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Original Article

An In Vitro Evaluation of the Effectiveness of Four Remineralizing Agents

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ABSTRACT

The study aimed to compare the effectiveness of four commercially available remineralizing products: GC Tooth Mousse, SHY-NM, Biomed Calcimax, and Bentodent, on demineralized human teeth. A total of 72 upper premolars, each bonded with a 3M premolar bracket, were randomly assigned to one of six groups, each containing 12 teeth. Group 1 was stored in de-ionized water (positive control), group 2 in Tencates demineralizing solution (negative control), group 3 in Tencates solution and coated with GC Tooth Mousse every 4 hours, group 4 was coated with SHY-NM, group 5 was coated with Biomed Calcimax, and group 6 was coated with Bentodent. After 96 hours, the teeth were sectioned using a diamond disc and thinned to 150-200 µm with a carborundum stone. The segments were examined under a polarized light microscope, and the images were analyzed using ImageJ software. A Bonferroni post-hoc test for multiple pairwise comparisons showed significant differences in the depth of demineralization between most groups, except between group 3 and group 4, as well as group 5 and group 4 for minimum demineralization depth. For maximum demineralization depth, significant differences were observed between group 2 and group 6, group 3 and group 4, group 4 and group 5, and group 5 and group 6. The results indicate that GC Tooth Mousse and SHY-NM are more effective in remineralizing artificially induced carious lesions compared to Biomed Calcimax and Bentodent.

Keywords: Polarized light microscopy, White spot lesion, Enamel demineralization, Medicaments.

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Introduction

Enamel demineralization is a common consequence of fixed orthodontic treatment, often exacerbated by poor oral hygiene, leading to the formation of white spot lesions (WSLs). These lesions are primarily caused by acidogenic bacteria such as Streptococcus mutans and Lactobacilli present in plaque. Fejerskov and Kidd *et al.* describe WSLs as "the initial visible sign of a carious lesion that appears in the enamel and can be observed with the naked eye" [1]. Typically, these lesions form around the brackets, especially near the gingival edge, as noted by Gorelick *et al.* (1982). The occurrence of WSLs among orthodontic patients varies widely, ranging from 2% to 96%. The labio-gingival region is the most common area for lateral incisors to develop WSLs, while the maxillary posterior segments are less frequently affected. Additionally, males are more prone to WSLs than females [2].

Enamel demineralization occurs in two stages. The first stage involves the softening of the enamel surface, characterized by the loss of interprismatic substances and mineral depletion. In the second stage, known as the active lesion phase, dissolution progresses deeper into the enamel, forming subsurface lesions. Despite this, the lesion remains covered by a mineral-rich, porous layer. Active lesions are considered to have a more favorable prognosis than arrested lesions because they allow for easier penetration of calcium and phosphate ions, promoting remineralization.

Remineralization is the body's natural repair process for non-cavitated lesions, relying on the presence of calcium and fluoride-supported phosphate ions to rebuild a new surface on the residual crystals left behind after demineralization [3]. These newly formed hydroxyapatite (HAP) crystals are less prone to acid dissolution compared to the original minerals. In natural remineralization, glycoproteins adsorb onto the tooth structure, forming a protective pellicle layer, while phosphoproteins regulate the calcium saturation of saliva. Key proteins, such as proline-rich proteins, statherin, histatin, and cystatin, play a crucial role in enamel remineralization by attracting calcium ions [4].

Shah *et al.* [5] demonstrated that orthodontic brackets coated with photocatalytic titanium oxide (TiO2) reduced bacterial adherence and biofilm accumulation on stainless steel. A year later, Hadler-Olsen *et al.* [6] reported that despite the use of preventive measures, 60% of orthodontic patients developed one or more white spot lesions by the end of their treatment. Therefore, modern orthodontics aims to manage WSLs non-invasively, effectively controlling disease progression while improving aesthetics, function, and strength.

Orthodontists must remain vigilant about the potential for decalcification and take proactive measures to prevent or slow down the demineralization process, promoting an environment favorable to remineralization through various remineralizing agents. Traditional remineralizing agents supply the carious lesion with necessary calcium and phosphate ions without causing calculus buildup or precipitation on the tooth surface. This study seeks to compare and evaluate the effectiveness of four remineralizing agents: CPP-ACP, NovaMin, calcium hydroxyapatite, and calcium bentonite.

Materials and Methods

The study received approval from the Institutional Review Board (approval number 200/IHEC/1-19). The sample size was determined using G*Power software (version 3.1.9.7), with an effect size f = 0.45, α err prob = 0.05, and a power (1-β err prob) of 0.8. The calculated sample size was 72 (12 per group), and the actual power was 0.8202265. Thus, the final sample consisted of 72 extracted maxillary first premolars, collected from patients undergoing therapeutic extractions for orthodontic treatment. The following exclusion criteria were applied: 1) teeth with caries, restorations, stains, hypoplasia, or white spot lesions, and 2) crowns with enamel fractures or structural defects. The inclusion criteria were: 1) patients aged thirteen to sixteen years, 2) teeth with intact buccal enamel and no signs of decalcification, 3) teeth that had not been previously bonded, 4) vital and fully erupted teeth at the time of extraction, 5) anatomically and morphologically described maxillary premolars, and 6) no cracks caused by the use of extraction forceps.

The teeth were randomly divided into six groups, with 12 teeth in each group. Group 1 (n=12) was stored in de-ionized water only (positive control), group 2 (n=12) was stored in Tencates demineralizing solution alone (negative control), group 3 (n = 12) was stored in Tencates demineralizing solution and coated with GC Tooth Mousse every four hours (CPP-ACP), group 4 (n=12) was stored in Tencates demineralizing solution and coated with SHY-NM every 4 hours (calcium sodium phosphosilicate or NovaMin), group 5 (n=12) was stored in Tencates demineralizing solution and coated with BioMed Calcimax every four hours (calcium hydroxyapatite and L-Arginine), and group 6 (n=12) was stored in Tencates demineralizing solution and coated with Bentodent every four hours (calcium bentonite).

The protocol for collecting, sterilizing, storing, and handling the extracted teeth was strictly followed, following OSHA and CDC guidelines. The teeth were thoroughly rinsed with de-ionized water, then bonded with 3M brackets using Scotch Bond etchant, Transbond XT adhesive, and primer (3M Unitek), following the manufacturer's instructions. A diamond disc was used to remove the apical third of each tooth. The teeth were then affixed to the inner surface of a twenty ml polypropylene jar lid with yellow adhesive wax, ensuring that only the crowns of the teeth were submerged in the corresponding solutions.

Groups 2, 3, 4, 5, and 6 were exposed to an artificial caries challenge. Groups 1 and 2 were not treated and served as positive and negative controls, respectively. Groups 3, 4, 5, and 6 received 0.5 ml of GC Tooth Mousse, SHY-NM, Biomed Calcimax, and Bentodent, applied with paintbrushes. To maintain consistent movement of the solution, all jars were placed on a vibrator for 30 seconds every 4 hours. After each 4 hours, the teeth were removed from the solution, rinsed with de-ionized water, and brushed for 5 seconds with a toothbrush to simulate regular brushing. Subsequently, GC Tooth Mousse, SHY-NM, Biomed Calcimax, and Bentodent were re-applied to groups 3, 4, 5, and 6 for 40 seconds every 4 hours over the 96-hour study period. After each application, the teeth were rinsed with de-ionized water, dried, and returned to the demineralizing solution.

After 96 hours, the teeth were removed from the solution and completely rinsed with de-ionized water. Longitudinal slices of the tooth surfaces were made in a linguobuccal direction, close to the brackets, using a diamond disc with de-ionized water cooling. The slices were then reduced to a thickness of 150–200 µm with a carborundum stone. The enamel sections were examined under an OLYMPUS CX41 microscope equipped with crossed polarizers and an analyzer plate to assess the depth of demineralization. Images were captured using a Canon 200D digital camera at 2x zoom, with magnifications of 5× to 10×. The demineralization depth was measured using ImageJ software, a Java-based image processing tool. For each sample, the linear distance from the tooth surface adjacent to the bracket to both the maximum and minimum demineralization depths was recorded in micrometers (**Figure 1**).

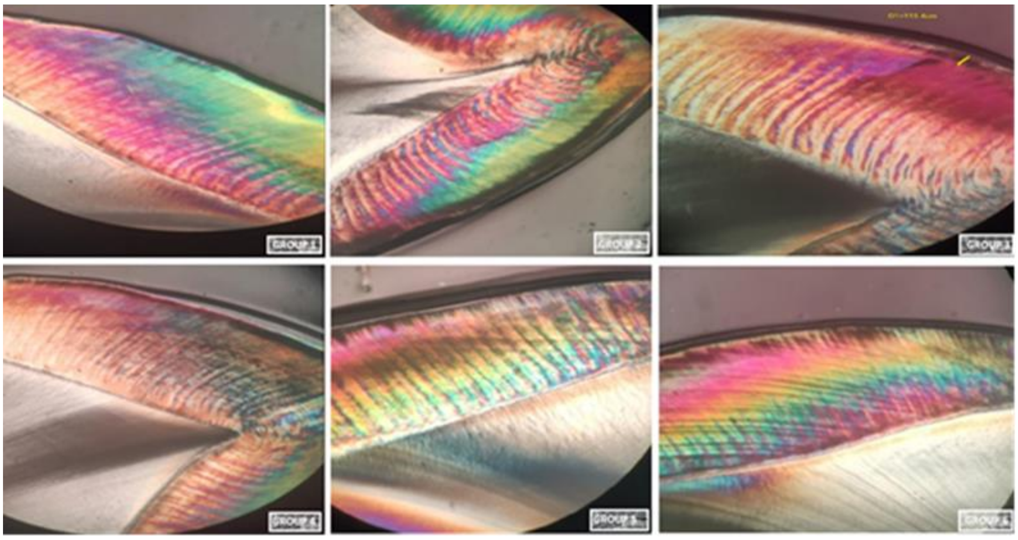

Figure 1. Demineralization depth measurement using ImageJ software

Statistical Analysis

Descriptive and inferential statistical analyses were performed using IBM SPSS version 20.0 (IBM Corp., 2011). Quantitative data are presented as mean values with standard deviations (SD). To compare parameters across the six groups, one-way ANOVA followed by a Bonferroni post-hoc test was employed. A P-value of less than 0.05 was considered to indicate statistical significance throughout the study.

Results and Discussion

A one-way ANOVA was conducted to compare the minimum and maximum demineralization depths between the groups, revealing significant differences in the minimum demineralization depth (**Table 1**). Following the ANOVA test, a post-hoc analysis was performed, which showed significant differences in the minimum demineralization depth between all group comparisons, except for group 3 vs. group 4, and group 5 vs. group 4 (**Table 2**). For maximum demineralization depth, significant changes were observed between the groups (**Table 3**). The post-hoc test indicated significant differences in maximum demineralization depth for all group comparisons, except for group 2 vs. group 6, group 3 vs. group 4, group 4 vs. group 5, and group 5 vs. group 6.

Table 1. Comparison of demineralization (minimum) between the groups

Variable	Group	Mean	Std. deviation	95 % Confidence interval		P-value
Max demineralization	1	32.73	8.12	27.56	37.89	0.041
	2	256.91	17.76	245.62	268.19	
	3	213.46	9.25	207.58	219.33	
	4	223.24	10.1	216.83	229.66	
	5	233.29	10.61	226.55	240.03	
	6	244.51	10.16	238.06	250.96	

Group 3 (CPP-ACP) demonstrated significant remineralization (P-value = 0.010) when compared to group 5 (Biomed Calcimax) and group 6 (Bentodent), but showed no significant remineralization (P-value = 0.561) when compared to group 4 (SHY-NM). Group 4 (SHY-NM) exhibited significant remineralization (P-value = 0.010) when compared to group 6 (Bentodent) but showed no significant differences in remineralization when compared to group 3 (CPP-ACP) (P-value=0.561) and group 5 (Biomed Calcimax) (P-value=1.000). Group 5 (Biomed Calcimax) showed significant remineralization (P-value=0.025) when compared to group 6 (Bentodent) (**Table 4**).

Table 2. Bonferroni post-hoc test for multiple pairwise comparisons (minimum)

(I) Group	(J) Group	Mean difference (I-J)	Std. error	P-value
1	2	-141.56667*	4.77399	0.01
	3	-88.47500*	4.77399	0.01
	4	-98.61667*	4.77399	0.01
	5	-105.80000*	4.77399	0.01
	6	-121.45000*	4.77399	0.01
2	1	141.56667*	4.77399	0.01
	3	53.09167*	4.77399	0.01
	4	42.95000*	4.77399	0.01
	5	35.76667*	4.77399	0.01
	6	20.11667*	4.77399	0.01
3	1	88.47500*	4.77399	0.01
	2	-53.09167*	4.77399	0.01
	4	-10.14167	4.77399	0.561
	5	-17.32500*	4.77399	0.008
	6	-32.97500*	4.77399	0.01
4	1	98.61667*	4.77399	0.01
	2	-42.95000*	4.77399	0.01
	3	10.14167	4.77399	0.561
	5	-7.18333	4.77399	1
	6	-22.83333*	4.77399	0.01
5	1	105.80000*	4.77399	0.01
	2	-35.76667*	4.77399	0.01
	3	17.32500*	4.77399	0.008
	4	7.18333	4.77399	1
	6	-15.65000*	4.77399	0.025
6	1	121.45000*	4.77399	0.01
	2	-20.11667*	4.77399	0.01
	3	32.97500*	4.77399	0.01
	4	22.83333*	4.77399	0.01
	5	15.65000*	4.77399	0.025

Table 3. Comparison of demineralization (maximum) between the groups

Variable	Group	Mean	Std. deviation	95 % Confidence interval		P-value
Max demineralization	1	32.73	8.12	27.56	37.89	0.041
	2	256.91	17.76	245.62	268.19	
	3	213.46	9.25	207.58	219.33	
	4	223.24	10.1	216.83	229.66	
	5	233.29	10.61	226.55	240.03	
	6	244.51	10.16	238.06	250.96	

Table 4. Bonferroni post-hoc test for multiple pairwise comparison (maximum)

(I) Group	(J) Group	Mean difference (I-J)	Std. error	Sig.
1	2	-224.18333*	4.66868	0.01
	3	-180.73333*	4.66868	0.01
	4	-190.51667*	4.66868	0.01
	5	-200.56667*	4.66868	0.01
	6	-211.78333*	4.66868	0.01
2	1	224.18333*	4.66868	0.01
	3	43.45000*	4.66868	0.01
	4	33.66667*	4.66868	0.01
	5	23.61667*	4.66868	0.01
	6	12.4	4.66868	0.149

3	1	180.73333*	4.66868	0.01
	2	-43.45000*	4.66868	0.01
	4	-9.78333	4.66868	0.599
	5	-19.83333*	4.66868	0.001
	6	-31.05000*	4.66868	0.01
4	1	190.51667*	4.66868	0.01
	2	-33.66667*	4.66868	0.01
	3	9.78333	4.66868	0.599
	5	-10.05	4.66868	0.525
	6	-21.26667*	4.66868	0.01
5	1	200.56667*	4.66868	0.01
	2	-23.61667*	4.66868	0.01
	3	19.83333*	4.66868	0.001
	4	10.05	4.66868	0.525
	6	-11.21667	4.66868	0.287
6	1	211.78333*	4.66868	0
	2	-12.4	4.66868	0.149
	3	31.05000*	4.66868	0.01
	4	21.26667*	4.66868	0.01
	5	11.21667	4.66868	0.287

The presence of fixed orthodontic appliances has been linked to an increased incidence of white spot lesions (WSLs), even with preventive measures in place, negatively affecting the aesthetics of the teeth [7]. A promising, non-invasive approach to caries management is enamel remineralization, which has garnered significant research interest over the past century. This method allows for the transformation of active lesions into inactive ones. Various professional delivery systems, including varnishes, toothpaste, gels, and fluoride-releasing products, are commonly used to remineralize high-risk areas. The key mechanism behind remineralization involves the diffusion of ions, primarily calcium and phosphate, from saliva and topical treatments to form a hypermineralized, acid-resistant layer on the remaining crystals, which then serve as nuclei for further remineralization [8]. Several methods are available to assess mineral changes in human enamel, such as microhardness testing of enamel cross-sections, microradiography, iodine absorptiometry, polarized light microscopy, and light scattering. In this research, polarized light microscopy (PLM) was chosen due to its high sensitivity to changes in hard tissues [9].

In recent years, numerous remineralizing agents have been introduced as additives to traditional fluoride-based systems [10]. The present in vitro study aimed to evaluate and compare the remineralizing effectiveness of 4 commercially available products: SHY-NM, Biomed Calcimax, GC Tooth Mousse, and Bentodent, all of which target demineralized human teeth. GC Tooth Mousse contains CPP-ACP, SHY-NM incorporates bioactive glass, Biomed Calcimax contains calcium hydroxyapatite and L-Arginine, and Bentodent toothpaste is composed of calcium bentonite. Seventy-two upper human premolar teeth, selected for therapeutic extraction, were collected and preserved in 10% formalin, a preferred disinfectant that also prevents demineralization by stabilizing proteins in the organic pellicle during storage [11]. In experimental caries research, spontaneously formed WSLs are typically used, though generating consistent carious lesions of uniform size in vivo is challenging. As a result, a demineralizing solution was employed in this research to create standard-sized incipient caries lesions, with the teeth submerged in the solution for 96 hours as per the method described by Reynolds [12].

In several studies, the pH cycling model has been utilized to replicate the dynamic processes of demineralization and remineralization. However, this model has limitations, such as its inability to replicate the actual intraoral conditions that lead to caries development, mimic the composition of plaque fluid and saliva found in vivo, and simulate the topical application and clearance of products from the oral cavity [13].

Saumya Kakkar *et al.* employed Tencates demineralization solution alone in their research [14]. While the limitations of chemical demineralization are similar, this approach was chosen for its simplicity and practicality. Group 1 was immersed in de-ionized water, as previous studies have indicated that de-ionized water does not significantly affect the microhardness of teeth for up to two months [15]. The other five groups were submerged in Tencates demineralizing solution for 96 hours at room temperature [16]. Lesion depth was assessed using polarized light microscopy, as it allows for better visualization of the histological features of enamel and dentin compared to transmitted light microscopy, due to its birefringent properties.

In this study, group 3, which was treated with 10% CPP-ACP (Recaldent–a water-based, lactose-free cream), demonstrated a higher level of remineralization compared to groups 4, 5, and 6. Recaldent technology was developed by Eric Reynolds and his colleagues at the University of Melbourne, Australia. Previous research has shown that Recaldent offers short-term remineralization benefits and long-term caries prevention in clinical and randomized control trials [17].

Two studies have demonstrated that CPP-ACP exhibits superior remineralizing potential compared to calcium sodium phosphosilicate, as evaluated through scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX) [18, 19]. A four-week application of CPP-ACP after debonding significantly reduced white spot lesions, suggesting that CPP-ACP is effective before, during, or after acid intervention [20, 21]. Additionally, casein phosphopeptide-amorphous calcium phosphate has been shown to reduce mutans streptococci and decrease bacterial counts [22].

Following CPP-ACP, the next most effective material in the research was SHY-NM, which contains bioactive glass (Novamin). Research using the Vickers microhardness test found that Novamin initially exhibited superior remineralizing properties compared to CPP-ACP; however, over time, both agents demonstrated similar remineralization potential [23]. In the case of bleached enamel, bioactive glass acts as an ion reservoir in demineralized areas. Another research concluded that Novamin formed a protective layer on enamel after ten days of the remineralization phase [24]. SEM analysis revealed that bioactive glass plugs are larger, more angular, and more closely attached to the enamel surface than the plugs formed by CPP-ACP [25].

The next remineralizing agent, after CPP-ACP and SHY-NM, was Biomed Calcimax toothpaste. In the absence of additional additives, synthetic hydroxyapatite (HAP) particles adhere to pellicle-covered enamel surfaces, with adhesion strength being directly proportional to the organic enamel content and inversely proportional to the particle size (1.3 µm). By filling small pores on demineralized surfaces, HAP crystals stimulate remineralization [26].

The dipole characteristics of hydroxyapatite (HAP) and the electrostatic interactions between its molecules are key to the cohesion of crystallite clusters on the tooth surface, contributing to its antibiofilm effect and reducing solubility. HAP replaces fluoride and antimicrobials in toothpaste formulations, making it particularly beneficial for patients with dry mouth (xerostomia) [27]. HAP crystals enhance enamel remineralization and improve acid resistance, with lasting effects even after 3 days or 3 months. A study utilizing the Vickers microhardness tester and scanning electron microscopy (SEM) revealed that n-HAP toothpaste had greater remineralizing efficacy compared to bioactive glass (BAG) toothpaste, because of the smaller particle size of nanohydroxyapatite (50–1000 nm) compared to regular hydroxyapatite (1.3 µm) [28].

Calcium bentonite is considered to have the least potential for remineralization. Bentonite, a member of the phyllosilicate group of minerals, is named after Montmorillon, France. It is composed of layered clay minerals formed by silicon oxide (tetrahedral structure) and aluminum hydroxide (octahedral structure in a 2:1 ratio). These particles are plate-like, with an average diameter of around one µm. The process of cation exchange capacity (CEC), which occurs when lower-valence cations are replaced, leaving behind a net negative charge on adjacent oxygen atoms, allows for the attraction of calcium and phosphate ions. This attraction leads to supersaturation and facilitates remineralization [29].

Recently, calcium montmorillonite has been utilized as a filler in dental composites to enhance remineralizing properties [30]. However, there is a lack of research evaluating the remineralizing potential of calcium bentonite in toothpaste formulations.

The limitations of this research include the inability to replicate the oral environment and assess the protective capabilities. The study only observed quantitative changes, and the application period for the remineralizing agents was shorter than the recommended duration.

To draw firm conclusions, well-designed, high-quality clinical studies are still needed. Therefore, additional in vitro and in vivo research with larger sample sizes is necessary to validate these findings.

Conclusion

GC Tooth Mousse and SHY-NM demonstrated greater effectiveness in remineralizing artificially induced caries lesions compared to Biomed Calcimax and Bentodent. The application of CPP–ACP resulted in a significant reduction in lesion depth when compared to both the negative and positive controls, as well as Biomed Calcimax and Bentodent. However, there was no statistically significant difference when compared to SHY-NM. No significant differences were observed between group 3 and group 4, group 4 and group 5, or group 5 and group 6. Therefore, GC Tooth Mousse containing CPP-ACP is considered superior to SHY-NM, Biomed Calcimax, and Bentodent toothpaste.

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Conflict of Interest: None

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