ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at:www.ijmacr.com

Volume – 8, Issue – 3, May - 2025, Page No.: 109 – 116

An-In Vitro Study Comparing Two Different Remineralizing Agents- A Scanning Electron Microscope Study

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How to citation this article: Dr. Piyush Gupta, "An-In Vitro Study Comparing Two Different Remineralizing Agents- A Scanning Electron Microscope Study", IJMACR- May - 2025, Volume – 8, Issue - 3, P. No. 109 – 116.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Objective: Through the use of a scanning electron microscope (SEM), energy dispersive X-ray (EDX) analysis, and the Vickers Microhardness (VMH) Test, the study sought to evaluate the remineralizing potential and dentinal tubule occlusion potential of two distinct commercially available agents.

Material and Methods: Twenty-two specimens were made from removed teeth (n = 11 each group). On each specimen, a 6 mm × 4 mm window representing the three zones of sound enamel, demineralized enamel and remineralized enamel was created. Two distinct remineralizing preparations, GC Tooth Mousse, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and Bioenamel from Prevest Denpro with Bioglass, were used to the demineralized enamel zone. VMH was used to evaluate REM, and SEM and EDX analysis, was used to evaluate the structural alterations. The specimens underwent a more recent demineralization treatment. A one-way ANOVA with a significance level of $P < 0.05$ was employed, followed by a post hoc Tukey test.

Results: Bioenamel showed significantly higher Vickers hardness values when compared to GC Tooth Mousse ($P = 0.011$).SEM showed higher degree of remineralization and dentinal tubule occlusion for Bioenamel as compared to GC Tooth Mousse.

Conclusion: Bioenamel from Prevest Denpro exhibited higher degree of remineralization and dentinal tubule occlusion than other remineralizing agent.

Keywords: SEM, Remineralization, Bioenamel, Dentin, Dentinal Tubule, Demineralization.

Introduction

Dental caries is one of the most common oral health issues globally, affecting people of all ages and causing significant dental problems. It is a condition where the enamel of the teeth undergoes progressive demineralization, which occurs due to the acids produced by bacteria when they break down dietary sugars. The loss of mineral content from enamel results in the softening and breakdown of the tooth structure, leading to the formation of cavities. Caries not only causes structural damage to the tooth but can also compromise the overall aesthetic and function of teeth, thereby influencing a person's oral health and quality of life. Consequently, the need for preventive strategies and effective remineralizing treatments has gained considerable attention in modern dentistry. Remineralization is the natural process that helps restore demineralized enamel by allowing minerals, primarily calcium and phosphate, to be deposited from the surrounding environment. In healthy conditions, this occurs continuously in a dynamic equilibrium with demineralization. However, when the demineralization rate exceeds the remineralization process, enamel integrity is compromised, leading to the progression of caries. Efforts to enhance this natural repair mechanism have led to the development of various remineralizing agents, which aim to deliver additional minerals to the enamel to promote its recovery and prevent further decay.[1] Among the numerous remineralizing agents available, Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) and Bioglass-based formulations have shown promise in enhancing enamel remineralization. CPP-ACP is a bioactive peptide derived from casein, a protein found in milk, which stabilizes calcium and phosphate ions and delivers them to the enamel surface, promoting remineralization. CPP-ACP-based products, such as GC Tooth Mousse, have demonstrated effective remineralizing properties in clinical and in-vitro studies.[2,5]These agents are commonly used in the management of early enamel demineralization, as they facilitate the repair of enamel by supplying bioavailable calcium and phosphate ions directly to the affected area. On the other hand, Bioglass, a material known for its bioactive properties, has been incorporated into dental products like Bioenamel from Prevest Denpro. Bioglass is a synthetic material that promotes the formation of a hydroxyapatite-like structure when exposed to physiological fluids, and it has been recognized for its ability to enhance enamel remineralization by providing calcium, phosphate and silica ions. Previous studies have shown that Bioglass can not only aid in remineralizing demineralized enamel but also contribute to the restoration of the structural integrity of enamel by forming a protective layer.[3] The effectiveness of these remineralizing agents can be assessed through various analytical techniques, such as Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX) analysis and Vickers Microhardness (VMH) testing. SEM provides high-resolution images of the enamel surface, allowing for the visualization of structural changes at the microscopic level. EDX analysis, in combination with SEM, can be used to determine the elemental composition of the enamel, providing insights into the degree of mineral deposition on the enamel surface. VMH testing is a reliable method to evaluate the hardness of enamel, which correlates with its mineral content and structural integrity. Collectively, these techniques enable a comprehensive assessment of the effectiveness of remineralizing agents in restoring the mineral content and mechanical properties of enamel.[4] The aim of this study was to compare the remineralizing potential and dentinal tubule occlusion potential of two commercially available agents: GC Tooth Mousse (CPP-ACP) and Bioenamel (Bioglass), using SEM, EDX, and VMH to evaluate structural changes and mechanical properties of remineralized enamel. By understanding the comparative efficacy of these agents, this study seeks to provide insights into the most effective approaches for enhancing enamel remineralization and preventing the progression of dental caries.

Material and Methodology

This study was conducted between year 2019-21 in Department of Conservative Dentistry and Endodontics Rural Dental College, Loni Maharashtra. Ethical Committee acceptance was obtained bearing approval number PMT/RDC/IEC/2019/02. Regardless of the arch, twenty- two (n=22) recently extracted non carious central incisor and third molars were gathered, cleansed of calculus, debris, and soft tissue, and preserved in a 10% formalin solution. A stereomicroscopic examination revealed unbroken enamel after the crown was sectioned 1 mm below the cementoenamel junction using a slow-speed diamond disc. With the buccal surface exposed, facing up, and parallel to the horizontal plane, each tooth crown was immersed in resin. Using abrasive paper ranging in grit from 400 to 1200, the buccal surface was ground smooth and polished in order. Following that, the samples were split into two groups, each with 11 members. Each sample's enamel SMH was assessed using the Vickers Microhardness test at the start of the investigation, following enamel demineralization, and at the conclusion of the study following REM. Furthermore, a small number of samples were chosen at random, examined using SEM, and then exposed to EDX.

Enamel surface microhardness test

The sample surface was covered with a 6 mm × 4 mm window of adhesive tape, and a consistent layer of nail varnish was put all around the sample to make it resistant to acid attack. After the samples had dried, the adhesive tape was taken off, and three locations spaced 100 µm apart were indented with a Vickers diamond indenter (SchimadzuTM) at a weight of 100 g for 10 s. The indentations' diagonal length was measured using an integrated microscope, which also showed the Vickers hardness number (VHN). To protect the undemineralized enamel, a 2 mm x 4 mm section was chosen and covered with nail varnish. To create white spot caries-like lesions, the specimens were subsequently submerged in 300 ml of demineralization solution (pH 4.4) for 96 hours. There was 2.2 mM $CaCl_2$, 2.2 mM NaH_2 PO_4, 0.05M acetic acid, and 1M KOH in the solution. At 48 hours, a new solution was added to replace the old one. Each specimen was cleaned with deionized water, allowed to air dry and the microhardness of demineralized enamel was measured for each group after 96 hours. Before exposing the specimen to remineralizing agents, the demineralized enamel measuring 2 mm × 4 mm was once more covered with an acid-resistant nail varnish layer. For four weeks, pH-cycling was used to reproduce daily variations in the oral cavity. The samples were exposed to 3 hours of DEM twice a day, with 2 hours of REM in between the DEM periods, after the REM agent was applied for 5 minutes to the final demineralized area of 2 mm × 4 mm.[6] For the remainder of the day, the teeth were submerged in 50 milliliters of artificial saliva.[7] 2.2 g/L gastric mucin, 0.381 g/L sodium chloride, 0.213 g/L CaCl2.2H2O, 0.738 g/L potassium hydrogen phosphate and 1.114 g/L potassium chloride make up the artificial saliva employed in this investigation. Ultimately, the pH of artificial saliva was adjusted to 7.00 using 85% lactic acid at 37°C.[8]

The remaining 2 mm x 4 mm samples were subjected to the REM method using remineralizing agent tailored to each group. The samples were arranged as follows based

on the REM agents employed in this investigation:
Group I: CPP ACP (GC Tooth Mousse)
Group II: Bioglass (Bioenamel from Prevest Denpro Limited)

A uniform application technique was used for each of the remineralizing agent. Using a cotton applicator, a pea-sized amount of remineralizing agent was pertained to the tooth surface, kept undisturbed for five minutes, and then rinsed with deionized water. The remineralizing solution had a pH of 7. It comprised 0.15M KCl, 0.9mM NaH_2PO_4, and 1.5mM $CaCl_2$.[9] This application was completed within the study's 28 days. Following REM, the enamel specimen's microhardness was once more measured. Three randomly chosen samples from each group were subjected to a SEM analysis using ×2000 in order to assess microscopic differences between the groups that received various remineralizing agents. Both positive and negative controls were used to compare these. Since the quantity of calcium and potassium in treated specimens was used to gauge the degree of REM, the specimens were also exposed to EDX analysis in addition to SEM to ascertain the amount of minerals in tooth specimens. SPSS for Windows was used to statistically analyze the data (SPSS version 22.0, IBM Corp., Armonk, NY, USA). The mean microhardness values for demineralized and remineralized enamel were compared among the groups by means of one-way ANOVA, and then a post hoc test was performed. The mean microhardness values between demineralized and baseline enamel, as well as between demineralized and remineralized enamel, were assessed using an unpaired t-test. A considerable threshold of $P < 0.05$ was established.

Results

Surface microhardness tests

After REM agent application, statistically significant variations in the groups' mean microhardness values were discovered. It was found that VHN values were drastically superior for Bioenamel as compared to GC Tooth Mousse.

SEM and energy dispersive X-ray analysis

Standard enamel showed a distinctive fish-scale look with a even, undamaged surface when examined under a scanning electron microscope. After DEM, SEM of the enamel revealed a little honeycomb pattern in both groups along with rough, uneven and increasing porosities. After 4 weeks both the groups showed signs of remineralization. The rods and interrod area were filled with an uneven but uniform coating of minerals that appeared to be erasing the DEM flaw. Additionally, although regions of unfilled defects continued to exist, areas of calcified deposits made up of irregularly shaped fluoro-hydroxyapatite crystals were also visible. The proportion of calcium and potassium content that was gone as a consequence of DEM and then gain as a result of REM in each group is shown in Table 1. It was found that Bioenamel had a greater percentage of REM and dentinal tubule occlusion than GC Tooth Mousse.

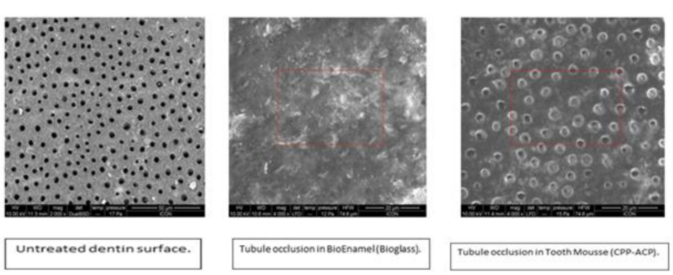

Figure 1: SEM images of untreated dentin surface , Bioenamel and GC Tooth Mousse

Table 1: Calcium and potassium ions as assessed by energy dispersing X-ray analysis for sound enamel, demineralized enamel and remineralized enamel in atomic percentage

Ca K^+	Sound enamel	Demineralized	Remineralized
GC Tooth Mousse	18.95	15.48	20.85
Bioenamel	20.15	16.76	23.49

Table 2: Comparison of mean microhardness values of normal enamel

Remineralizing Agent	n	Mean±SD	F	P
GC Tooth Mousse	11	285±33.34	0.92	0.56(NS)
Bioenamel	11	302±38.65		

NS using one-way ANOVA. SD: Standard deviation

Table 3: Comparison of mean microhardness values of demineralized enamel

Remineralizing Agent	n	Mean±SD	F	P
GC Tooth Mousse	11	107.98±34.9	2.9	0.34(NS)
Bioenamel	11	116.62±45.2		

NS using one-way ANOVA. SD: Standard deviation

Table 4: Comparison of mean microhardness values of remineralized enamel

Remineralizing Agent	n	Mean±SD	F	P
GC Tooth Mousse	11	145.87±21.4	5.2	0.11*
Bioenamel	11	171.64±47.7		

NS using one-way ANOVA. SD: Standard deviation

Comparison using post hoc test	P
GC Tooth Mousse in opposition to Bioenamel	0.650

Discussion

The primary objective of this study was to evaluate the remineralizing prospective of two commercially available agents - GC Tooth Mousse (casein phosphopeptide-amorphous calcium phosphate, CPP-ACP) and Bioenamel (Bioglass) on human enamel. The results of this study demonstrated that both agents are capable of promoting enamel remineralization, but Bioenamel exhibited superior performance when evaluated through multiple analytical methods, including Vickers Microhardness (VMH) testing, Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX) analysis. Bioglass, the primary component of Bioenamel, is known for its bioactive properties that promote the formation of a hydroxyapatite (HA)-like layer on demineralized surfaces, a critical process for remineralization .[10] In addition to its remineralizing effects, Bioglass has been shown to stimulate the production of hydroxyapatite-like crystals, which are essential for the restoration of enamel’s natural composition.[11] This supports the finding that Bioenamel-treated surfaces exhibited a more pronounced recovery of enamel's structural integrity, with mineral deposits that closely resemble the original enamel structure. Such findings align with earlier studies that demonstrate Bioglass's ability to effectively remineralize enamel, enhancing both its microhardness and resistance to carious lesions.[3,12]

Effectiveness of Remineralizing Agents

Vickers Microhardness Test: The Vickers hardness test serve as a reliable marker of the mineral content and structural integrity of enamel. After remineralization, the Bioenamel-treated group exhibited significantly higher hardness values compared to the GC Tooth Mousse group. This result suggests that Bioenamel, with its Bioglass content, is more effective at enhancing the mechanical properties of remineralized enamel. The increase in microhardness observed in the Bioenamel group could be attributed to the additional minerals provided by Bioglass, which promotes the formation of a hydroxyapatite-like structure on the enamel surface.[3]The release of these ions also plays a critical role in the formation of a stable mineral layer, which is less prone to dissolution in acidic environments.[10] This property is consistent with findings in other studies that have demonstrated Bioglass's ability to enhance enamel remineralization through the deposition of minerals like calcium and phosphate.[3,4] In contrast, the GC Tooth Mousse group showed a more modest increase in hardness. While CPP-ACP has demonstrated beneficial effects in remineralizing early enamel lesions, the less pronounced increase in hardness in this study may suggest that CPP-ACP is less effective in fully restoring the mineral content and mechanical properties of enamel when compared to Bioglass-based products.[2,5] Furthermore, while CPP-ACP is often used in combination with fluoride to maximize remineralization.[14] This study assessed the agents independently, and the lack of synergy with fluoride could have contributed to the relatively modest results observed for GC Tooth Mousse. Scanning Electron Microscopy (SEM): SEM analysis discovered diverse difference in the surface morphology of the enamel treated with each remineralizing agent. Normal enamel showed a smooth surface with a characteristic fish-scale appearance, while demineralized enamel exhibited increased porosity and a honeycomb-like pattern, indicative of mineral loss.[4] After four weeks of remineralization, both GC Tooth Mousse and Bioenamel showed some evidence of remineralization, with mineral deposits filling the pores in a somewhat uniform manner. However, the Bioenamel-treated group exhibited a more pronounced and uniform coverage of the enamel surface. The remineralized enamel surfaces in the Bioenamel group showed a more complete recovery of the enamel's natural structure, with the formation of irregular fluor-hydroxyapatite crystals. These findings suggest that Bioenamel facilitates more efficient remineralization by promoting the deposition of minerals that are crucial for the restoration of enamel's integrity.[3]

Energy Dispersive X-ray (EDX) Analysis

EDX analysis provided further insight into the elemental composition of the enamel surface, revealing that the calcium and potassium ion content in the Bioenamel-treated specimens was significantly higher than that of the GC Tooth Mousse group. This finding suggests that Bioenamel not only provides a elevated concentration of calcium and phosphate ions for remineralization but may also contribute to the development of a more stable and durable mineral coating on the enamel surface. The higher calcium and potassium content in the Bioenamel group is consistent with previous research that highlights the superior remineralizing properties of Bioglass, which releases a variety of ions (calcium, phosphate, and silica) to enhance enamel remineralization and restore its mechanical properties.[3,8] On the other hand, while GC Tooth Mousse was able to deposit calcium and phosphate ions onto the enamel surface, the overall mineral content was lower compared to Bioenamel. This discrepancy could be accredited to the nature of CPP-ACP, which, although effective in stabilizing calcium and phosphate ions, may not be as efficient in driving the formation of a durable mineralized layer on the enamel.[2,5] Another study conducted at Dicle Faculty of Dentistry, Turkey concluded at GC Tooth Mousee showed less remineralization when compared to herbal agents.[15]

Conclusion

The findings of this study hold significant clinical relevance for the prevention and treatment of dental caries and hypersensitivity. Although both GC Tooth Mousse and Bioenamel demonstrated some degree of remineralization, Bioenamel proved to be more effective in restoring the mineral content and mechanical properties of demineralized enamel. As enamel remineralization is fundamental for reversing early carious lesions and preventing the progression of dental caries, the result suggest that Bioglass-based products like Bioenamel may offer a more effective solution for enamel repair, hypersensitivity and caries prevention. Furthermore, given that dental caries remains one of the most common oral diseases worldwide, the development of remineralizing agents that can efficiently restore enamel and halt the progression of caries is of paramount importance.[1] The results of this study show up the potential of Bioglass-based formulations to serve as a valuable tool in clinical settings, offering a promising alternative to traditional fluoride treatments for enamel remineralization.

Limitations and Future Research

While this learning provides valuable insights into the comparative effectiveness of two remineralizing agents, there are some limitations that should be considered. First, the study was conducted in vitro, and the results may not fully reflect the vibrant situation of the oral cavity, where factors such as saliva flow, pH fluctuations, and microbial activity play significant roles in remineralization.[13] Additionally, the study focused only on two specific remineralizing agents, and other products, such as fluoride-based formulations or nano-hydroxyapatite treatments, could offer additional insights into the comparative efficacy of remineralizing agents. Future research should aim to evaluate the long-standing usefulness of these remineralizing agents in clinical trials, taking into account factors such as patient compliance, dietary habits, and the potential for synergistic effects when combined with other preventive measures. Furthermore, studies exploring the effect of different concentrations of Bioglass and CPP-ACP, as well as their combination with fluoride, could provide a more comprehensive understanding of their relative efficacy.

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