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EVALUATION OF THE REMINERALIZING AGENTS AFTER ORTHODONTIC BRACKET DEBONDING: A SEM-EDX INVESTIGATION

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Objectives: To compare the remineralizing efficacy of (Fluoride (Fl) Varnish, Nanohydroxyapatite (n-HAp) serum and NovaMin-containing toothpaste) on premolars subjected to orthodontic debonding and a pH-cycling model.

Methods: 60 human premolars were divided into six groups (n=10). Group IA, sound enamel, IB, orthodontically treated premolars, IC, orthodontically treated premolars subjected to demineralization cycles. Three experimental groups included orthodontically treated premolars subjected to demineralization cycles and treated with Fl varnish (IIA), n-HAp serum (IIB), and NovaMin toothpaste (IIC). Surface topography and mineral content were evaluated using SEM-EDX analysis, and data were statistically analyzed using ANOVA test and Tukey's post-hoc test.

Results: SEM images of the experimental groups showed signs of surface precipitants and enamel prism reorganization. EDX analysis showed increased Ca and P where NovaMin recorded the highest Ca/P ratio followed by n-HAp serum, and then Fl varnish.

Conclusions: NovaMin demonstrated the highest remineralization potential increasing Ca and P content. n-HAp serum exhibited comparable remineralization efficacy to NovaMin, while Fl varnish demonstrated the lowest remineralization potential.

Keywords: NovaMin, Hydroxyapatites, Orthodontic appliances.

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Conflicts of interest:
The authors declare no conflicts of interest.

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ÉVALUATION DES AGENTS REMINÉRALISANTS APRÈS DEPOSE DE BRACKET ORTHODONTIQUES: UNE ÉTUDE SEM-EDX

Objectifs: Comparer l'efficacité reminéralisante du vernis fluoré (Fl), du sérum de nanohydroxyapatite (n-HAp) et du dentifrice contenant du NovaMin sur des prémolaires soumises à un décollement orthodontique et à un modèle de cycle de pH.

Méthodes: 60 prémolaires humaines ont été réparties en six groupes (n = 10) : groupe IA (émail sain), groupe IB (prémolaires traitées orthodontiquement) et groupe IC (prémolaires traitées orthodontiquement et soumises à des cycles de déminéralisation). Trois groupes expérimentaux comprenaient des prémolaires traitées orthodontiquement, soumises à des cycles de déminéralisation et traitées avec du vernis Fl (IIA), du sérum n-HAp (IIB) et du dentifrice NovaMin (IIC). La topographie de surface et la teneur en minéraux ont été évaluées par analyse SEM-EDX, et les données ont été analysées statistiquement par ANOVA et test post-hoc de Tukey.

Résultats: Les images SEM des groupes expérimentaux ont montré des signes de précipitations de surface et de réorganisation du prisme de l'émail. L'analyse EDX a montré une augmentation des concentrations de Ca et de P, NovaMin enregistrant le rapport Ca/P le plus élevé, suivi du sérum n-HAp, puis du vernis Fl.

Conclusion: NovaMin a démontré le potentiel de reminéralisation le plus élevé, augmentant la teneur en Ca et en P. Le sérum n-HAp a montré une efficacité de reminéralisation comparable à celle de NovaMin, tandis que le vernis Fl a montré le potentiel de reminéralisation le plus faible.

Mots-clés: NovaMin, hydroxyapatites, Appareils orthodontiques.

Introduction

Fixed orthodontic treatment is one of the treatment options used in the correction of malocclusion. Compared to removable orthodontic appliances, their fixed counterparts represent the preferred option for both orthodontists and patients as it provides better results, produces more complex tooth movement and most importantly does not require full patient compliance [1].

The final procedure in the fixed orthodontic therapy is the removal of the bonded attachments on the teeth. The enamel surface should then be restored as closely as possible to its original pretreatment condition. Yet, the use of adhesive material to attach the bracket to the tooth surface creates a problem upon treatment termination. This is because during the procedure of bracket debonding and removal of the residual adhesive material, injury to the enamel can occur [2].

Comprehensive review studies have concluded that most of the orthodontic debonding methods could produce severe iatrogenic damage to the enamel surface Among the potential sequela of removing orthodontic appliances is the loss of enamel surface layer and increased surface roughness leaving enamel more susceptible to demineralization in the oral medium [3, 4].

Scientists have investigated numerous materials with the potential to remineralize tooth enamel. Fluoride (Fl) has always been known to represent the gold standard in preventive dentistry. But due to its various drawbacks like limited penetration depth, pH dependency and fluorosis risk, there is a growing variety of non-fluoridated alternatives that show promising results [5]. Calcium (Ca) phosphate systems like nanohydroxyapatite (n-HAp) and bioactive glass materials like Ca sodium phosphosilicate (NovaMin) mimicking the natural composition of enamel, are now garnering increasing attraction .

The purpose of this study was, therefore, to compare the remineralizing efficacy of topical Fl varnish with n-HAp serum and NovaMin toothpaste following orthodontic bracket debonding under a pH cycling regimen, utilizing Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX).

The null hypothesis proposes that the remineralization efficacy of NovaMin toothpaste and n-Hap serum following orthodontic debonding will be higher than that of Fl varnish.

Materials and methods

This experimental design was revised by the Research Ethics Committee of faculty of dentistry, Ain-Shams University and received exemption number: FDASU-Rec EMO12228.

Sixty freshly sound premolar teeth recently extracted for orthodontic reasons were collected from the oral surgery clinic, MSA university. Teeth with surface cracks, hypoplastic or carious lesions and restorations on the buccal surfaces of the teeth were excluded. The samples were cleaned and stored in 0.9% saline solution under refrigeration until testing was commenced. The saline solution was changed weekly to avoid bacterial colonization.

Sample grouping

The teeth were randomly divided into a total of 6 groups with 10 samples each:

a. **Group I (Control groups):**

Consisting of 3 sub-groups;

- **Group IA (sound enamel):** sound premolars acted as –ve control.
- **Group IB (post-orthodontic debonding):** orthodontically treated premolars acted as +ve control.
- **Group IC (post-orthodontic debonding + DC):** orthodontically treated premolars were exposed to Demineralization Cycles (DC).

b. **Group II (Experimental Groups):**

Consisting of 3 sub-groups of orthodontically treated premolars exposed to DC:

- **Group IIA (Fl varnish):** were treated with Topical Fl Varnish (VOCO Profluoride Varnish® manufactured by VOCO, Germany) .
- **Group IIB (n-HAp serum):** were treated with n-HAp Serum (10% n-HAp Repairing Serum® manufactured by Prevdent international BV, Netherlands).
- **Group IIC (NovaMin toothpaste):** were treated with NovaMin-containing toothpaste (Sensodyne Repair and Protect® manufactured by GalaxoSmithKline, USA).

Sample Preparation

In the middle third of the buccal surfaces of all teeth a window 4 x 5 mm corresponding to the bracket bases was created by covering the selected area with pink wax then the remaining of the buccal surfaces was covered by nail varnish (Luna 280). After the varnish was applied and set, the pink wax was removed.

Simulated bonding and debonding procedures

Groups IB, IC, IIA, IIB & IIC samples were subjected to a 37% phosphoric acid gel (Etching agent) for 30 seconds, then were rinsed and dried with oil-free air spray for 15 seconds. An orthodontic adhesive resin was placed on the premolar bracket base then firmly pressed on the enamel windows of the buccal surfaces of the teeth. The samples were then stored in artificial saliva for 1 month at 37 C before the process of debonding. During this period, the artificial saliva was replaced once every 5 days. Debonding of the metal brackets was done by bracket removal with hand pliers and then remnant resin was removed by a low speed tungsten carbide bur followed by smoothing and polishing with a rubber cup and slurry of fine pumice [6].

Application of the remineralizing agents

Group IIA: Topical Fl varnish was applied to the enamel surfaces of Group IIA samples following the manufacturer's instruction in a single stroke painting motion for one time. The varnish was left on enamel for 4 minutes to dry before being placed in artificial saliva [7].

Group IIB: n-HAp repairing serum was applied to the enamel surfaces for 3 minutes and rinsed after 20 minutes with water. The process was performed once a day for 10 days [8].

Group IIC: NovaMin toothpaste was applied to the enamel surfaces using a Oral B PRO Trizone 500-800 with oscillating movements of 7600.0 rpm and pulsating movements of 20000.0 rpm with precision clean head brush for 3 minutes. The process was repeated twice a day for 10 days [9] .

In all groups, teeth were stored in artificial saliva. Finally, the specimens were washed thoroughly before entering the simulated pH cycling.

Simulated pH cycling

To accurately evaluate the remineralization of all experimental groups, pH cycling was performed to simulate the cycle of demineralization and remineralization of the enamel in the oral cavity. Groups 1C, IIA, IIB, & IIC were subjected to demineralization solution for 2 hours, three times per day, and returned to artificial saliva between the 2-hour cycles. Samples were thoroughly rinsed for 30-60 seconds with distilled water before placing them in counter solutions, to prevent any cross-reaction of chemical ingredients and affect the process. The artificial saliva and demineralizing solution were replaced daily [10].

SEM and EDX Examination

Examination was done to the middle third of buccal surface, after cleaning from any remaining solution. Teeth were refrigerated until examined by SEM .

Grand Egyptian Museum Conservation Center Scanning electron microscope unit (SEM) (Model FEI Quanta 3D 200i) attached with EDX Unit (Energy Dispersive X-ray Analyses / thermofisher pathfinder) was employed to investigate the morphological structures and elemental composition of the samples under operating conditions of accelerating voltage 20~30 K.V, resolution for Gun.1nm, at different magnifications using SED (secondary electron detector) and BSED (Back scattered electron detector) .Samples were fixed on aluminum stubs with standard diameter using a carbon double sticky tape. Representative images of different samples were selected .

Energy Dispersive X-ray (EDX) analysis was used to evaluate the surface mineral content of Calcium (Ca) and Phosphorus (P).

Results

SEM examination results

a. Group IA (intact enamel): The enamel surface presented the characteristic fish-scale pattern defined by enamel rod ends (EREs) appearing as shallow, saucer-shaped depressions with relatively regular margins (Figure 1).

b. Group IB (post-orthodontic debonding) exhibited numerous scratches and grooves, with residual adhesive resin present both superficially and within enamel rod ends (resin tags). Prism core dissolution was evident, accompanied by irregular enamel rod end morphology (Figure 1).

c. Group IC (post-orthodontic debonding + DC) showed preferential dissolution of prism core with elevated prism peripheries resulting in honey-comb like appearance, while other

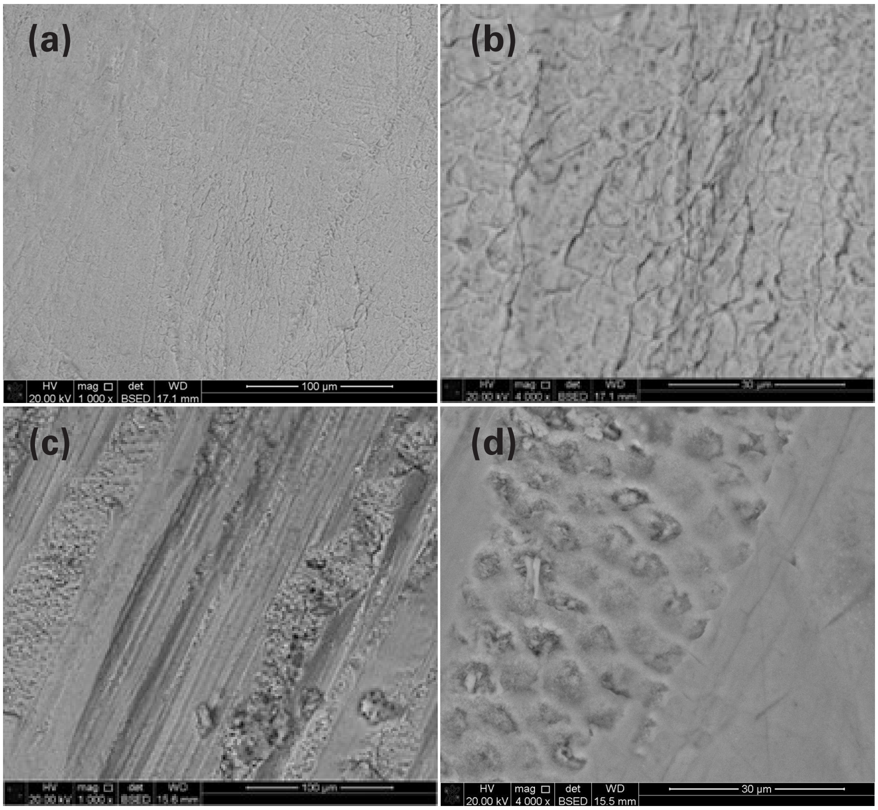

Figure 1. (a) SEM of IA (intact enamel) showing EREs in fish scale pattern (red arrows) (1000x); (b) A higher magnification of IA showing the characteristic fish-scale pattern of EREs with almost regular margins (red arrows) (4000x). (c) SEM of IB (post-orthodontic debonding) showing grooves (yellow arrows) and remnants of adhesive resin (red arrows) (1000x); (d) A higher magnification showing resin tags remnants in the concavities of the EREs (red arrows) (4000x).

areas showed dissolution of prism peripheries. Numerous EREs were observed on the enamel surface with irregular boundaries (Figure 2).

d. Group IIA (FI varnish) showed few scratches on the enamel surface. Some EREs were observed on the enamel surface with regular boundaries, while others were found with irregular boundaries. Preferential dissolution of prism core was also observed. Evidence of precipitates within the interprismatic area were observed leading to increased thickness of interrod region. Precipitates were also found on top of the EREs which presumably lead to their partial occlusion (Figure 2).

e. Group IIB (n-HAp serum) showed precipitation of rod-like crystallites within the inter-prismatic area and on top of the EREs. Precipitates were apparently more than in group IIA. Some EREs were observed on the enamel surface with irregular boundaries. Demineralization of prism peripheries and surface scratches were observed, although less pronounced compared to Group IIA (Figure 3).

f. Group IIC (NovaMin toothpaste) exhibited a higher prevalence of mineral deposits located both on the EREs within the inter-prismatic area. Some EREs were observed on the enamel surface with regular and irregular boundaries. Areas of demineralization and surface scratches were less pronounced compared to subgroups IIA and IIB (Figure 3).

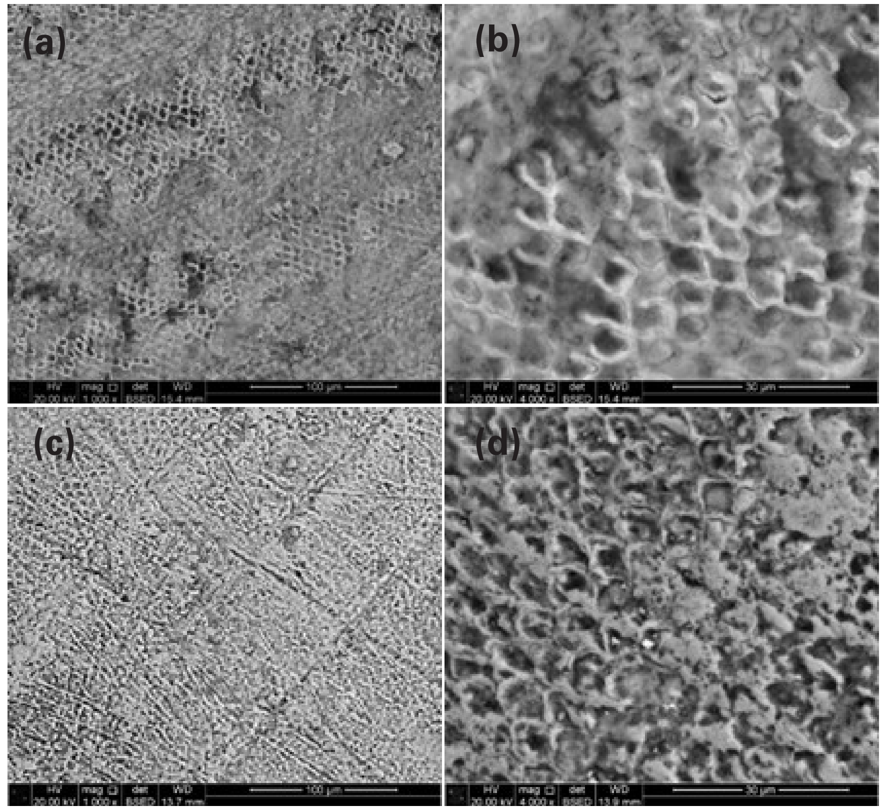

Figure 2. (a) SEM of IC (post-orthodontic debonding +DC) showing dissolution of prism cores resulting in honey-comb like appearance (yellow bracket) (1000×); (b) A higher magnification showing irregular prism boundaries (red arrows), dissolution of prism core and peripheries (yellow arrows) at (4000x). (c) SEM of IIA (FI Varnish) showing few scratches (yellow arrows), some remnants of adhesive resin (red arrows), areas with regular prism margins (yellow brackets) and other with irregular margins (red brackets) (1000×); (d) A higher magnification showing precipitate on top of the EREs and in the interprismatic area (yellow arrows) (4000x).

Statistical results (EDX Analysis)

To compare calcium and phosphate measurements across six distinct groups, the normality of the data was assessed using the Shapiro-Wilk test. For calcium measurements, the results showed that five groups (IA, IB, 2A, 2B, and 2C) were normally distributed ($p > 0.05$), while IC was not ($p < 0.05$). For phosphate measurements, the results showed that five groups (IA, IC, 2A, 2B, and 2C) were normally distributed ($p > 0.05$), while IB was not ($p < 0.05$). Given that the majority of the groups were normally distributed, an ANOVA was performed. The ANOVA results indicated a significant difference among the groups for both calcium ($F(5,54)=75.97$, $p < 0.001$) and phosphate ($F(5,54)=12.22$, $p < 0.001$).

Calcium (Ca)

The results in Table 1 showed that among the control groups, the highest value was recorded in subgroup IA (43.317) followed by subgroup IB (39.067) while subgroup IC was the lowest one (25.635). On the other hand, among the experimental groups, the lowest value was recorded in subgroup IIA (30.446) followed by subgroup IIB (35.239) while subgroup IIC was the highest one (38.363) (Figure 4). To further investigate specific Ca group differences, Tukey's HSD post hoc test was conducted with a significance level of 0.05. The post hoc analysis revealed significant differences between several pairs of groups (Table 2).

Table 1. The mean Ca values for each group.

Group	Mean Ca
IA	43.317
IB	39.067
IC	25.635
IIA	30.446
IIB	35.239
IIC	38.363

Table 2. The significant pairwise comparisons from the Tukey's HSD post hoc test following ANOVA, highlighting the Z-values, P-values, and significance of each comparison among the calcium measurement groups.

Comparison	Z-value	P-value	Significance
1A vs 1B	1.664	= 0.0011	Significant
1A vs 1C	6.159	< 0.001	Significant
1A vs IIA	4.955	< 0.001	Significant
1A vs IIB	3.393	0.010	Significant
1B vs 1C	4.494	< 0.001	Significant
1B vs IIA	3.291	0.015	Significant
1C vs IIC	4.276	< 0.001	Significant
2A vs IIC	3.073	0.032	Significant
2B vs 1C	6.837	< 0.001	Significant

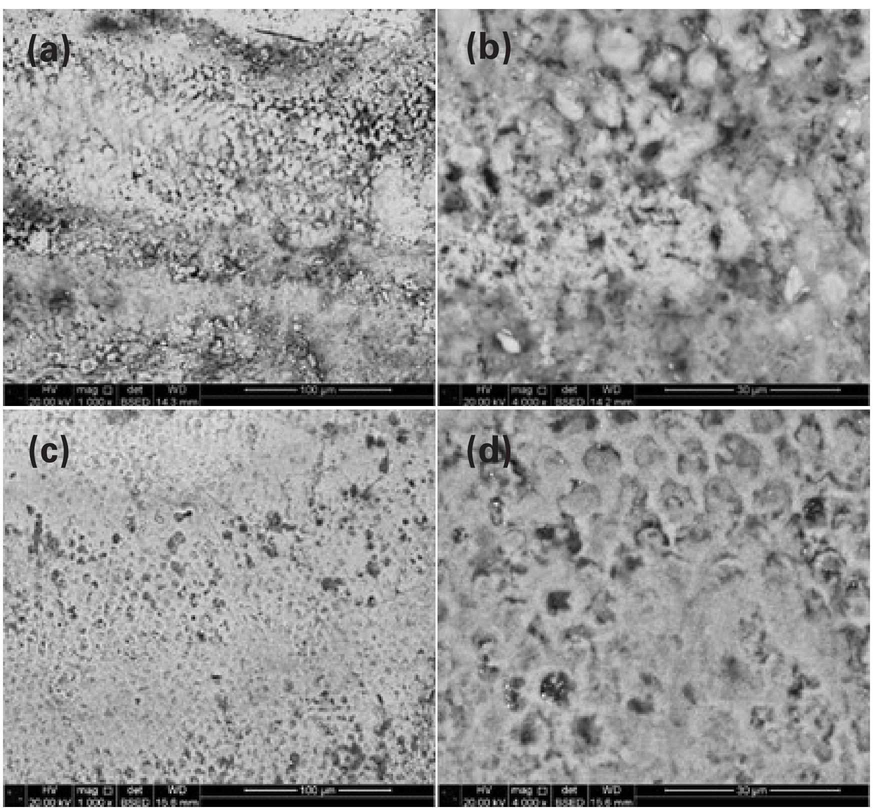

Figure 3. (a) SEM of IIB (n-HAP serum) showing crystallite precipitates on the enamel surface (red arrow), precipitates on top of the EREs (red bracket) and dissolution of prism core and peripheries (yellow arrow) (1000×); (b) A higher magnification showing precipitate on top of the EREs and inter-prismatic areas (red arrows) (4000x). (c) SEM of IIC (NovaMin toothpaste) showing surface precipitation (red brackets) and dissolution of prism core and peripheries (yellow arrows) (1000×); (d) A higher magnification showing precipitates on top of the EREs (red arrows), regular prism margins (yellow arrows) and irregular prism margins (green arrow) (4000x).

Phosphate (P)

The results in Table 3 showed that among the control groups, the highest value was recorded in subgroup IA (19.056) followed by subgroup IB (18.464) while subgroup IC was the lowest one (12.471). On the other hand, among the experimental groups, the lowest value was recorded in subgroup IIA (15.142) followed by subgroup IIB (17.501) while subgroup IIC was the highest one (18.091) (Figure 4). To further investigate specific P group differences, Tukey's HSD post hoc test was conducted with a significance level of 0.05 and significant differences were noted (Table 4).

Table 3. The mean Ca values for each group.

Group	Mean P
IA	19.056
IB	18.464
IC	12.471
IIA	15.142
IIB	17.501
IIC	18.091

These results indicate substantial variability in both calcium and phosphate levels among the groups, with certain groups showing more pronounced differences. The significant differences identified in the pairwise comparisons underscore the heterogeneity in both calcium and phosphate measurements across the groups.

Calcium/phosphate (Ca/P) ratio

To further understand the relationship between calcium and phosphate levels across the six groups, the calcium/phosphate (Ca/P) ratio was calculated by dividing the mean calcium value by the mean phosphate value for each group (Table 5). The calculated Ca/P ratios indicate a consistent relationship between Ca and P levels, with all groups exhibiting Ca/P ratios around 2. Specifically, the ratios ranged from 2.011 to 2.273. This consistency suggests a stable proportional relationship between Ca and P across the different groups, despite the variations in individual Ca and P measurements. This further underscores the importance of considering both minerals together when analyzing their levels and potential impacts on biological processes.

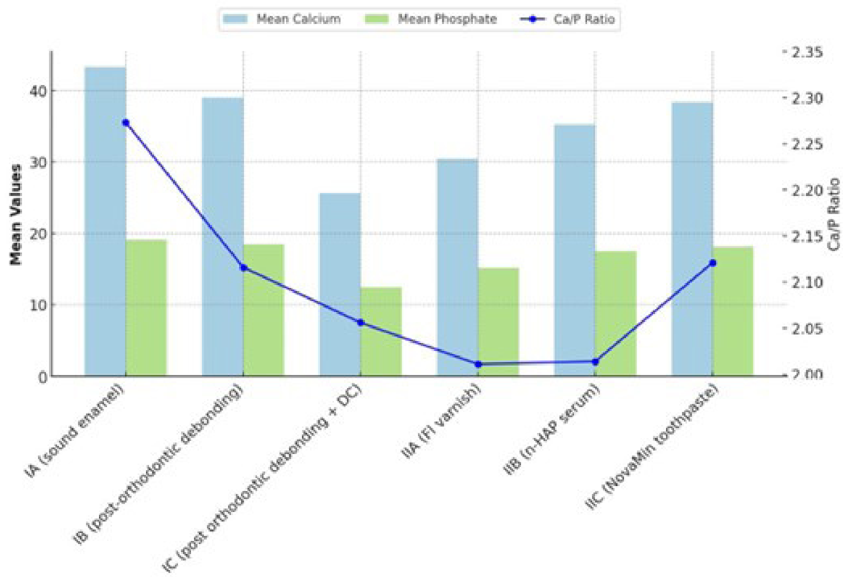

Figure 4. Bar chart showing mean calcium and phosphate values, and line chart showing Ca/P ratio for each group.

Table 4. The significant pairwise comparisons from the Tukey's HSD post hoc test following ANOVA, highlighting the Z-values, P-values, and significance of each comparison among the phosphate measurement groups.

Comparison	Z-value	P-value	Significance
1A vs 1C	6.585	< 0.001	Significant
1B vs 1C	7.177	< 0.001	Significant
1B vs IIA	4.505	0.015	Significant
1C vs IIB	-5.030	< 0.001	Significant
1C vs IIC	-5.620	< 0.001	Significant

Table 5. Ca/P ratios calculated by dividing the mean calcium values by the mean phosphate values for each group. The ratios suggest a consistent proportional relationship between calcium and phosphate across the groups.

Group	Mean Ca	Mean P	Ca/P Ratio
IA	43.317	19.056	2.273
IB	39.067	18.464	2.116
IC	25.635	12.471	2.056
IIA	30.446	15.142	2.011
IIB	35.239	17.501	2.014
IIC	38.363	18.091	2.121

The calcium/phosphate (Ca/P) ratios were subjected to statistical analysis to determine if there were significant differences across the six groups. The Shapiro-Wilk test was first used to assess the normality of the Ca/P ratios, resulting in a p-value of 0.217, indicating that the data were normally distributed ($p > 0.05$). Given the normal distribution of the ratios, a one-way ANOVA was conducted to compare the means of the Ca/P ratios among the groups. The results showed no significant differences among the groups, suggesting that the proportional relationship between calcium and phosphate levels is consistent across the different groups. The mean Ca/P ratio was 2.099 with a standard deviation of 0.089, further indicating the stability and consistency of these ratios across the groups (Figure 4).

Discussion

Orthodontic treatment, particularly the debonding process, has been associated with iatrogenic enamel damage. This damage can be attributed to various factors, including abrasive dental prophylaxis, acid etching, and the mechanical removal of adhesive resin. These procedures compromise enamel integrity, rendering it more susceptible to acid erosion and subsequent decalcification [11-13].

Fl has been established as the gold standard in preventive dentistry. However, its efficacy is limited by factors such as depth of penetration, pH dependency, and the risk of fluorosis. Consequently, a growing interest in alternative remineralization agents has emerged. Ca phosphate-based systems, including nano-hydroxyapatite (n-HAp) and Ca phosphosilicate, have gained significant attention due to their structural similarity to natural enamel. These materials have demonstrated promising remineralization properties in various studies [14].

There are few studies in literature that evaluated the effects of remineralizing agents on enamel surface morphology post-orthodontic debonding. Thus the aim of the present study was to evaluate and compare the efficacy of n-HAp serum and NovaMin toothpaste with Fl varnish on premolars subjected to orthodontic debonding and exposed to a pH-cycling model using SEM-EDX analysis.

SEM examination of Group IB (post-orthodontic debonding) revealed the presence of grooves on the enamel surface. Residual adhesive resin particles were also observed, likely due to the incomplete removal of resin tags embedded within the enamel structure. Irregular and poorly defined enamel rod ends were evident, along with evidence of demineralization. This demineralization could be attributed to the residual effects of acid-etching during the bonding process.

The previous results came in line with Fan [15] who observed scratches, deep grooves and scars on the enamel surface produced by diamond finishing burs after the adhesive remnant removal of orthodontic brackets of human maxillary premolars.

SEM analysis of Group IC (post-orthodontic debonding + DC) revealed cracks on the enamel surface, potentially resulting from the use of adhesive removal pliers. Dissolution affecting the enamel prism cores was observed. This could be explained by the implementation of a pH-cycling protocol, which induced more enamel demineralization in comparison to group IB. The findings of the present study align with those of Fan [15], who reported enamel surface fractures following adhesive removal using an enamel chisel. However, unlike the current investigation, Fan [15] did not employ a pH cycling model in their experimental design.

Evaluation of Fl Varnish group showed minimal presence of precipitates within the interprismatic regions and on top of the enamel rod ends. These findings align with those of Elewa [16], who reported similar mineral deposit formation following the application of Enamelast Fl varnish to artificial white spot lesions.

The n-HAp serum group exhibited the formation of rod-like crystallites within the interprismatic regions. These results coincided with Ragab [17] who attributed the enhanced remineralization capacity of n-HAp paste to its nanoscale dimensions, enabling penetration and occlusion of subsurface defects within the enamel matrix.

However, the present study disagreed with Ajami [8] who found no statistically significant reduction in enamel surface roughness after a 10-day application of the n-HAp serum following orthodontic debonding procedure. The authors attributed these results to the potential interference of residual resin with n-HAp particle adsorption. The divergent outcomes of Ajami [8] may also be explained by their exclusive focus on surface roughness assessment which may have precluded the evaluation of remineralization efficacy.

Evaluation of the NovaMin treated group revealed minimal surface scratches and irregular shaped, spherical deposits. Consistent with the present study, Hsu [18] SEM analysis revealed the presence of granular deposits within the intercrystalline spaces of NovaMin treated HA discs. The authors attributed this phenomenon to the interaction between NovaMin and HA crystals, resulting in morphological alterations and a reduction in intercrystalline gaps, thereby accelerating the remineralization process.

Our EDX analysis revealed a reduction in mass % of both Ca and P content within Group IB (positive control: post-orthodontic debonding) relative to Group IA (negative control: intact enamel). These findings confirm the established principle that the weight percentage of Ca and P diminishes progressively from the superficial to deeper enamel layers [19]. Notably, this depletion of mineral content was pronounced in Group IC (post-orthodontic debonding + DC), resulting in an exacerbated demineralization process where the Ca and P content exhibited significantly lower levels relative to Group IA (negative control: intact enamel). This could be attributed to the removal of the enamel's superficial layer, increasing the enamel's susceptibility to subsequent demineralization processes.

Conversely, results showed an increase in mineral content (Ca and P) among the treatment groups where NovaMin recorded the highest Ca/P ratio, followed by n-HAp serum , whereas the least Ca/P ratio was recorded in the Fl varnish group. These findings align with those of Chaudhary [20], who reported a lower remineralization potential for fluoridated toothpaste compared to calcium sodium phosphosilicate. The authors attributed this to Fl's self-limiting surface phenomenon which limits its penetration into the lesion depth following fluorapatite formation.

Moreover, Samineni [21] reported that n-HAp significantly increased Ca and P levels in demineralized enamel around orthodontic brackets. The authors proposed that the observed increase in mineral levels could be attributed to the role of n-HAp as a scaffold facilitating the deposition of additional minerals from the surrounding saliva onto the outer layer of enamel.

Furthermore, similar to the present study, Ergucu [22] demonstrated a significant elevation in Ca and P content within bleached enamel following treatment with Ca fluorosilicate glass (BioMin). The authors attributed this enhancement to the reactivity of the bioactive glass particles within the oral environment, leading to the formation of a hydroxycarbonate apatite (HCA) layer through a surface chemical reaction.

Howoever, factors like occlusal loads, pH changes and masticatory movements present *in vivo*, can influence the penetration and effectiveness of remineralization agents over time. Another possible limitation for this study is the fact that the oral cavity experiences temperature variations due to food and drink intake, which can affect the kinetics of chemical reactions involved in remineralization. This is typically controlled and constant in an *in vitro* study.

Conclusion

1- NovaMin demonstrated the highest remineralization potential by increasing enamel Ca and P content.
2- n-HAp serum exhibited comparable remineralization efficacy to NovaMin.
3- Fl varnish demonstrated the lowest remineralization potential in enamel following orthodontic debonding and demineralization cycling.

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