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IN VITRO EVALUATION OF ENAMEL MICROHARDNESS AFTER APPLICATION OF THREE DIFFERENT REMINERALIZING AGENTS ON PRIMARY TEETH

1.Prajakta Bhaskar Mahajan, 2. Kirti Pattanshetti, 3. Jayatri Mondal, 4. Tejaswini Kalaskar, 5. Dipaneeta Sarkar, 6. Rupashree Chatterjee

[1]Post graduate student, Department of Pedodontics& Preventive Dentistry, Maitri college of Dentistry & Research Centre, Chhattisgarh.
[2]Professor & Head of the Department, Department of Pedodontics& Preventive Dentistry, Maitri college of Dentistry & Research Centre, Chhattisgarh.
[3]Post graduate student, Department of Pedodontics& Preventive Dentistry, Maitri college of Dentistry & Research Centre, Chhattisgarh.
[4]Post graduate student, Department of Pedodontics& Preventive Dentistry, Triveni Institute of Dental sciences Hospital & Research Centre, Chhattisgarh
[5]Consultant Pedodontics, Umerkot, Nabarangpur, Odisha
[6]Post graduate student, Department of Pedodontics& Preventive Dentistry, Triveni Institute of Dental sciences Hospital & Research Centre, Chhattisgarh

Corresponding author
Jayatri Mondal,
Post graduate student, Department of Pedodontics& Preventive Dentistry, Maitri college of Dentistry & Research Centre, Chhattisgarh.
mondaljayatri@gmail.com

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INTRODUCTION

Despite recent advancements in oral healthcare, dental caries remains a significant health problem for individuals of all age-group.Ernest Newbrun best explained dental caries as a cyclic process, with periods of demineralization interspersed with periods of remineralization.[1]Remineralization and demineralization are routine processes that dental hard tissues undergo. The presence of cariogenic dental plaque, imbalance of remineralization and demineralization, and fermentable carbohydrates in the host give rise to dental caries.[2] Enamel white spot lesions are the earliest macroscopic evidence of enamel caries in which the enamel surface layer stays intact during subsurface demineralization, but without any intervention, cavitation will take place. In the first stage of enamel demineralization, removal of interprismatic mineral content takes place subsequently followed by a well-defined surface layer formation that constitutes an early carious lesion. The progression of the early enamel lesion is a slow process, and this early lesion is reversible through the process of remineralization (RML).[3] Nowadays, various novel calcium phosphate-based materials are developed and clinically accessible for remineralization of tooth in the deficiency of fluoride, such as bioactive glass, amorphous calcium phosphate, and milk-derived protein complexes. These nonfluorinatedremineralizing agents create supersaturated state for calcium and phosphate ions for remineralization in absence of fluoride.GC Tooth Mousse Plus (CPP-ACPF) has been introduced which contains CPP-ACP and fluoride in which the level of fluoride is 0.2% or 900 ppm. CPP-ACP when added with fluoride provides with synergistic outcome on early carious lesions remineralization processes. This CPP-ACP enables enamel remineralization by adhering to the plaque pellicle, hydroxyapatite as well as soft tissues, and supplying bioavailable calcium and phosphate into saliva fluids and plaque pellicle. More recently, a new product was launched as Clinpro Tooth Crème [CTC, 950ppm sodium F with 500ppm functionalized tri-calcium phosphate, 3M ESPE Dental, Minnesota, USA] has been introduced, which contains functionalized tricalcium phosphate with 950 ppm of fluoride. While manufacturing the product, placement of a protective barrier around calcium particles is ensured so as allowing its coexistence with the fluoride ions. Advantages of this product are unlike other calcium-based additives; only

low levels of functionalized TCP are needed to produce strong, acid-resistant mineral nucleation without negatively affecting fluoride's proven benefits.[4]With the focus on preventive strategies and minimally invasive dentistry for caries management in young children, silver diamine fluoride (SDF) has emerged as a novel method on the horizon. One of the hassles encountered by a dental practitioner when dealing with very young children or children with special needs is the risk of triggering anxiety and fear with the use of rotary handpieces or sharp spoon excavators. For such patients, SDF can be a successful alternative. Aptly named a "silver-fluoride bullet," SDF acts by the synergic effect of silver and fluoride ions. Rosenblatt et al. advocated that the silver ions inhibit the colonization of cariogenic bacteria either by blocking the electron transport system in bacteria or by deactivating the thiol group of enzymes or by mutation of bacterial DNA. Its anti-enzymatic property can also be attributed to the silver ions. SDF penetrates the enamel and accumulates more fluoride ions in the subsurface layer than any other fluoride solution. It also increases the microhardness of the enamel surface by the formation of fluorapatite. Owing to its benefits, 38% SDF (44800 ppm) has been proposed for the prevention of new carious lesions and management of existing carious lesions in both primary and permanent dentition.[1]Surface microhardness indentation used for demineralization and remineralization studies provides with a relatively simple, non-destructive and rapid method. A relationship of enamel hardness values with mineral content of the tissue on a weight basis exists.[4]

Earlier studies to compare the remineralization potential of white-spot enamel lesions by Tooth Mousse Plus and functionalized tricalcium phosphate have been done on permanent teeth. But those comparing their remineralization potential on primary teeth are not yet being done. Because of the above consideration, the present study is designed to investigate the remineralization GC Tooth Mousse Plus (CPP-ACPF), Clinpro Tooth Creme (functionalized tricalcium phosphate paste) (fTCP) and SDF on artificially created white spot lesions in human primary enamel through microhardness assessment.

MATERIALS AND METHOD

Inclusion criteria:

- Primary molars

Exclusion criteria:

- Carious teeth
- Crazed teeth / Fractured teeth
- Congenital defects
- Previously restored teeth

Sample size estimation:

Sample size calculation was performed using G*Power Version 3.1.9.4. 36 molars were selected using an alpha (α) level of 0.05 with a confidence interval (CI) 95%. The minimum estimated sample size is 10 samples per group.

Sample preparation

All teeth were free of biofilm and blood stains afterward they were kept in distilled water. The 1.5 cm length ×1.5cm width ×2.5cm height dimensional enamel blocks obtained from the labial surface of the tooth sample were mounted in the mold with epoxy resin exposing 3 × 3 mm of the enamel surface (Fig 1). The labial enamel surfaces were wet polished with no. 400, 800, and 1000 Sic paper. Nail varnish was applied all over the samples except the specified window area.

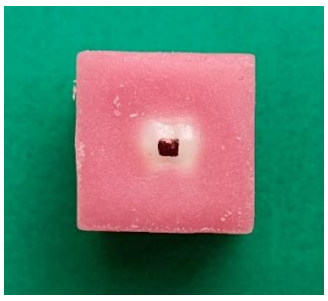


Fig 1: Sample preparation

Formation of White Spot Lesion and pH cycling: Each specimen was placed in a demineralization solution (2.2 mM CaCl2·2H2O, 2.2 mM KH2PO2, 50 mM acetate, pH 4.4) for 96 hr at 37 °C to create lesions (Fig 2)
The specimens were then randomly divided (10 specimens/group) into four treatment groups. (Fig 3)

Group I: Control group where no treatment was performed.
Group II: GC tooth mousse plus was used as a remineralizing agent.
Group III: SDF was applied as a remineralizing agent.
Group IV: Clinpro tooth creme was used as a remineralizing agent.
All samples were immersed in the demineralized solution for 96 hrs at pH 4.4 at 37 °C. After the formation of Enamel white spot lesion specified remineralizing agents were applied. Demineralization was continued for a further 3 hrs to obtain the acidic ph i.e. 4.4. Then the samples were stored on artificial saliva for 2 hrs. The same ph cycles were continued till 10 days.

Fig 2: lesion formation

Fig 3: Sample grouping

Method of preparation of artificial saliva
The artificial saliva used in this present study was prepared according to Macknight-Hane and Whitford's (1992) formula. Sorbitol was not used because artificial saliva would be more viscous than normal saliva when sorbitol is mixed with sodium carboxymethyl cellulose (Levine, et al., 1987). Composition (in grams per litter) – The artificial saliva contained 2.0 g of methyl p-hydroxybenzoate, 10.0 g of sodium carboxymethyl cellulose, 0.625 g of KCl, 0.059 g of MgCl2 .6H2 O, 0.166 g of CaCl2 .2H2 O, 0.804 g of K2 HPO4, and 0.326 g of KH2 PO4 per liter of solution. The pH of artificial saliva was adjusted to 6.75 with KOH.

Post-treatment analysis
After pH cycling, samples were washed with deionized water. All specimens of each group were subjected to microhardness test.

Vickers Microhardness
Surface micro-hardness was measured by a microhardness tester under a load of 100 gm/10s. Three indentations were done on each specimen and the average values were calculated. Then the average values for all groups were compared. (Fig 4)

Fig 4: Microhardness test

STATISTICAL ANALYSIS

The microhardness data was reported as the mean for each experimental group and statistically analyzed via a one-way analysis of variance (ANOVA) in which the mean microhardness of the tooth enamel surface was compared for the three treatment groups

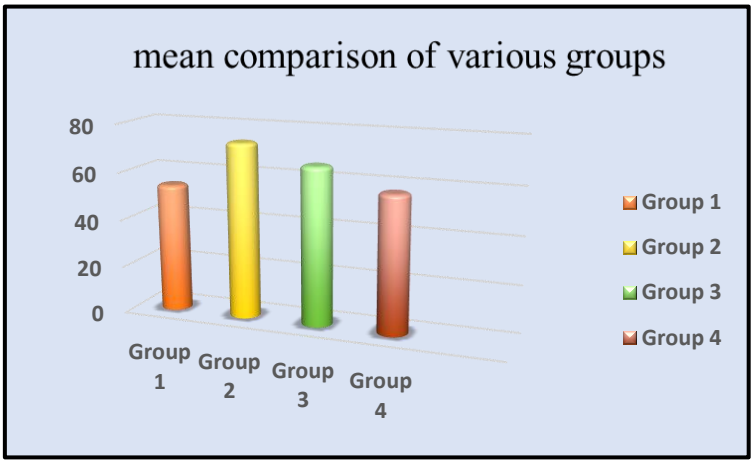
RESULTS

The following observations were made based on microhardness, there was a mean increase in microhardness following remineralization in GC Tooth Mousse Plus (73.4883±3.42888)>SDF (66.0380±4.00293)>Clinpro tooth crème (57.409757±3.78969) compared to the control group (54.0263±2.13244). The increase in microhardness was statistically significant in GC Tooth Mousse plus. The results depicted in Table 1 showed a significant difference between the above-mentioned group (p-value 0.001)

Table 1: Mean comparison of various groups

Sample	Mean	Sd	f-value	p-value
Group 1	54.0263	2.13244	197.63	0.001 (s)
Group 2	73.4883	3.42888		
Group 3	66.0380	4.00293		
Group 4	57.4097	3.78969		

Statistical test: ANOVA; (p<0.05- significant, CI=95%), N= number of study subjects s- Significant, n.s: Significant



DISCUSSION

Remineralization is a novel non-invasive management of early carious lesions, which bridges the traditional gap between preventive and invasive dentistry. The successful management using this technique requires the detection of caries lesions at an early stage and correctly quantifying the degree of mineral loss to ensure correct intervention.[5]

Ideal characteristics of the use of a remineralizing agent are white spot lesions formation or subsurface caries formation. So, in my study, I have used a combination of these materials as my demineralizing solutions.

High Ca and PO4 3- concentrations in the saliva is maintained by various salivary proteins, which may account for remineralization and development of enamel.[6]Darshan and Shashikiran[7] used artificial saliva in their study, and they believed that artificial saliva contributed to a slight increase in microhardness, after demineralization. Hap is the main component of enamel. To simulate conditions within the oral cavity, the pH cycling model was used in various studies. In this present study, the pH-cycling protocol used was given by Ten Cate and Duijsters (1995). first, initial caries formation was induced in the samples by keeping the samples in demineralizing solution for 96 hours at a pH of 4.4. The cariogenic challenge was conducted using the same demineralizing and remineralizing solutions described for inducing initial caries development. Application of the remineralizing agent was made for 2 minutes every day. This cycle was repeated for 10 days. A change in solution was done daily to prevent depletion or saturation of the solution and accumulation of enamel dissolution agents. before each immersion in the demineralization solution, the pastes were applied according to each group. Microhardness measurements can be evaluated by many different parameters like Knoop hardness number and Vickers hardness number, quantitative energy dispersive X-ray analysis. Polarized light microscopy, X-ray diffraction, scanning electron machine, optical coherence tomography, light-induced fluorescence, diagnodent.

Structural equation modeling (SEM) is one of the parameters which is used by many researchers. SEM was not chosen due to its high cost in imaging all the samples. That's why we chose Vicker's Indenter as one of the parameters. Vicker's hardness method was used to evaluate microhardness because it is very reliable, rapid, and economical test compared to hardness tests. The square-shaped indent obtained was easier and more accurate to measure and detect visually and digitally.[8,9]Accurate measurement of SMH by Vickers microhardness test can be made on flat surfaces only. Therefore, the prepared enamel blocks were polished and smoothened using 400, 800, and 1000-grit emery paper to achieve a flat and homogenous surface.[10,11]

The organic content of the primary tooth enamel is higher than that of the permanent tooth making the primary tooth enamel softer and more porous and consequently more susceptible to caries compared to permanent enamel. Zhang Q etal (2011)[12], VeerittaYimcharoen (2011)[13], Mirkarimi M et al (2013)[14] N Agrawal et al (2014)[15]AminabadiNAet al (2015)[16] performed a similar study on primary teeth. The present study compared the change in microhardness following treatment with CPP-ACPF, SDF and f-TCP over 10 days of in vitro pH Cycling. The results showed a statistically significant difference in the microhardness between the control group and the treatment groups at the end of the experimental design. This depicted that with the application of CPP-ACPF, SDF, and f-TCP, the net remineralization process in primary teeth was significantly enhanced in comparison to when no agent is applied.CPP-ACPF is a supersaturated solution of amorphous and crystalline calcium phosphate phases. It has added fluoride content. It is a stabilized composition so that spontaneous precipitation of calcium phosphate is stopped. The remineralizing capacity is directly proportional to the levels of free calcium and phosphate ions that are stabilized by CPP. When CPP-ACPF is applied on the tooth surface, its sticky CPP part readily mixes with enamel and biofilm releasing the calcium and phosphate ions. The free calcium and phosphate ions enter the enamel rods and form the apatite crystals again.[17]Since penetration of fluoride into enamel layer is limited and decreases with the depth, TCP may help to increase the depth of fluoride penetration and remineralization of the lesion as a result. Combining fluoride with TCP could provide more preventive effect than the topical application of fluoride alone. The anticariogenic effect of TCP has the advantage of providing calcium and phosphate in the enamel-surrounding medium, this process is more important when there is an acid challenge around. TCP is a good delivery vehicle that can release calcium and phosphate slowly at the tooth surface. Clinpro tooth Creme contains three main remineralizing elements that are calcium, phosphate and fluoride ions. Calcium and phosphate ions penetrate the surface of enamel and repair the destroyed crystals of hydroxyapatite. Fluoride ions accelerate the penetration of previous ions and permeate into hydroxyapatite crystals to form fluorapatite crystals that are more stable and resistant to acids.[18,19]In the present study, GC Tooth Mousse Plus exhibited more remineralizing potential in comparison to SDF. This could be because the effect of SDF is more pronounced on dentinal carious lesions. The silver in SDF inhibits the dentin collagenase, cathepsin B and K that help mediate the degradation of collagen during a caries attack. Silver ions have an affinity for proteins due to their large ionic radius, adding to their strong inhibitory effect on matrix metalloproteins and cathepsins. Since the protein content, carbonates, and phosphates in enamel are much less in comparison to dentin, SDF does not have this protective effect on enamel.[20,21]In the present study, GC Tooth Mousse plus group showed a statistically significant increase in microhardness. The results were following the studies conducted by AykutYetkiner et al (2014)[22]. Rallan et al. (2013)[23] had suggested that combining fluoride along with CPP-ACP can have a synergistic effect on enamel remineralization. Conversely, Vanichvatana and Auychai (2013)[24] reported that f-TCP provided similar benefits as the F toothpaste; however, no additional benefit of GC Tooth Mousse plus was observed.

Limitation of study

The oral cavity is a complex entity and several factors influence the remineralization and demineralization cycles in the oral environment. Although the pH cycling regime was used in the present study to best simulate the oral environment, it is difficult to attain the oral conditions in vitro. It is also not possible to adequately simulate the topical use and clearance of the remineralizing agents from the oral cavity under in vitro conditions. Hence, conditions similar to the oral environment may not have been reproduced which might have affected the results. Moreover, the sample size in the present study was limited. Hence, studies with larger sample sizes are needed to further compare the remineralizing effect on primary teeth. The period of remineralization used in the study was 10 days, which could not remineralize artificial caries completely. Although surface remineralization was confirmed, enamel subsurface remineralization was not evaluated in the study. Thus, direct extrapolations to clinical conditions must be exercised with caution because of the obvious limitations of in vitro studies.

CONCLUSION

- In the current study, it was observed that CPP-ACPF showed comparatively more remineralization potential than f-TCP and SDF when applied to treating white spot lesions.
- Casein phosphopeptide amorphous calcium phosphate (CPP-ACPF) can be suggested as an effective material for clinical use in minimally invasive dentistry due to its potential remineralization properties and also due to easy availability and economical nature.

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