Enamel remineralization efficacy of three widely used remineralizing tooth pastes: An in-vitro study

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Abstract

Background: With introduction of newer enamel remineralization products in the market, it becomes important to evaluate and compare their remineralization efficacy. Hence the objective of the study was to assess and compare the enamel remineralization efficacy of Casein phosphopeptide amorphous calcium phosphate cream, Nano-hydroxyapatite containing paste and Xylitol containing paste on artificially induced enamel carious lesions using surface micro hardness analysis.

Methods: In-vitro study was conducted using 50 enamel specimens, randomly allocated to 5 groups of 10 samples each after standardization of Surface micro hardnes. Except Group 1, specimens were subjected to demineralization for 4 days at 37°C following remineralization with slurry of remineralizing agents; Tooth Mousse Plus, nanoHAP and Remin Pro. Specimens were subjected to pH cycling for 28 days followed by assessment of surface micro hardness (PISMH). Student’s paired t-test, One-way analysis of variance test (ANOVA) and post hoc Tukey test were used for statistical analysis.

Results: The surface harness of Tooth Mousse plus (333.62±38.95VHN) and Remin Pro (342.24±27) groups were significantly higher than the sound enamel group, nanoHAP group (275.97±67) and demineralized enamel group (p<0.05). There was a significant rise in micro hardnes of enamel post intervention in Remin Pro group (p<0.0001) and Tooth Mousse group (p<0.05). Mean surface hardness of Remin Pro group was found to be more than GC Tooth Mousse plus group (p>0.05). Conclusion: Tooth Mousse plus and Remin Pro showed promising enamel remineralizing potential.

Keywords: Casein phosphopeptide, amorphous calcium phosphate, fluoride, nanohydroxyapatite, enamel remineralization, xylitol

1. Introduction

Dental caries is a multifactorial disease spread across populations regardless of gender, age and ethnicity [1]. It is the localized destruction of tooth tissue by specific dental plaque bacteria that ferment dietary sugar to organic acid [2]. One of the early stages in the formation of carious lesion involves the demineralization of hydroxyapatite crystals in the enamel. The process of de- and remineralization is governed by the degree of saturation of oral fluids (saliva and plaque) with respect to apatite minerals. Given an appropriate change in conditions, remineralization may become the predominant process, thus leading to lesion repair. Therefore, an agent that can reduce the rate of dissolution of hydroxyapatite when the teeth are subjected to demineralization may have some inhibiting effect on caries formation [3].

Dentifrices containing fluoride have been demonstrated to reduce caries experience significantly in randomized, controlled clinical trials [4]. The efficacy of these dentifrices have been attributed to their ability to incorporate fluoride ions into plaque and enamel. Several researchers have suggested an inverse relationship between plaque fluoride levels and caries incidence [3]. Toothpastes containing casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP), with or without fluoride, have shown potential to prevent enamel demineralization and increase remineralization in vitro and in-vivo studies [6]. Casein Phosphopeptide-Amorphous Calcium Phosphate with 900ppm fluoride (Tooth Mousse Plus - MI Paste Plus; GC Corporation, Tokyo, Japan) cream is being widely used as a remineralization agent. The CPP-ACP has also been shown to remineralize enamel subsurface lesions in situ when delivered in oral care products [2]. Nano-hydroxyapatite (nanoHAP) is considered one of the most biocompatible and bioactive materials.
Evidence has demonstrated that nano-sized particles have similar morphology and crystal structure compared with dental apatite. It contains nano-structured calcium phosphate, organized in a crystalline form of hydroxyapatite-mineral that constitute the tooth structure. Recently, reports have shown that nanoHAP has potential to remineralize enamel carious lesions when added to tooth pastes and mouth washes [6, 7]. Nano HAP paste (Desensibilize Nano P, FGM, Joinville, Santa Catarina, Brazil) acts as a promising source of free calcium providing protection against caries and dental erosion [8]. Remin Pro remineralizing paste (VOCO GmbH, Cuxhaven, Germany) contains hydroxyapatite along with fluoride and xylitol [9]. Hydroxyapatite fills eroded enamel, fluoride seals dentinal tubules and xylitol acts as an antibacterial agent [10]. It is claimed to prevent demineralization and erosion of tooth structure. It aids in neutralization of acids in plaque, thereby balances oral microflora [11]. In Remin Pro, the hydroxyapatite helps to fill superficial enamel lesions and the tiniest irregularities, and fluoride (1450ppm) prevents acid attack, thereby help in remineralization [12].

Literature search revealed very few studies which have compared the remineralizing effects of Nano-HAP and Remin Pro pastes. Considering the importance of the surface layer in caries progression, the evaluation of changes in this region following remineralisation with various remineralizing agents is relevant. Surface micro hardness (SMH) measurement is a suitable technique for this purpose. Surface micro hardness indentation provides a relatively simple, non-destructive and rapid method in demineralization and remineralization studies [13]. Hence a study was planned with the aim of assessing and comparing the remineralizing effect of Tooth Mousse Plus, nano-HAP and Remin Pro pastes on artificially induced enamel carious lesions using surface micro hardness analysis. Study tested the hypothesis that there is a difference in enamel remineralization effect of these three agents on artificially induced carious lesions.

2. Methods

Study design was in –vitro, randomized controlled trial with parallel group design. Freshly extracted human molar teeth were selected which were non-caries, devoid of any restorations, fractures, hypoplasia, abrasion and erosion signs. The probability of type I error was fixed at 5%, type II error was fixed at 20%. power of the study was 80%. d was fixed based on the significant difference between the Nano phosphate group and CPP ACP groups observed in the study done by Carvalho FG et al. [14]. The formula used for determining the sample size was as shown in Eq. 1.

\[ n = \frac{2(Z_\alpha + Z_\beta)^2 \times \text{SD}^2}{d^2} \]

Where, \( Z_\alpha = 1.96, Z_\beta = 0.84, \text{SD} = 6, \) Pooled Variance (\( S_d \)) = 36, \( d = 10. \) Substituting the values in the formula, the sample size obtained was 6 in each group. Anticipating any damage to specimens during the processing cycle, 10 specimens were considered in each group with 50 in total.

2.1 Storage of extracted teeth and infection control

Freshly extracted molar teeth were collected and cleansed of visible blood and gross debris and maintained in a hydrated state in sodium hypochlorite solution diluted with saline in the ratio of 1:10 in a container which was sealed and labelled. Elimination of microbial growth was achieved by using an autoclave cycle for 40 min.

2.2 Preparation of enamel samples

Teeth were sectioned 1mm below the Cemento-Enamel Junction (CEJ) with a slow speed diamond disc (NSK micro motor handpiece). Roots were discarded and the crowns were used for the study. The crowns were sectioned mesio-distally into buccal and lingual halves using a diamond disc bur (NSK micro motor handpiece) under cool water spray. The sectioned enamel samples were mounted on acrylic blocks. The mounted enamel sections were flattened and polished using a series of silicon carbide grit papers (200, 400, 600, 800, 1000 and 1200 grits). Following polishing of the samples, a 4x4mm working window was marked on the sectioned enamel surfaces of all the samples using adhesive tape. The area of the crown other than the working window was covered with nail varnish (Colorama nail varnish, Maybellene) making it resistant to acid attack.

2.3 Baseline Surface Micro Hardness assessment

The baseline surface micro hardness of the specimens was determined using digital micro hardness tester (Reichert 3637- Austria) with a Vickers elongated diamond pyramid indenter and an X40 objective lens. A load of 50 grams was applied to the surface in the 4x4 working window for 10 seconds. Two indentations were placed on the surface and the average value was considered. Precision microscopes of magnification of X400 were used to measure the indentations. The diagonal length of the indentation was measured by built in scaled microscope and Vickers Hardness values (VHN) were converted to micro hardness values.

2.4 Standardization and random allocation of enamel samples to five groups

After baseline hardness test, the enamel hardness was standardized to a range between 219 and 350 VHN. Specimens whose values were within standardized range were considered and others were discarded. Fifty samples selected were labeled and randomly allocated to five groups with ten samples in each group by a person not involved in the study. Random numbers were generated utilizing online software.

Group 1: Enamel specimens were kept in deionized water only.

Group 2: Enamel specimens were demineralized and treated with slurry of Tooth Mousse Plus (MI Paste Plus; GC Corporation, Tokyo, Japan).

Group 3: Enamel specimens were demineralized and treated with slurry of nano-HAP paste (Desensibilize Nano P, FGM, Joinville, Santa Catarina, Brazil).

Group 4: Enamel specimens were demineralized and treated with slurry of Remin Pro paste (VOCO GmbH, Cuxhaven, Germany).

Group 5: Enamel specimens were demineralized and kept in deionized water.

2.5 Artificial Caries Lesion Preparation

Each of the enamel samples (except group 1) were immersed in 40 ml of demineralizing solution (2.2mM calcium chloride, 2.2mM sodium phosphate, and 0.05 M acetic acid; pH adjusted with 1M potassium hydroxide to 4.4), for a period of 4 days at a constant temperature of 37°C, in an incubator to induce artificial caries formation, simulating an active area of demineralization.

2.6 Slurry preparation of CPP-ACPF, Nano-Hap and Remin Pro

Slurry of Tooth Mousse Plus, nano-HAP and Remin Pro were prepared by suspending 12g of respective agent in 36ml of deionized water to create a 1:3 dilution. The suspensions were
thoroughly stirred with a stirring rod and mechanically agitated by means of vortex mixer for 1 minute. The suspensions were centrifuged at 3500 rpm for 20 minutes at room temperature, once daily before starting the pH-cycling.

2.7 Method of pH –Cycling
Each of the enamel samples were treated with respective treatment slurries for 1 minute. After the first treatment, samples were rinsed with deionized water followed by immersion in 40 ml of remineralizing solution for 6 hours. After demineralization, the samples were rinsed in deionized water, and were treated again with treatment slurries for one minute followed by rinsing with deionized water next the enamel samples were immersed in 20mL of remineralizing solution for 16 hours. The remineralizing solution was replaced for every 48 hours and demineralizing solution for every 5 days. The entire cycling procedure was carried out for 28 days.

2.8 Post Intervention Surface Micro hardness assessment
PISMH was assessed after 28 days using Vickers micro hardness tester (Reichert 3637-Austria).

2.9 Statistical Analyses
Statistical analyses was performed using Statistical Package for Social Sciences software (SPSS version 20.0). Data was normally distributed.

Student’s paired t-test and One way Analysis of variance followed by post hoc Tukey’s test were used for data analysis. Significance level was fixed at p ≤ 0.05

3. Results
At baseline, there was no statistically significant difference in microhardness values of enamel specimens observed between the five interventional groups (p=0.546) which allowed for valid comparisons between groups post intervention. Significant difference (p<0.0001) in surface hardness was observed between five interventional groups post intervention. (Table 1). Mean surface hardness of MI Paste plus and Remin Pro groups was significantly higher than the sound enamel group, nano HAP group and demineralized enamel group (p<0.05).

No significant difference was observed between Remin Pro and MI paste plus groups. (p>0.05) There was significant rise in micro hardness values (VHN) of enamel post intervention in Remin Pro group (p=0.0001) and MI Paste plus group (p=0.019). There was significant reduction (p<0.05) in the micro hardness levels (VHN) post intervention in Nano-HAP group and demineralized enamel group (Table 1).

4. Discussion
Considering the limited research on comparison of remineralization efficacy of Nano HAP (Desensibilize Nano P, FGM, Brazil) and Remin Pro (VOCO GmbH, Cuxhaven, Germany) pastes, the present study was planned with the aim of assessing and comparing the remineralizing effect of MI Paste plus, Nano HAP and Remin Pro pastes on artificially induced enamel lesions using surface micro hardness analysis. Results revealed that, enamel surface microhardness in MI Paste Plus group specimens was significantly higher compared to Nano-HAP group. Contrary to this finding in a study conducted by Carvalho F G et al., Nano HAP group showed higher surface hardness compared to CPP-ACPF group in which Nano HAP paste was directly applied to the enamel samples [13]. No significant difference in remineralization potential was seen in study conducted by Livia P et al. between Nano HAP and MI Paste plus groups [10]. The low SMH of Nano HAP group may perhaps be attributed to the physical form of paste which is dispensed through syringe which demonstrated poor solubility in deionized water during slurry preparation. In a previous study the experimental Nano HAP paste presented low calcium and phosphate content in water [6]. Perhaps, experimental Nano HAP paste could not react appropriately with the enamel samples while Remin Pro is a water-based cream in which the hydroxyapatite fills the superficial enamel lesions that arise from demineralization [10]. The mean surface hardness of MI Paste plus group specimens was higher compared to sound enamel and demineralized enamel group. Contrary to this, in a study conducted by S Shetty et al., surface hardness of sound enamel group was found to be higher compared to other two interventions [17]. MI Paste plus group demonstrated significant remineralization efficacy. Few studies have shown similar results [13, 16]. Perhaps casein phosphopeptide incorporated in CPP-ACPF easily bonds to the biofilm and saturates calcium and phosphorus ions exactly at the required spot. These ions penetrate into enamel crystals and increase the density of hydroxyapatite crystals. Remin Pro group showed significant remineralization efficacy. Similar results were observed in few studies [9, 16]. Remin Pro contains hydroxyapatite particles similar to calcium and phosphate ions in CPP-ACPF that are deposited on demineralized enamel thus increasing the surface micro hardness of enamel.

In the Nano HAP group, significant difference in mean

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Table 1: Comparison of enamel surface hardness across interventional groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline surface hardness (VHN) (Mean ± SD)</th>
<th>Post intervention Surface hardness (VHN) (Mean ± SD)</th>
<th>t test value (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound enamel</td>
<td>257.9±40.7</td>
<td>264.2±40.2</td>
<td><del>394</del></td>
</tr>
<tr>
<td>GC Tooth Mousse group</td>
<td>271.4±34.01</td>
<td>333.6±38.9</td>
<td><del>394</del></td>
</tr>
<tr>
<td>Nano HAP group</td>
<td>286.5±40</td>
<td>275.97±67</td>
<td><del>394</del></td>
</tr>
<tr>
<td>Remin Pro group</td>
<td>267.5±32.82</td>
<td>342.24±27.8</td>
<td><del>394</del></td>
</tr>
<tr>
<td>Demineralized enamel</td>
<td>276.1±41.06</td>
<td>210.8±16.88</td>
<td><del>394</del></td>
</tr>
<tr>
<td>One-way Anova Value (P Value)</td>
<td>0.776 (0.54)</td>
<td>16.754 (0.0001)</td>
<td><del>394</del></td>
</tr>
</tbody>
</table>

VHN-Vickers Hardness number, *statistically significant,
Similar alphabets represent significant difference between groups with post Hoc Tukey’s test
surface micro hardness was not evident post-intervention from baseline. This result is in line with results of study done by Livia LP Comar et al. [19] Contrary findings were seen in the studies conducted by decarvalho FG et al. [14] Huang et al. [19] Ithagarun et al. [20] where Nano HAP group exhibited significant remineralizing effect.

There was decrease in mean SMH from baseline to post pH cycling in demineralized enamel group (p<0.01) which was similar to result of study conducted by Lata S et al. [13] The deionized water (negative control) which was used as a vehicle to carry interventional pastes did not produce any remineralizing action on the samples. This result validates the efficacy of demineralizing solution and deionized solution used.

Considering the importance of the surface layer in caries progression, the evaluation of changes in this region was relevant. Surface micro hardness (SMH) measurement is a suitable technique for this purpose. Surface micro hardness indentation provides a relatively simple, non-destructive and rapid method in demineralization and remineralization studies [13]. The surface micro hardness of enamel can be measured by many techniques including Spherical, Knoop or Vickers indenters. In the present study, Vickers hardness test was employed. The test is suitable for determining the hardness of very brittle materials, such as tooth structure [21]. Various composition of demineralizing solutions are available in the vast literature available. Majority of them are composed of calcium and phosphate with acetic acid/ lactate. The main variation lies in concentrations of each component, which influences the final pH and the sample exposure time. The pH employed varies from 3.5 to 5 and the time differs from 2 hours to 21 days [22]. In this study, demineralization similar to enamel subsurface lesion was sought. An intermediate pH 4.4 was therefore employed for 4 days as done in a study by S.Shetty et al. [17] The composition of the solution was similar to the one employed by Tencate J.M et al. [23] In spite of the progress of in situ and in vivo research in cariology, lab tests are still widely used to evaluate dental caries, mainly the effect of fluoride on inhibition of enamel-dentin demineralization and enhancement of remineralization. Among these protocols, there is consensus that pH cycling models may be used because they mimic caries development in vivo [24]. For the effects of caries preventive substances to be studied, de and remineralization can best be examined with a pH cycling system in which the pH depressions occurring in the oral environment are mimicked in a lab model [25]. Hence, pH cycling model was adopted to simulate the dynamic process of demineralization and remineralization that occurs in the oral cavity and determine the effects of three remineralizing agents in comparison to deionized water (negative control). pH cycling can be done for 7, 10, 14 and 28 days [26]. Hence, 28 days cycle was considered in the present study to observe the long-term effects. Limitation of the study includes simulated oral cavity conditions. Remineralization in vitro may be quite different when compared to dynamic complex biological system which usually occurs in oral cavity. Scanning electron microscopy and micro radiographic assessment of enamel samples can explain the pattern of deposition of the remineralizing agent on demineralized enamel. However, in our study, we could not employ such assessments as the specimens had to remain intact before demineralization and after the intervention since they were used as controls also. The present study design did not take into account all oral factors; like the complexity of tooth-pellicle-plaque-saliva interface which was not simulated. Lack of bacteria in the demineralizing solution used, absence of saliva also adds to the limitations. Hence, further studies are needed to explore the remineralizing potential of these agents in f conditions where, enamel slabs incorporated into oral appliances can be utilized to compare the efficacy of different remineralizing agents in oral environment.

5. Conclusion
MI Paste Plus and Remin Pro showed promising enamel remineralizing potential.

6. References


