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Original Research

Evaluation of the remineralizing efficacy of GC Tooth Mousse Plus and Enafix using surface microhardness and surface roughness analysis in deciduous molars: an in vitro study

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ABSTRACT

Aim: To compare and evaluate using surface microhardness measurement, and surface roughness analysis in deciduous molars using GC Tooth Mousse Plus and Enafix.

Material and methodology: A four-equal window acid resistant varnish was placed around the exposed enamel surface of forty removed deciduous molars before they were submerged in a demineralizing solution and remineralized for weeks using Tooth Mousse Plus and Enafix.

Statistical Analysis Used: One Way ANOVA and Post Hoc Turkey test ($P<0.05$) were used

Results: Following 4 weeks of remineralization, all specimens underwent SEM analysis and revealed indications of thickening of their inter-rod material. Tooth Mousse Plus also revealed pronounced remineralization evidence. Enafix demonstrated greater resistance to breakdown during the last acid exposure. Surface micro hardness (SMH) and surface roughness (Ra) both showed comparable results, with a noticeable decrease in roughness values and an increase in microhardness values, while Enafix showed a greater source of remineralization and Tooth Mousse Plus showed a greater resistance to the final acid challenge.

Conclusion: Enafix has demonstrated superior resistance to the final acid trial, whereas superior remineralization property was seen in GC Tooth Mousse Plus.

Key words: *Remineralising agent, Surface Hardness, Tooth Mousse Plus, Enafix*

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INTRODUCTION

Teeth structure, dental biofilm, nutritional factors, and salivary factors combine intricately to cause dental caries, which is a multifactorial illness. This is due to bacterial acid production as well as the buffering effects of oral cavity constituents. Therefore, the tooth surface is in a dynamic balance with the oral environment around it being extremely important. The earliest clinical sign of dental caries of the enamel is a so-called "white spot lesion," which is a tiny region of sub-surface demineralization, located under the tooth plaque. Due to a shift in refractive index from that of the transparent enamel around it, the lesion looks white. In many cases, sub-surface lesion retains "apparently intact surface layer" above it despite having lost up to 50% of its original mineral content.[1] Every time a carbohydrate is consumed and the bacteria in the mouth break it down, the demineralization process continues. Among its many functions, saliva buffers (neutralises) the acidic pH and supplies mineral deposits that can trade those that are lost during a demineralization from tooth. Remineralization is the process of replacing minerals. Remineralization is the process by which lost ions of calcium and phosphate are delivered to the tooth to facilitate deposition again. Enamel, dentine, or cementum get demineralized when the pH falls below a threshold level, while these materials become remineralized when the pH rises. Therefore, dental caries is a long-term, diet-related, microbiological, site-specific illness brought on by changes from variables protecting teeth that favour tooth remineralization to factors damaging teeth that bring about the demineralization process. During the day, the process of demineralization and remineralization occurs more often. This procedure eventually results in either the healing and reversal of a lesion or caries lesions.[2] One of the most well-known remineralizing agents is fluoride, which interacts with oral fluids on the enamel-dentine interface and in subsurface areas of teeth before forming fluorapatite by joining calcium and phosphate ions.[3] A milk product called CPP-ACP aids in remineralization and guards against tooth cavities. Casein phosphopeptide can be used to supply amorphous calcium phosphate and can help the ACP adhere to tooth structure. Streptococcus Mutans can be significantly decreased by casein phosphopeptide because it may combine with the pellicle. At Melbourne, Eric Reynolds and associates developed chewing gums and a tooth cream called GC TOOTH MOUSSE PLUS™ that contains fluoride at a concentration of 900 ppm (GC Tooth Mousse Plus TM).[4] With the introduction of ENAFIX (Group Pharmaceuticals) as a remineralizing agent in the Indian market, a fresh method of remineralization without fluoride that incorporated calcium sucrose phosphate (CaSP) entered the market. This substance typically breaks down into calcium, phosphate, and sucrose ions, which accelerates the process of remineralization.[5]

Only a small number of studies have evaluated the effects of different calcium and phosphate delivery methods, as well as fluoride ions of different compositions, on the ability of developing carious lesions to remineralize. In order to compare the impact of various calcium-phosphorous and fluoride administration techniques on enamel remineralization, this study was carried out utilising surface micro-hardness and surface roughness methodologies.

MATERIALS & METHODS

This study protocol was approved by the Institutional Review Board (IEC No: MCDRC/2021/OCT/421) and this randomized controlled in vitro trial was conducted at Department of Pedodontics and Preventive Dentistry, Maitri College of Dentistry and Research Centre.

Extracted first and second human primary molars with no developmental defects, caries, white spot lesion or those subjected to previous treatment were included in the study.

***Preparation of specimen*:**

For two weeks, sixty extracted human mandibular molars that had no clinically obvious abnormalities were kept in an aqueous solution of saturated thymol. The teeth were carefully cleaned before being inspected with a stereomicroscope (made by Nikon, Japan) to look for any teeth that had apparent stains, erosions, or microcracks on their enamel surfaces. Self-cured acrylic resin was poured into each of the specially constructed, 1 inch by 1 inch plastic cylinder moulds. The mandibular molar section's buccal were placed horizontally in acrylic resin and allowed to dry overnight. On the buccal surface of the teeth, an acid-resistant nail varnish was applied using an applicator tip, leaving four equal portions of wax sheet that are roughly (2 mm x 2 mm) each.

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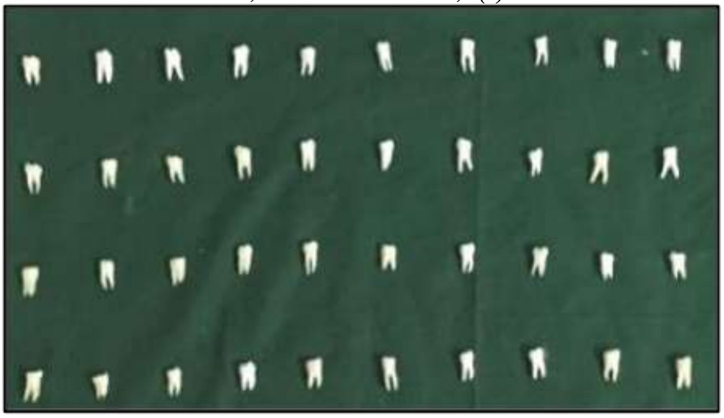

Figure 1 shows extracted deciduous teeth

***The demineralization process*:** The solution of pH 4 was created using MKOH, $CaCl_2$ $2H_2O$, KH_2PO_4, and acetic acid. A homogeneous white spot lesion was produced on the window's surface after 5 days of soaking the mounted teeth in this solution.

Figure 2 shows samples immersed in demineralizing solution

***Process of Remineralization*:** Until use, all samples were kept in deionized water. Sixty samples of enamel entrenched in acrylic block were created in total, and the samples were split randomly in two groups of thirty samples each for further remineralization.All specimens were sorted into the two groups below at random:

GROUP A: Tooth Mousse Plus (GC INDIA) – Caesin Phosphopeptide Amorphous Calcium Phosphate containing 900ppm of Fluoride.

GROUP B: Enafix (GROUP PHARMACEUTICALS) – Calcium Sucrose Phosphate and amorphous calcium phosphate.

Figure 3 shows group A Tooth Mousse plus and **Figure 4** group B Enafix remineralizing agent

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The samples in each group were treated with the respective remineralizing agent that were continuously applied onto the remaining 3 windows of the tooth surface with the help of a disposable cotton applicator tip for 3 minutes once in every 24 hours for a period of 4weeks. Samples after application of the remineralizing agents were then washed with deionized water and then placed in artificial saliva, that was changed once in every 24 hours until the final acid challenge. Remineralization process was continued for a period of 2 weeks and the second window was coated with acid resistant nail varnish, the remaining two windows were continued remineralization and the third window was closed after a period of 4 weeks. After these times, the samples were once more submerged for five days in the demineralizing solution previously described in order to test the treated surfaces' acid resistance. The fourth window was then painted with acid-resistant nail polish.

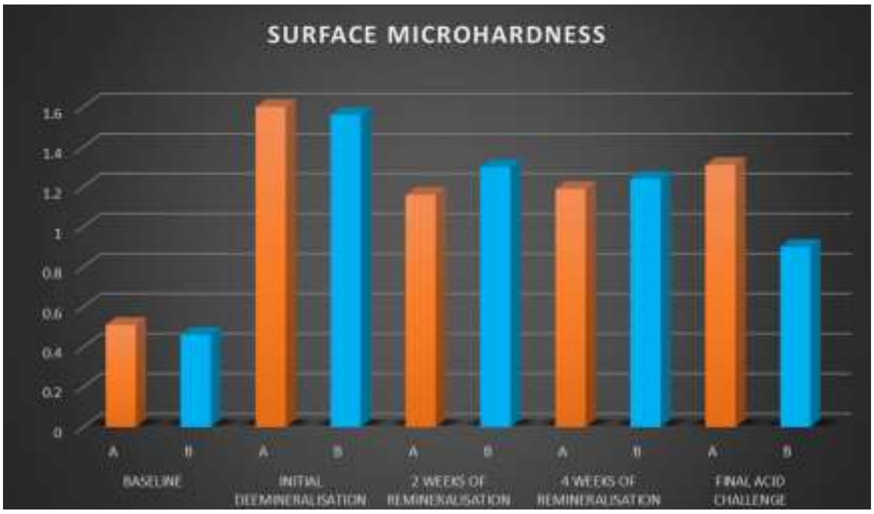
Figure 5 shows samples after application of remineralising agent; sample again were immersed in demineralising solution.

The 30 samples from each group were then split into three groups and put to the test using the techniques listed below: Vickers elongated diamond pyramid indenter with a 100 g load was used to measure the surface micro-hardness on 15 samples at baseline, after demineralization, and after remineralization utilising the Mityuto SJ 410 Contact Profilometer.

STATISTICAL ANALYSIS

The data were subjected to a one-way ANOVA [$P<0.05$] after analysing the surface microhardness and surface roughness of both groups. Intergroup assessment was done using Post hoc test.

RESULTS



Graph 1: Surface Microhardness

In Graph 1, a visual depiction of the mean SMH values for all the surfaces treated at various enamel treatment sites is shown. At the outset, SMH was considerably higher in all groups (p 0.05). All of the specimens' demineralized enamel surfaces had a substantial drop in SMH values (p 0.05). After 2 and 4 weeks of using the various remineralization regimens, the SMH values of all treated groups showed a noticeable significance in the SMH values.

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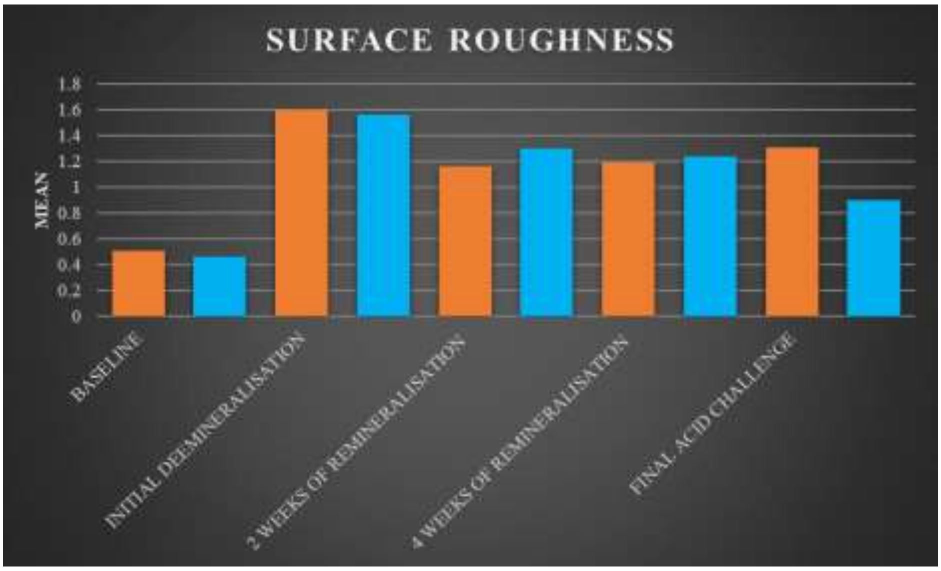
The SMH values for both groups significantly decreased, indicating mineral loss as a result of the acid exposure. SMH values in Group A were much higher than those in Group B early on in the two-week remineralization process, which has been shown in Graph 1. The greatest SMH value was discovered in Group A even after 4 weeks of remineralization. The findings between Group B were also not statistically significant following the 4 weeks of remineralization ($p > 0.05$). Following the acid test, Group B had statistically significantly higher SMH values than did Group A.



Graph 2: Surface Roughness

In Graph 2, the mean Ra values of surfaces at various enamel treatment sites are represented graphically. The mean Ra levels of the samples were low at the baseline measurement. All groups examined showed a substantial rise in Ra levels following demineralization (p 0.05). All experimental groups demonstrated a substantial decrease in their individual Ra levels after two weeks of remineralization. Ra readings in Group A were substantially lower than those in Group B following the first two weeks of remineralization. Further considerable reductions in the Ra levels were evident in Groups A and B after 4 weeks of remineralization. Comparing Group B and Group A's Ra readings after 4 weeks of remineralization revealed an interesting difference ($p > 0.05$). When compared to 4 weeks of remineralization, all examined groups after the acid challenge exhibited a considerable rise in Ra levels. Following exposure to the acid test, Group B displayed the lowest Ra levels that were statistically significant, followed by Group A. It was clear from comparing the remineralizing agents tested at various time points that Group B showed considerably lower Ra levels after two weeks of remineralization, while they also showed significantly lower Ra values after being exposed to acid challenge.

DISCUSSION

Histologically speaking, subsurface carious lesions of the enamel are what are known as early enamel caries, also known as White Spot Lesions (WSL) or Incipient Lesions. a prominent demineralized zone under the enamel surface that is both intact and unharmed. When compared to healthy enamel, the surface may be intact but the mineral content may be lacking. Ca2+, OH, PO4, 2, F, CO3, Na+, and Mg2+ are moved from the enamel surface to the outside during the demineralization process. The outflow of ions increases with increasing acidity of the environment. The surface of the lesion has a larger mineral concentration than the lesion's body, though.[6]

The goal of the current investigation was to examine the effectiveness of four remineralizing regimens on enamel lesions created to mimic caries in terms of changes to surface microhardness and surface roughness. The samples were then kept in the demineralizing solution for a total of 5 days, following the Gangrade A et al. (2018)[7] methodology, until a consistent white spot lesion was produced on the surface.

Micro-hardness testing is thought to be a reasonably easy and accurate approach for giving indirect information on changes in the mineral content of hard dental tissues.[8] Micro-hardness testing is thought to be a reasonably easy and accurate approach for giving indirect information on changes in the mineral content of hard dental tissues.[9]

When in contact with saliva, the ACP and fluoride compounds in Tooth Mousse Plus quickly solubilize to

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calcium, phosphoric, and fluoride ions, generating a supersaturated calcium, phosphate, and fluoride state surrounding the tooth enamel.[10]ACP is stabilised by CPP, which is a peptide derived from casein, in the CPP-ACPF technology. Casein phosphopeptide (CPP) have the amino acid cluster sequence Ser(P)-Ser(P)-Ser(P)-Glu-Glu, and it has been observed that these amino acids bind calcium phosphate to create tiny groups of casein phosphopeptide (CPP-ACP). This aids in keeping these clusters from growing large enough to precipitate, stabilising calcium phosphates in solution nearby the surface of teeth, and facilitating its accessibility whenever required. When these nanocomplexes are applied to the tooth structure and dental plaque, they serve as calcium and phosphate reservoirs.[11] To prevent the calcium and phosphorus components from interacting with one another prior to usage, this method uses a two-phase delivery mechanism. Two salts, calcium sulphate and dipotassium phosphate, make up the supply of calcium and phosphates. These two salts quickly combine to create CPP-ACPF, which precipitates on tooth surface. For the purpose of remineralizing the tooth surface, the precipitated component easily dissolves into saliva.[12]

Calcium salts of sucrose and phosphate esters combined with inorganic calcium make up CaSP (Calcium Sucrose Phosphate) ions are easily broken down and released into the saliva. Its weight percentages of calcium and phosphorus are 10%–12% and 8%–10%, respectively. In aqueous media, calcium and phosphate ions produce an insoluble precipitate. Without precipitation, CaSP creates an aqueous solution with a high calcium and phosphate content. For the calcium and phosphate in water, it serves as the perfect transporter. Enafix works by quickly adhering sucrose phosphate ions to the enamel surface, slowing the rate at which hydroxyapatite dissolves in acids, and hastening the remineralization of calcium and phosphate ions through the common ion effect.[13]

The amount of enamel that is remineralized, the amount of crystal gaps that are repaired, and the amount of a restored crystalline phase that is produced all depend on the amount of bioavailable fluoride, calcium, and phosphate ions. Until now, numerous studies revealed that the bioavailability of active fluoride ions is essential for promoting remineralization and preventing demineralization in conditions when there is a high concentration of calcium and phosphate.[14]

At acidic environment and at neutral pH levels, enamel solubility decreases, and remineralization rates increase as a result of calcium and phosphate ions' rapid adhesion to the enamel. The adsorbed sucrose phosphate anions that cling to the tooth's surface also slow the pace at which acid dissolves. This inorganic fluoride has some disadvantages when compared to the organic fluoride in Enafix, prominent among them the propensity to react with the hydroxyapatite on the surface enamel and generate a thick layer of calcium fluoride. And because a thick coating of calcium fluoride has developed, the fluoride ions are less bioavailable. However, they came to the conclusion that even though Amflor and Enafix did not show statistically significant differences from the control group, they still work to prevent enamel demineralization and are also reasonably priced.[15]

Results of our study are in accordance with studies conducted by Sharma S et al. (2017)[16], Kakkar S et al. (2018)[15], Tashmasbi S et al. (2016)[17] and results of study by Thakur S et al. (2015)[18] didn't coincide with our study.

CONCLUSION

When compared to artificial saliva, remineralizing agents made of calcium phosphate have superior remineralization effects, increasing surface hardness, decreasing surface roughness, and increasing resistance to acid softening. All treated surfaces also preserved calcified deposits. Enafix can be utilised as an option for improved remineralization, particularly for the Indian population, as it is more affordable than GC Tooth Mousse Plus.

ABBREVATIONS

1. WSL: White Spot Lesions
2. CPP-ACP: Casein phosphopeptide
3. CaSP: Calcium Sucrose Phosphate

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CONFLICTS OF INTEREST - There are no conflicts of interest

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