In Vitro Assessment of Two Agents for Their Ability to Remineralize Human Enamel

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Abstract

Background: The continuous remineralization demineralization taking place in the oral cavity results in incipient caries which can be reversed back to normal with the use of remineralizing agents. Recent cariology research led to application of remineralizing agents on demineralized areas of teeth fills in the microspores and helps in stopping mineral loss.

Aim: This study was carried out to assess the remineralization potential of two different commercially available agents using Vickers Microhardness (VMH) Test.

Method and Material: Enamel specimens were prepared from forty caries-free premolars and were kept for demineralization for 4 days. Following demineralization, enamel specimens were assigned to four groups: (1) Control group, (2) GC Tooth Mousse, (3) SHY-NM and (4) Combination of the two. Enamel specimen of the experimental groups were then subjected to Vickers Microhardness Testing to check remineralization potential

Statistical Analysis: One way ANOVA and Tukeys Post hoc test were undertaken.

Results: The GC Tooth Mousse group gave highest remineralization followed by Combination, Novamin and Control.

Keywords: Casein phosphopeptide-amorphous calcium fluoride phosphate, Demineralization, Novamin, Remineralization, Vickers microhardness testing

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Introduction: Chalky white enamel defects represent initial visible signs of demineralized enamel, typically restricted to the outer enamel layer. At this stage, the enamel surface remains intact without any visible structural breakdown. However, continued mineral loss without intervention can eventually result in cavitation.[1]

The demineralization–remineralization (DEM–REM) cycle is a continuous and dynamic process heavily impacted by various elements, including salivary pH. Oral fluid contributes to oral health by neutralizing acids in dental plaque and supplying essential minerals near the enamel surface. However, the natural remineralization capacity of saliva is relatively limited—it proceeds slowly, often fails to restore a total mineral gain, and minimally effects the cosmetic and configural recovery of extended enamel defects.[1]

Even though fluoride continues to be most effective entity for enhancing remineralization, alternative calcium–phosphate-based compounds are increasingly utilized in clinical practice. Products with (CPP–ACPF) and bioactive materials like NovaMin have shown promise in facilitating repair of early-stage enamel demineralization.

NovaMin, a bioactive compound composed of calcium sodium phosphosilicate, facilitates enamel remineralization through its unique mechanism of action. Upon contact with saliva, it dispenses calcium, sodium, phosphate, along with silicates, which bind to tooth surface and initiate the remineralization process.[2]

Another effective remineralizing agent is casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), which secures high amount of calcium, phosphate, and fluoride ions in liquid state, creating

favorable environment for mineral deposition and enamel repair.[1]

To evaluate enamel demineralization (DEM) and remineralization (REM), various analytical techniques are employed. The current in vitro study compares remineralization ability of various distinct materials tested on human tooth enamel following controlled demineralization using surface microhardness (SMH) as the primary assessment tool.

Material & Methods: The study was carried out following consent from the institutional ethics review committee. A total of 40 freshly extracted, non-carious central incisors and third molars were collected. All specimens were thoroughly cleaned to remove any residual calculus, debris, or soft tissue.

Each tooth crown was segmented approximately one milimeter below the CEJ (Figure a). The segmented crowns were encased in acrylic resin keeping buccal face up, exposed, also aligned parallely to horizontal plane.To prepare a standardized surface for testing, the buccal enamel was sequentially flattened and polished using abrasive papers of 400 to 1200 grit.[2]

The samples were assigned to four groups at random [10 per group(n)]. Surface microhardness (SMH) of the enamel was assessed with the Vickers microhardness tester after initial demineralization and again at the conclusion of the study following remineralization.

A standardized window measuring 4 mm by 4 mm was made on the buccal surface per specimen to serve as the test area. Samples were then placed in 300 ml of a demineralizing liquid with pH of 4.4 (Figure b) upto 96 hours simulating initial carious defects. Solution being prepared used 2.2 mM calcium chloride ($CaCl_2$), 2.2 mM sodium dihydrogen phosphate (NaH_2PO_4), 0.05 M acetic acid, adjusted to the desired pH using 1 M potassium hydroxide (KOH). Solution efficacy was maintained by replacing it with a fresh batch every 48 hours.[3]

After the demineralization phase, the specimens were thoroughly rinsed with deionized water and allowed to air-dry. The surface microhardness (SMH) of the demineralized enamel was then measured across all groups. To maintain a reference region for later remineralization assessment, a 2 mm × 2 mm section within the demineralized area was covered with an acid-resistant nail varnish. (figure c)

Simulating the natural fluctuations of pH in oral environment, a pH-cycling regimen implementation over period of four weeks followed. Remineralizing toothpaste was applied to the exposed 2 mm × 2 mm demineralized enamel window for five minutes daily (figure d). Following each application, the specimens were stored in 50 mL artificially prepared saliva for remainder of day to mimic intraoral conditions.[4]

Artificial saliva formulation carried out in this study consisted of 2.2 g/L gastric mucin, 0.381 g/L sodium chloride, 0.213 g/L calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), 0.738 g/L potassium hydrogen phosphate, and 1.114 g/L potassium chloride[3].With pH adjusted to 7.0 using 85% lactic acid followed by maintaining a constant temperature of 37°C physiological conditions got simulated.

The remineralization protocol was applied to the remaining demineralized surface area (2 mm × 4 mm) using the designated remineralizing dentifrice specific to each experimental group. The specimens were categorized into four groups based on the remineralizing agent applied, as outlined below:

- **Group I – CPP-ACP**: Treated with GC Tooth Mousse having CPP-ACP
- **Group II – NovaMin**: Applied with SHY-NM toothpaste containing bioactive glass (calcium sodium phosphosilicate).
- **Group III – Combination**: Treated with a combination of CPP-ACP (GC Tooth Mousse) and NovaMin (SHY-NM) applied sequentially.
- **Group IV – Control**: No remineralizing agent was applied; specimens were only stored in artificial saliva.

Each remineralizing agent was applied according to manufacturer instructions and as per the standardized remineralization protocol. Following the four-week pH cycling and treatment regimen, surface microhardness (SMH) measurements were repeated to assess the efficacy of remineralization across all groups.

Pea-sized quantity of the respective dentifrice got dispensed on a sterile cotton applicator and spread uniformly over exposed enamel surface. The material remained undisturbed for five minutes to allow adequate contact, after which specimens were completely rinsed using deionized water. This procedure was performed daily for the entire 28 day duration of the study.[5]

Following the completion of the remineralization phase, the surface microhardness (SMH) of each enamel sample measurement was again done evaluating the effect of treatment.

Statistical Analysis: All data got analyzed using Statistical Package for the Social Sciences (SPSS), version [insert version]. ANOVA compared the mean SMH values among four groups both for demineralized and remineralized enamel. When significant differences were found, a post hoc test got conducted determining intergroup comparisons. Differences were deemed statistically significant when p-values were below 0.05.

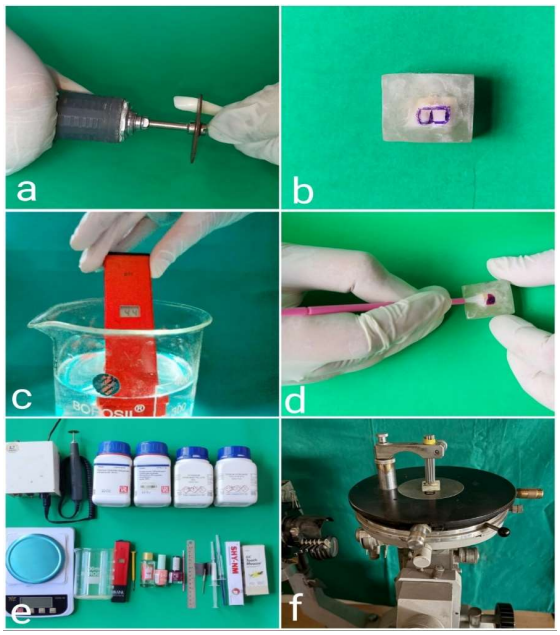

Figure 1a. Crown sectioning below the CEJ; **Figure 1b.** Standardized enamel window preparation; **Figure 1c.** pH measurement of the demineralizing solution; **Figure 1d.** Application of the remineralizing dentifrice; **Figure 1e.** Materials and armamentarium used, **Figure 1f.** Surface microhardness testing using the Vickers tester.

Results

Surface Microhardness Analysis: Surface microhardness (SMH) values comparsion across groups used (ANOVA).

No significant variation found in the average microhardness values between sound and demineralized enamel across all groups ($p > 0.05$), confirming uniform demineralization.

However, following remineralization, variations that were significant statistically have been noticed among the groups ($p < 0.05$) (Table 1). The Vickers Hardness Number (VHN) was highest in the CPP-ACP group (Group I)**,** followed by the Combination group (Group III) and the NovaMin group (Group II)**.** The Control group (Group IV) showed the lowest post-treatment microhardness values.

Post hoc analysis further confirmed that the variations in mean microhardness values amoung three test units (CPP-ACP, NovaMin, and Combination) were statistically significant, indicating varying levels of remineralization potential among the agents tested (Table 2).

Discussion: CPP-ACP ensures well-established reservoir of calcium and phosphate ions that are bioavailable. It not only inhibits demineralization but also promote remineralization of enamel surfaces.[6]

In the current study, CPP-ACP demonstrated highest remineralization ability among the tested agents. Interestingly, although the combination of CPP-ACP and bioactive glass (NovaMin) had not been extensively studied previously, the results suggest that their synergistic effect appeared beneficial, though not superior to CPP-ACP alone.

The trend observed in this study, with CPP-ACP showing the highest increase in surface microhardness, followed by the Combination group followed by NovaMin, is consistent with previous findings. Notably, our results coincide with that reported by Soares et al. (2011).

These findings underscore the clinical relevance of using targeted remineralizing agents in managing early enamel lesions and suggest that CPP-ACP remains a gold standard in non-invasive caries management.[6]

To simulate an oral condition with diminished salivary clearance, the amount of phosphate and calcium in demineralization solution were deliberately reduced, based on in vitro pH cycling model established by Ogata et al. (2010). In this study, the low concentrations of calcium and phosphate mimicked conditions where salivary clearance is limited, providing a more realistic scenario for remineralization.

Artificial initial caries-like defects in enamel exhibit all principal histological characteristics of natural cavitated lesions and are being widely used in in vitro studies to investigate enamel demineralization under controlled experimental conditions. Soumya et al. (2011)

CPP-ACP exists in a dissolved state, biologically active, and lacking crystalline structure, that contributes to its higher remineralization potential.[6] In contrast, the bioactivity of solid NovaMin glass is triggered only after it dissolves. CPP-ACP acts as a pool of phosphate and calcium ions, effectively inhibiting enamel demineralization and promoting remineralization, or potentially both. The presence of CPP-ACP in plaque reduces the rate of calcium loss during cariogenic attacks, allowing for a quicker return to baseline calcium concentrations, thereby accelerating remineralization (Yamaguchi et al. ,2006).Casein plays a key role in buffering plaque acid through bacterial catabolism, releasing amino acids that accept protons and act as a buffer, thus maintaining pH homeostasis in the oral environment (Rose et al., 2000).[6] Additionally, casein releases more amount of phosphate and calcium ions across a wide pH range, further contributing to enamel remineralization. CPP-ACP also facilitates the integration of fluoride into the biofilm, significantly enhancing enamel remineralization.[7]

In the group applied with CPP-ACP, remineralizing occurred primarily through the deposition of calcium and phosphate, forming an amorphous calcium-phosphate layer on the enamel surface. Previous studies have demonstrated that CPP-ACP promotes remineralizing of artificially induced dentinal defects.[7] The proposed method behind the effect is stabilization of calcium phosphates on tooth surface by casein phosphopeptides, creating increased concentration slopes of calcium and phosphate ions, thereby fostering remineralization of hard tissues (de Oliveira et al., 2020).

Combination group produced greater remineralization than NovaMin group, but it was less effective than GC Tooth Mousse (CPP-ACP) group. The additive effect of CPP-ACP and NovaMin in combination group yielded better results than NovaMin alone. However, the combination treatment did not surpass the efficacy of GC Tooth Mousse, likely due to shorter duration of application. Research has shown that remineralization increases with longer exposure times and higher dosage levels, which may explain the superior results observed with GC Tooth Mousse (Patil et al., 2013).

The NovaMin group exhibited less remineralization compared to the GC Tooth Mousse (CPP-ACP) and Combination groups. NovaMin, which consists of bioactived glass particles, has been studied for its efficiency in promoting remineralization of dental tissues and in sealing dentinal tubules.[3] Upon exposure to an aqueous environment, NovaMin releases ions of silica, calcium, phosphate, and sodium, triggering remineralization hence forming a calcium-phosphate complex that crystallizes to hydroxycarbonate apatite.[8]

Calcium sodium phosphosilicate (NovaMin) is provided as a solid bioactived glass that must dissolve prior becoming active. In contrast, CPP-ACP exists in a active, dissolved and amorphous form, that boosts its remineralization ability justifying superior performance of CPP-ACP. [9]

Containing nanometric bioactive glass (NovaMin), SHY NM is a toothpaste that does not include fluoride.[10] While fluoride is widely recognized for its beneficial effects on precluding tooth decay, excessive fluoride ingestion can have adverse health effects.[11] SHY NM avoids supplementing its formula with fluoride, relying instead on the bioactive glass particles to enhance remineralization.[12]

In contrary to the findings of this present study, Narayana et al. assessed the remineralizing effectiveness of bioactived glass, fluoride containing toothpaste, CPP-ACP (Tooth Mousse), and CPP-ACPF, finding bioactive glass to be more effective.[13] When different elements are integrated in enamel structure, an outer layer chemical reaction occurs, creating hydroxycarbonate apatite (HCA) layer. This layer closely resembles biological apatite both chemically and structurally, without the intermediate formation of amorphous calcium phosphate (ACP).[14] However, a notable limitation of NovaMin is its relatively slow action, making it less effective compared to other remineralizing agents, as observed in previous studies[15] (Rai et al., 2016).

One limitation of this study was its relatively less sample size and the in vitro nature of analysis. Additionally, duration (21 days) may have been insufficient for complete remineralization to occur.[16] Therefore, More extensive in vivo research, incorporating larger sample sizes and advanced techniques like scanning electron microscopy (SEM), is needed to comprehensively analyze the remineralization capacity of these agents.

Conclusion: From the current findings, it can be inferred that CPP-ACP as well as NovaMin exhibit remineralization capacity. However, the remineralizing efficacy of GC Tooth Mousse (CPP-ACP) was found to be superior compared to SHY NM and the Combination group. To evaluate the influence of these remineralizing agents on clinical outcomes, larger-scale clinical trials with extended monitoring periods are recommended for more robust evidence.

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