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EVALUATION OF REMINERALIZING POTENTIAL OF ENAFIX, CLINPRO5000 AND GC TOOTH MOUSSE AND THEIR EFFECT ON MICROHARDNESS OF ENAMEL USING VICKERS MICROHARDNESS TEST: AN *IN VITRO* STUDY

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ABSTRACT

Introduction: Dental caries, etiologically is a multifactorial disease because of both bacterial acid production along with the buffering action from saliva and the surrounding surface of the tooth structure earlier visible as White Spot Lesions (WSLs). Remineralization is the process whereby calcium ions and phosphate ions are supplied from an external source to the tooth to promote ion deposition into crystal voids. Evidence shows that early caries lesions can be mineralized in-vitro and in-vivo, with fluoride as one of the most significant agents for promoting remineralization. **Aim:** To compare and evaluate the of remineralizing potential of Enafix, Clinpro5000 and GC Tooth Mousse on artificially demineralized human enamel- An in vitro study. **Materials and Methods:** 40 enamel samples were divided into 4 groups. Group A: CaSP (Enafix); Group B: Tricalcium phosphate (Clinpro5000); Group C: CPP-ACP (GC Tooth mousse) and Group D (control) containing dentifrice. All teeth were subjected to demineralization for 24hours followed by remineralization with respective dentifrice for 30 days and the VHN was assessed. **Results:** The result of this study concludes that surface microhardness of enamel decreased after demineralization procedure and increased after application of all the remineralizing agents. The highest increase was seen in group tested with fTCP followed by CPP-ACP and least for CaSP. The values for all the three groups were higher than the control so we can conclude that all the agents can be used as remineralizing agent with f-TCP being the best among the three. **Conclusion:** All remineralizing agents showed improved surface remineralization. However, Tri-calcium phospahte showed a better potential for remineralization compared to CPP-ACP and CaSP.

KEYWORDS: Dental caries, demineralization, remineralization, Tricalcium phosphate, CPP-ACP, Calcium sucrose phosphate, Vickers hardness number.

INTRODUCTION

Dental caries is a world-wide chronic disease, easily detectable and occurs when the demineralization process exceeds remineralization. The progression of dental caries lesion is a slow process and during the early stages, non-invasive intervention converts lesion from an active state to an inactive state. The process of caries formation is a cycle of remineralization and demineralization.[1] Demineralization- Remineralization" are two process which control progression or reversal of caries. This depends mainly on Saliva: pH, composition, flow rate, Diet, Tooth: composition. It should be noted that the extent to which fluoride & whole saliva remineralize the enamel is limited.[2,3]

Early diagnosis of incipient lesions can lead to a new era in preventive dentistry in the form of remineralization. One of the best modes for caries management is the use of remineralizing products. Various remineralizing agents have been introduced in treating initial carious lesions. They create supersaturated environment around the early lesion; thus, preventing mineral loss and forces calcium and phosphate ions in the vacant areas.[4]

Calcium sucrose phosphate (Enafix) remineralizing agent is available only as a dentifrice. Its quicklybreaks down and release calcium, phosphate, and sucrose phosphate ions into the saliva, calcium and phosphate ions, rapidly absorb onto the enamel, decrease the rate of enamel solubility under acidic conditions.[5]

Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP–ACP) (GC Tooth Mousse, India) was introduced as a remineralizing agent in the year 1998. It contains nanocomplexes of milk protein Casein phosphopeptide with ACP.[6] It has been claimed that it enhances remineralization of the early carious lesions by maintaining a supersaturated environment for essential minerals, at the same time it also hinders colonization of dental surfaces by cariogenic bacteria.[7]

Tri-Calcium Phosphate is a new technology that delivers calcium and phosphate ions as. It contains 1.1 % sodium fluoride and delivers phosphate and calcium to the teeth which work synergistically with fluorides without any unfavourable interactions during storage of the product.[8] It has been introduced by 3M ESPE and is incorporated into many products that are available in the market such as 36 Clinpro 5000 toothpaste with 5000 ppm F, Clinpro White Varnish with 22600 ppm F, and Clinpro Tooth Crème with 950 ppm F.[9]

The combination of fluoride with beta-TCP provides greater remineralization in terms of fluoride absorption and microhardness.[10]

Tooth structure microhardness can be measured a technique known as the Vickers microhardness test (VMHT).[11] VMHT uses a diamond indent area to impress a small area on the tooth surface with a predefined set load for a specified amount of time. The microhardness number is later computed after microscopic examination of the indentation in relation to the used indentation load and the area of the remaining impression. Therefore, the present study aimed to assess and compare the effects of remineralization on artificial enamel lesions by agents containing Tricalcium phosphate (CLINPRO5000), CPP-ACP (GC Tooth Mousse), Calcium sucrose phosphate (ENAFIX) microhardness testing using Vicker's hardness test.

MATERIALS AND METHOD

Forty caries-free freshly extracted premolars for Orthodontic reasons without any obvious defects, restorations, intrinsic stains and developmental defects were obtained for the present study from the Department of Oral and Maxillofacial Surgery, SDM Dental College & Hospital and other private dental clinics in Dharwad and Mangalore. The teeth were stored in 10% formalin immediately after extraction and were thoroughly cleaned of its debris, calculus, and soft tissues. They were washed in 0.1 M phosphate buffer, rinsed with deionised water and were stored in distilled water at a temperature of 4^0C. The polished extracted teeth were randomly grouped into four group each containing ten samples using simple randomized sampling method.

ENAMEL SPECIMEN PREPARATION AND GROUPING

The teeth were decoronated at CEJ and the crown portions were divided into four segments of two buccal and two palatal halves each, using a diamond disc bur at slow speed with water as coolant. Custom made cylindrical moulds were prepared and self-cure acrylic resin was sprinkled into them. The buccal fragment of each enamel specimen was then embedded onto the top of partially set acrylic resin and was allowed to set completely. The buccal surfaces were then ground flat and hand polished progressively using silicon carbide paper. All enamel specimens were subjected to analysis using Vickers microhardness test (VHN) under 100 g load applied for 15s and the data was recorded at baseline.

Grouping of the Specimen

Group I: Calcium sucrose phosphate tooth paste (Enafix)
Group II : Tricalcium phosphate (Clinpro5000 F)
Group III : CPP-ACP (GC Tooth Mousse)
Group IV : Control group

Demineralizing solution

- Phosphorous (as monopotassium phosphate-KH2PO4)- 2.2mM,
- Acetic acid- 50mM,
- CaCl2 - 2.2mM

The pH of the solution was measured with digital pH meter and adjusted to 4.5

CYCLES OF DEMINERALIZATION

All the samples of Groups A, B, C and D were then immersed into a glass container containing 50 ml of prepared demineralizing solution for 48 h at 37°C inside a Universal Incubator. This demineralizing procedure intended to produce a consistent subsurface lesion.

After 48 h of incubation in the demineralizing solution, the teeth were washed with deionized water, dried with the help of an air syringe, and placed in different clean glass containers until further evaluation.

CYCLES OF REMINERALIZATION

The samples in Groups A, B and C were treated with respective remineralizing agents at every 24 h for 7 days. Samples were rubbed with respective remineralizing agent with the help of polishing cup attached to a contra-angle handpiece for 4 min washed with deionized water, and then placed in artificial saliva. All samples were placed in the Universal Incubator at 37°C between each remineralizing cycle. In the control groups, samples were only washed with deionized water and placed in artificial saliva. Artificial saliva was renewed every 24 h just before immersion of freshly treated samples.

TESTING OF SPECIMENS

VICKERS MICROHARDNESS TEST: Microhardness was tested using Vickers micro hardness tester. The test specimens were placed on the stage of the tester and stabilized. Then the area to indent was selected by focusing with 10x objective lens and further focused with a 50x objective lens. After this, test was carried out where the indentations were made with a rate of 100g load for 10s. The indentation formed was viewed and measured on the display monitor with 50x objective lens. The average micro hardness of the specimen was determined from 4 indentations to avoid any discrepancy. After baseline surface microhardness determination, enamel was demineralized by immersing the specimen into glass container containing 20 mL of demineralization solution for 72 hours in anincubator at a temperature of 37°C. Surface microhardness wasevaluated for all samples after demineralization.

After application of remineralizing agent the specimens were washed and stored in artificial saliva. This treatment was done twicedaily for 28 days and again the surface microhardness was assessed(Fig. 2—Normal sample, Fig. 3—Sample after remineralizationof Group A, Fig. 4—Sample after remineralization of Group B &Fig. 5— Sample after remineralization of Group C).

After getting the microhardness values the microhardnessrecovery in percentage was calculated for each tooth sample in all groups by using following formula:

Microhardness recovery (%)

$$= \frac{\text{Remineralized hardness - Demineralized hardness}}{\text{Baseline hardness - Demineralized hardness}} \cdot 100$$

RESULTS

Baseline surface microhardness measurements of the specimen is shown in Table 1.

Samples in all the groups showed decreased surfacehardness values after demineralization as compared to thebaseline values. The surface microhardness value of group B after remineralization is highest among all groups while it is lowest for group D. By comparing values in the Table 1, it is seen that the surface microhardness of all specimens except group D increases after remineralization (Figs 6 and 7).

From the Table 2, it is observed that value of $p > 0.05$, inother words, 0.301, thus it can be concluded that there is not anystatistically significant difference between Group A, Group B, and Group C.

Fig 1: Vickers hardness tester.

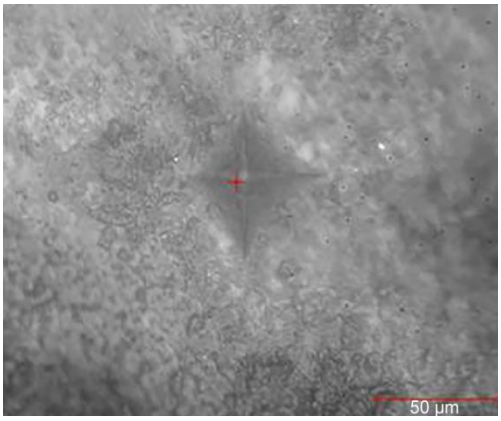

Fig 2: Normal sample.

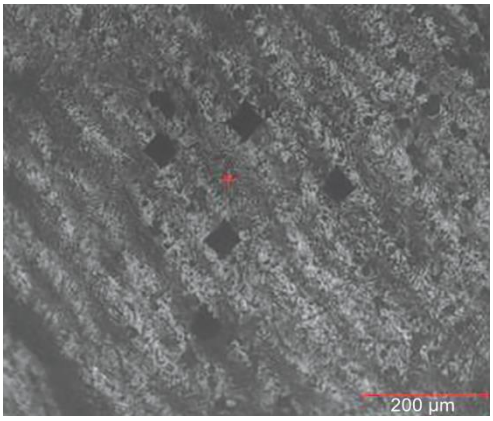

Fig 3: Sample after remineralization Group A.

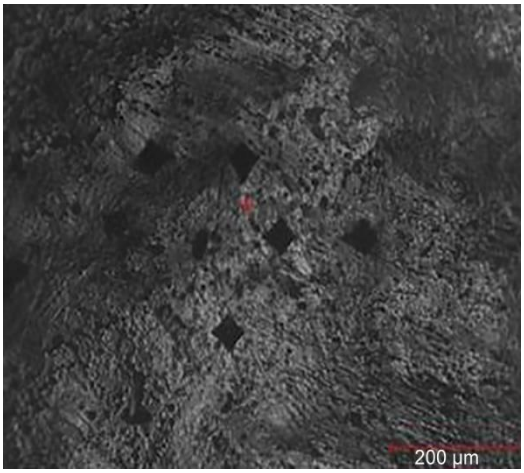

Fig 4: Sample after remineralization Group B.

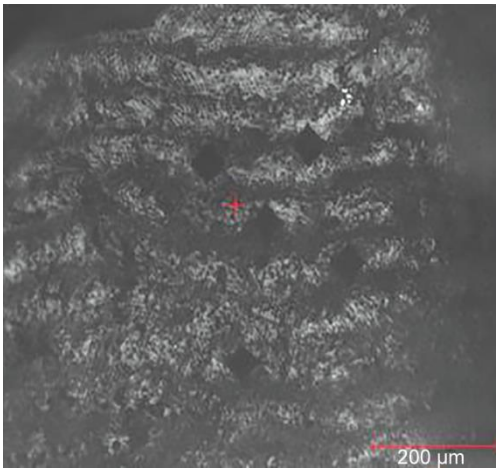

Fig 4: Sample after remineralization Group B.

Table 1: Showing mean and standard deviation value of baseline surface microhardness, surface microhardness after demineralization, surface microhardness after remineralization and percentage microhardness recovery of specimens in the four groups

Procedure	*Specimens*	*Group A*	*Group B*	*Group C*	*Group D*
1. Baseline surface microhardness	Mean	251.81	305.10	282.20	257.74
	Standard deviation	± 39.70	± 29.75	± 43.53	± 42.61
2. Surface microhardness after demineralization	Mean	156.59	167.01	157.99	159.84
	Standard deviation	± 12.34	± 16.59	± 20.73	± 30.73
3. Surface microhardness after remineralization	Mean	197.90	240.78	214.29	152.70
	Standard deviation	± 19.16	± 20.34	± 40.58	± 31.87
4. Percentage microhardness recovery	Mean	45.0403	53.0650	50.9873	-7.285
	Standard deviation	± 13.21	± 11.10	± 10.80	± 1.82

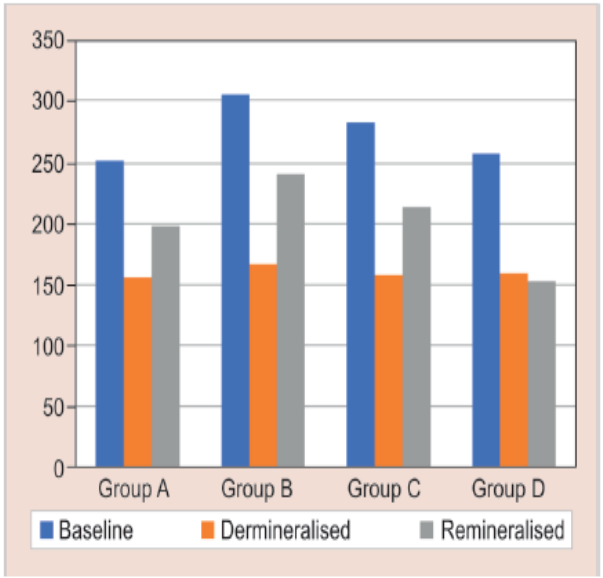

Fig. 6: Graphical representation of baseline surface microhardness compared with surface microhardness of enamel after demineralization and remineralization procedures in various groups

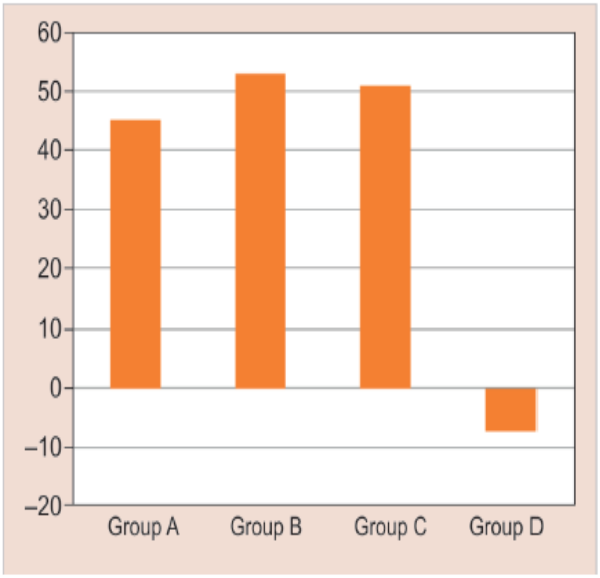

Fig. 7: Graph shows percentage surface microhardness recovery of enamel after the remineralization procedures in various groups

DISCUSSION

Dental caries is a prevalent chronic disease that has been a concernfor human beings.[12] First sign of caries is a white spot lesion. Iftreated at this stage it can be remineralized but if left untreated it may progress to form a frank cavity. White spot lesion is the firstclinical presentation of dental caries (Margolis et al.)[13] Enamel remineralization is not a new topic but nowadaysremineralization of both noncavitated and cavitated lesion is attempted.

The goal of modern dentistry is to manage non-cavitated caries lesions non-invasively through remineralization in an attempt to prevent disease progression and improve aesthetics, strength, and function.[14] Though remineralization has been a major area of investigation, it is still difficult to exactly define the efficacy of various remineralization methods.[15] Currently, preventive and minimally invasive dentistry are offering a wide variety of methods to detect and care for even the tiniest changes in hard tooth tissue. Selecting an appropriate treatment to remineralize white spot lesions and accurately and reliably monitoring the lesion progression or regression is the key to avoid the need for invasive treatments.

Though clinical studies are bet ter for determining remineralizing efficacy of agents but *in vitro* studies are also areliable alternative.[16] Featherstone in the year 1981 used Surface microhardness profiles of enamel to compare changes during demineralization and remineralization.[17]

In vitro surface microhardness studies have the advantage ofbeing simple, easy to conduct, noninvasive and is not affected bypatient burnout, also the same specimen can be used for multipletimes which reduces the chances of experimental error.[16,18]

In Vickers hardness method, the length of indentation suggestsmineral loss or gain. Increase in length of indentation suggeststhat the tissue has lost mineral and on the other hand decrease in length indicates gain of mineral. To calculate the Vickers Diamond Pyramid hardness number, length of diagonals of the indentationare measured and the mean is calculated. Mean value is used with load to determine the value of microhardness.[19]

Initially, in this study baseline surface microhardnessvalues were obtained for all specimens which were in therange of VHN 245–310. These values satisf ies the VHNrange of normal enamel tissue according to studies doneby S Priyadarshini et al.[20]

In this study, we used artificial saliva to store specimens during remineralization treatment which mimic the oral environment since the remineralizing agents need to be in contact with saliva to enhance the remineralization.[21]

In the present study microhardness mean values increasedafter remineralization for Group A, in other words, 197.90 ± 19.16,for Group B, in other words, 240.78 ± 20.34 and for Group C, In other words, 214.29 ± 40.58 but no increase in microhardness was seen for Group D, in other words, 152.70 ± 31.87 which actas a negative control. No mineral regain occurs in group D. This may be because of equilibrium of calcium and phosphate ioncontent of the specimen and remineralizing agent, in other words,artificial saliva.[22]

The mean value for group B, in other words, 240.78 ± 20.34 ismore than group A, in other words, 197.90 ± 19.16. so we can saythat efficacy of group B, in other words, f TCP is better than group A, in other words, CPP-ACP, as Clinpro5000 is a new prospective calcium system that is prepared by reacting the soluble tricalcium phosphate with a surfactant to form a functionalized tricalcium phosphate (fTCP). It uses tricalcium phosphate particles which have been ball milled along with sodium lauryl sulphate. This technology has been included in a tooth cream with sodium fluoride in different concentrations. These components naturally absorbed by teeth, therefore helps in preventing the initiation and further progression of demineralization and allowing remineralization to occur.[23]

The mean value of group B, in other words, 240.78 ±20.34 is more than group C, in other words, 214.29 ± 40.58 therefore remineralizing potential of group B, in other words, (f TCP)is more than group C, in other words, (CPP-ACP).

In the present study group A (CaSP) showed slightly lesser than GC Tooth mousse (CPP-ACP) and fTCP (Clinpro5000). This is because the anti-cariogenic mechanism of CPP-ACP is achieved by the incorporation of the nano-complexes of the amorphous calcium phosphate (ACP) into plaque and onto the tooth surface. The casein phosphopeptides (CPP) have an important role as an ACP carrier localizing the highly soluble calcium phosphate phase at the tooth surface. This localization maintains high concentration gradients of calcium and phosphate ions in the subsurface enamel, thereby facilitating remineralization.[23]

Percentage microhardness recovery was calculated to evaluateregain in microhardness after remineralization and thus the efficacy of different remineralizing agents. Increased in microhardnessrecovery with Group B, in other words, 53.06 ± 11.10 and Group C, in other words, 50.98 ± 10.80 than Group A, in other words, 45.04± 13.21 can be attributed to the presence of fluoride in group B. Fluoride improves the crystalline tooth structure with less internal crystalline stress and strain. The results of the present study were consistent with the results found by Karlinsey et al (2009) where they compared Functionalised tricalcium phosphate with MI paste in terms of remineralization and observed that the combination of functionalized tricalcium phosphate and

NaF at different fluoride levels provides a successful dose response remineralizing effect. Karlinsey et al (2008) also concluded that 1,000 ppm fluoride fTCP dentifrice showed fluoride uptakes and mean Vickers hardness value is higher than those of the paste with CPP-ACP and 900 ppm fluoride in remineralization of white-spot enamel lesions.

The reason for the enhanced remineralizing potential of fTCP when compared to other remineralizing agents as stated by manufacturer is that during manufacturing of the toothpaste, a protective barrier is created around the calcium ions thereby allowing it to coexist with the fluoride ions. During brushing when the toothpaste comes into contact with saliva, the barrier dissolves and allows the release of calcium, phosphate and fluoride ions on the tooth surfaces to help prevent tooth decay and remineralize demineralized enamel. The exclusive manufacturing of fTCP in terms of milling process protects the TCP during storage so that the calcium does not degrade the fluoride. Various organic materials can be used to tailor the fTCP system to a different topically applied oral care preparations, such as toothpaste, oral rinses and varnishes.

The findings of the present in vitro study suggested that Clinpro5000 (fTCP) with 5000 ppm of fluoride resulted in enhanced remineralization compared to GC tooth mousse (CPP-ACP) and Enafix paste. This is in accordance with the study conducted by Karlinsey et al (2009).

CONCLUSION

Following conclusions can be drawn from the results:

- After demineralization surface microhardness of enamel get reduced, in other words, loss of minerals from the surface of enamel takes place.
- After application of remineralizing agents surface microhardness of enamel increased, in other words, regain of minerals on the surface of enamel takes place.
- Among three experimental remineralizing agents used in the present study tricalcium phosphate(Clinpro5000) containing 5000ppm of fluoride has better remineralization when compared to other groups.
- Enafix being a cost effective material when compared to Clinpro5000 and GC Tooth Mousse, can be used as an alternative for better remineralization especially for the Indian population.

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