three different remineralizing agents.

Materials and methods: Twenty-five intact caries free maxillary premolar were taken for the study. The tooth was decoronated 2 mm below cemento-enamel junction (CEJ). Enamel samples of 2 mm width were prepared, the enamel slices were taken from the buccal and lingual surfaces of the teeth using a diamond disc. Following sample preparation, windows were created with a dimension of 5 mm × 5 mm. Adhesive tape was placed over it to resist acid attack. The specimens were demineralized using a demineralizing solution for ten days and then subjected to microhardness testing. Teeth were divided into five groups.

- **Group 1:** Negative control in which no therapy was done
- **Group 2:** Demineralized enamel specimen
- **Group 3:** Remineralization with potassium nitrate containing toothpaste
- **Group 4:** Remineralization with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) containing toothpaste,
- **Group 5:** Remineralization with hydroxyapatite containing toothpaste. The remineralizing agents were applied using cotton applicator tips for 3 minutes twice daily and they were washed under distilled water. The samples were then stored in artificial saliva for 10 days and subjected to microhardness testing.

Statistical analysis: One-way analysis of variance (ANOVA) test. A post hoc comparison showed that done using a paired t-test.

Results: The result showed that there has been statistically significant difference in the microhardness for control samples and experimental samples. Within the experimental samples, group which used hydroxyapatite paste as remineralizing paste showed better results when compared to paste which contain calcium and potassium ions and sodium nitrate.

Keywords: Dental caries, Microhardness, Remineralizing agents.

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INTRODUCTION

An ounce of prevention is worth a pound of cure!!

Dental caries is the most common disease affecting a large number of communities. Dental caries is a process that takes place on any tooth surface where the dental plaque is accumulated for a long period.¹ The occurrence of caries is dependent on the pH of saliva. Enamel dissolution starts when the pH drops below 5.5, thus initiating demineralization. It marks the beginning of early enamel caries as well. During repeated falls in pH, the subsurface layer gets demineralized while the enamel surface layer stays consistently unmineralized.² Neutralizing the oral pH opposes the process of demineralization.³ This can be achieved by intensifying calcium and phosphate ions supply. This process is called as remineralization, which involves the rebuilding of partly dissolved apatite crystals. Demineralization is naturally reversed to remineralization by incorporation of minerals from saliva into the lesion.⁴

The emphasis on caries management has been recently modified with the development of strategies for early detection of caries lesions and the practice of non-invasive treatment for these lesions.⁵ Various remineralization agents have been introduced to enhance this mechanism. Numerous mechanisms are available for accelerated remineralization. This demands the availability of calcium and phosphate along with fluoride ions to rebuild a new surface on existing affected structure. It involves a delivery mechanism of ions to the affected area mainly the subsurface lesion area than merely on the surface. Topical fluorides have been well known and proven as a

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delivery system for the prevention of caries.⁶ Restriction of initiation of dental caries can be achieved by milk and its products. Casein, calcium, and phosphate provide this anti-cariogenic property to milk, imparting its protective action.^{7,8} Recently, hydroxyapatite was introduced as a promising remineralizing agent.⁶⁻⁸

To date, no study has compared the remineralization potential of hydroxyapatite (Aclaim), CPP-ACP), GC Tooth Mousse, Leuven, Belgium) and Potassium nitrate (KNO_3 , Colgate sensitive clove oil). Therefore, the present in vitro study was designed to assess and compare the remineralizing capacity of the above agents on artificial enamel lesions, through Vicker's microhardness testing.

MATERIALS AND METHODS

Twenty-five freshly extracted sound maxillary premolars, extracted for therapeutic reasons, were used. Teeth with any caries or white spot lesions were excluded (Fig. 1). The teeth were decoronated and the crown portions were divided into two segments of one buccal and one palatal half using a diamond disc mounted on a contra-angle

handpiece. Enamel samples were fixed on the self-cure acrylic mold with the enamel surface exposed. These samples were stored in deionized water until further use. A total of fifty enamel samples embedded in partially set acrylic cylinders were produced and then they were randomly divided into five groups of 10 samples each.

- *Group 1:* Negative control in which no therapy was done (Fig. 2)
- *Group 2:* Demineralized enamel specimens (Fig. 3)
- *Group 3:* Potassium nitrate containing toothpaste (Fig. 4)
- *Group 4:* CCP-ACP containing toothpaste (Fig. 5)
- *Group 5:* Hydroxyapatite containing toothpaste (Fig. 6)

The demineralizing solution was prepared in the Department of Biochemistry.⁹ The pH of the demineralizing solution was checked during and after preparation of solution using digital pH meter. The composition of the demineralizing solution used was as follows:

- 2.2 mM calcium chloride, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
- 2.2 mM monosodium phosphate, $\text{NaH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$
- 0.05 M lactic acid, $\text{C}_3\text{H}_6\text{O}_3$



Fig. 1: Sectioning of tooth



Fig. 2: Negative control

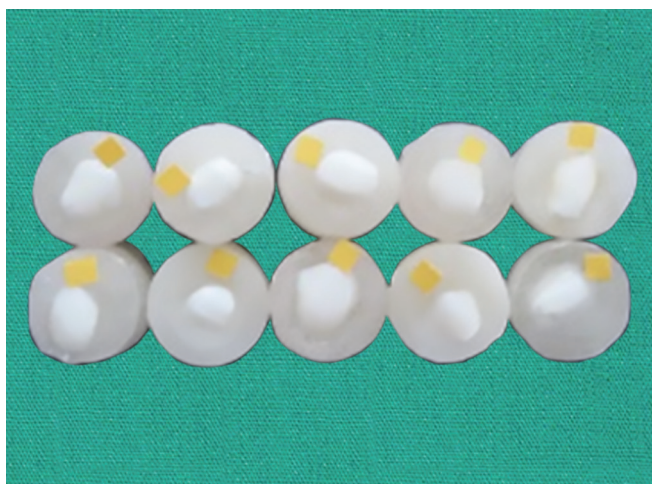


Fig. 3: Demineralized enamel specimen



Fig. 4: Remineralization with Hydroxyapatite paste



Fig. 5: Remineralization with CCP-ACP paste

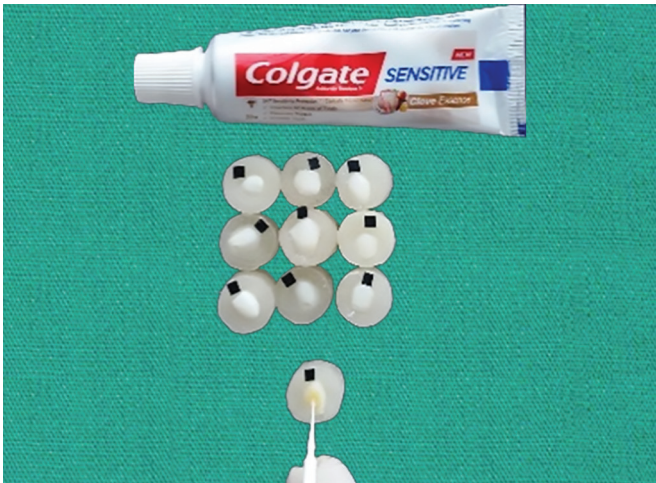


Fig. 6: Remineralization with potassium nitrate paste

The final pH was adjusted to 4.5 with 50% sodium hydroxide, NaOH. All the samples except group 1 were then immersed into a glass container containing 50 mL of prepared demineralizing solution for a period of 6 h at 37°C. This demineralizing procedure was considered to produce a consistent subsurface enamel lesion in all samples.¹⁰ After demineralization, the teeth were washed with deionized water and dried.

The samples in groups 3 to 5 were treated with respective remineralizing agents for 3 minutes twice daily for 10 days¹¹ (Table 1). Samples were applied with a respective remineralizing agent with the help of cotton tip applicator for 3 minutes twice daily, washed with deionized water and then stored in artificial saliva. All samples were placed in a universal incubator at 37°C between each remineralizing cycle. In the control groups, samples were only washed with deionized water and stored in artificial saliva. Artificial saliva (Moi Stir) was renewed every 24 hours.

After 10 cycles of remineralization, the surface microhardness of the specimens was determined using Vickers microhardness testing machine (Fig. 7). A load of 100 g was applied steadily to the surface of specimens for a period of 10s using Vickers elongated diamond pyramid indenter under a $\times 40$ objective lens.¹² The accuracy of values of diagonal length of indentations was determined

under high power magnification of $\times 400$. The depth of the indentations was measured, and the values were converted to Vickers microhardness values. Five indentations were placed on the surface and the mean value was considered for each specimen.

STATISTICAL ANALYSIS

One-way ANOVA test, Tukey simultaneous comparison test was done for statistical analysis. One-way ANOVA test showed there has been an overall statistically significant difference between the demineralized and remineralized samples with a p-value less than 0.001 (Table 2). It also showed there is difference in mean values between the groups. Tukey’s comparison test has been used for experimental groups to compare group’s mean (Table 3). (compared with each other) are different (Graph 1).

Of all the groups the group in which no treatment was done showed the highest microhardness value. The statistical analysis was done comparing the experimental groups. To examine the difference post hoc test was done. Group 5 (HA) showed highest microhardness when compared with

Table:1 Details of product

Product	Manufacturer	Composition
Aclaim	Aclaim™, group pharmaceuticals, Bengaluru, India	1% Nano hydroxyapaptite
GC tooth mousse	Recaldent	10% calcium phosphopeptide - amorphous calcium phosphate (CPP-ACP)
Colgate sensitive clove essence toothpaste	Colgate company	Potassium nitrate Clove oil



Fig. 7: Microhardness testing machine

Table 2: ANOVA test

One factor ANOVA					
	Mean	n	Std. dev		
	304.0173	10	6.21974	Group 2	
	306.1380	10	6.72149	Group 3	
	324.1812	10	32.38933	Group 4	
	350.2408	10	23.53393	Group 5	
	321.1443	40	27.23333	Total	
ANOVA table					
Source	SS	df	MS	F	p-value
Treatment	13,743.52243	3	4,581.174142	10.86	0.000
Error	15,181.00433	36	421.694565		
Total	28,924.52675	39			

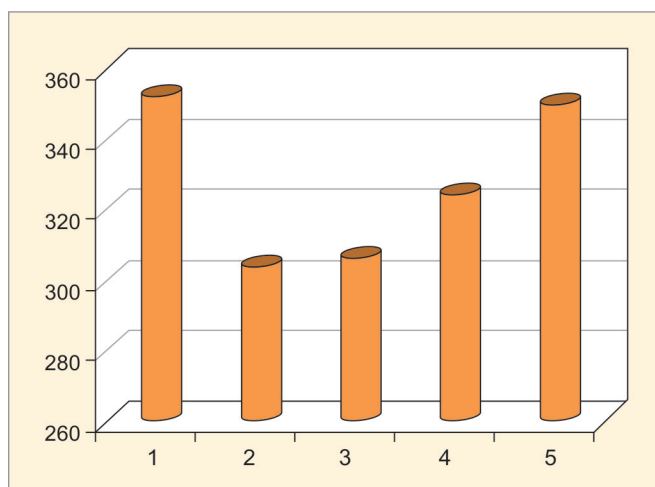
Table 3: Tukey's simultaneous test

Post-hoc analysis					
Tukey simultaneous comparison t-values (d.f. = 36)					
		Group 2	Group 3	Group 4	Group 5
		304.0173	306.1380	324.1812	350.2408
Group 2	304.0173				
Group 3	306.1380	0.23			
Group 4	324.1812	2.20	1.96		
Group 5	350.2408	5.03	4.80	2.84	
Critical values for experiment wise error rate					
		0.05	2.70		
		0.01	3.35		

group 4 (CCP-ACP) and group 3 (potassium nitrate) and showed the highest microhardness. Groups 3 and 4 also showed statistically significant values in which group 4 (CCP-ACP) performed better compared to group 3.

RESULTS

The result showed there has been statistically significant difference in the microhardness for control samples and experimental samples. Within the experimental samples group which used hydroxyapatite paste as remineralizing paste showed better results than compared to paste which contain calcium and potassium ions and sodium nitrate (Graph 1).



Graph 1: Results chart

DISCUSSION

This study highlights the remineralization effects of different toothpaste on demineralized enamel of extracted teeth. Dairy products such as milk and milk products like milk concentrates, powders, and cheeses have been demonstrated to have anti-cariogenic properties.^{9,10} In previous studies on rats have shown that a soluble form of casein or caseinate has the ability to reduce caries in rats.¹¹ However, for effective caries control, huge amount was necessary, and that form of casein was not commercially ideal.¹² The major tryptic peptide which has the anti-cariogenic nature of casein is casein phosphopeptides (CPP). Thus multiple phosphoserine residues, CPP can stabilize calcium phosphate in a CPP-amorphous calcium phosphate (ACP) complex.^{12,13}

Special milk-derived protein is present in recalcant called casein phosphopeptide (or CPP), and it carries calcium and phosphate ions in the form of amorphous calcium phosphate (ACP). This delivery system has bio-available calcium and phosphate ions, as a remineralizing ingredient that strengthens teeth.^{12,13}

Although the paste supplies calcium and phosphate ions for remineralization the microhardness was less than compared to nano hydroxyapatite paste. According to Galbiatti et al. following CCP-ACP application non-homogeneous deposits with adherent irregularities were formed and gets deposited as globular structures. These globular structures fill the interprismatic cavities and partially cover the prisms.¹⁴

Recently more studies are on biomimetic oral health products comprising of nano-sized hydroxyapatite particles. This manmade nano-hydroxyapatite (nHAP) has chemical-physical properties similar to the apatite structure within enamel, making it an interesting ingredient for oral health compounds.¹⁵ Studies have shown that this nHAP have a strong affinity to the tooth, and can get strongly adsorbed to enamel surfaces.¹⁵

Aclaim toothpaste contains nanoparticles of hydroxyapatite which is similar to natural hydroxyapatite of tooth. It was found to penetrate deeper into dentinal tubules and forms biomimetic apatite crystals on the dentin surface for additional protection. It was found to fill microcracks of enamel and helps in remineralization. Similar results were found in a study conducted by Najibfard et al. and he concluded that dentifrices containing nHAP have the potential for the same remineralizing capacity as a fluoride dentifrice and may be employed as an effective alternative to fluoride-containing dentifrices.¹⁶

According to Hannig et al. growth of organized, nano-apatite-based coatings closely resemble the hierarchical architecture of human enamel.¹⁶ Singh et al. studied the efficiency of four dentifrices which are Aclaim (n-HAP), Apagard (n-HAP), Clinpro Tooth Crème (tricalcium phosphate and fluoride), and Colgate Total (fluoride) to inhibit demineralization adjacent to orthodontic brackets. His study found out that the efficiency of aclaim and Apagard remineralizing pastes were found to be superior^{17,18} when compared with other toothpaste.

Nano hydroxyapatite has affinity to water which aid in wetting character that is capable of producing a thin but tightly bound layer on the tooth surface.¹⁹ This results in a high surface hardness of enamel. Ebadifar et al. in his study found out that fluoridated toothpaste can remineralize initial carious lesions. He also noted the synergistic effect when nanohydroxyapatite and fluoride toothpaste were used together in remineralization.²⁰

Clove oil belongs to class of essential oils, extracted from the clove plant called *Syzygium aromaticum*. It has health benefits and is also used in the production of perfume. Clove oil contains eugenol, acetyl eugenol, caryophyllene, and other compounds. Eugenol has many benefits, and it's been used as an antiseptic and anesthetic agent. This compound is contained in many dentistry products and used as a flavoring for medicines. Combination of clove oil with potassium nitrate in this new toothpaste offers everyday protection to the rampant sensitivity issues.

The mode of action of potassium nitrate is that the potassium ions enter the tubules and block the nerves of the pulp. It blocks A-fibers where repolarization of these fibers is prevented after initial depolarization. The

potassium levels block the action potential generated in the intradental nerves. If potassium nitrate is kept in high concentration the depolarized state decreases the perception of pain. It acts as a numbing effect on dentin hypersensitivity. Although in Colgate sensitive clove essence potassium nitrate and clove oil are present it didn't enhance any remineralizing property. This could be the reason for less microhardness than compared to hydroxyapatite and CCP-ACP paste.

Andréia Mara Andrade Pizani et al. studied the effect of different bleaching agent on microhardness of teeth. He concluded that the agent which contained calcium and potassium nitrate had better property than with the bleaching agent without calcium.²¹

Studies have reported more or comparable remineralizing effects for nanohydroxyapatite toothpastes in comparison to other toothpastes containing fluoride and CCP-ACP but not on potassium nitrate alone.

CONCLUSION

Remineralizing paste has various applications in dentistry. It can be used in primary teeth where there is a high risk of caries, as a preventive regimen to prevent caries for patients on chemotherapeutic treatment. It can also be used as a part of minimal intervention therapy in the early white lesion, prophylactic for orthodontic cases, erosive lesions, reducing dentin hypersensitivity.

From the present study, it was found the samples in which toothpaste containing hydroxyapatite showed better remineralizing than other two pastes.²²

Even though potassium nitrate widely used in reducing dentin hypersensitivity, it cannot show any remineralizing property.

CLINICAL SIGNIFICANCE

Due to change in lifestyle and dietary habits, there is increase in incidence of caries in young children and adults. The balance of remineralization and demineralization will determine the non-cavitated caries lesion. With diet counseling, diet modifications and judicious use of remineralizing paste, caries can be prevented.

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