Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2025 June; 58(2): 157–162

Original article

The effect of Novamin on enamel content after bracket removal

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ABSTRACT

Background: *Orthodontics is one of the dental rehabilitation treatments for malocclusion correction, such as fixed orthodontic treatment using brackets, as the use of orthodontic brackets for a prolonged period can damage the enamel. Novamin contains casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), which can be used to prevent enamel erosion.* ***Purpose:*** *The aim of this study is to elucidate the effect of Novamin on the enamel content and microstructure surface after bracket removal.* ***Methods:*** *This study used 25 human premolar teeth randomly divided into five groups: I (Control); II (Biofix etching and bracket mounting); III (Biofix-Novamin); IV (Fuji Ortho); and V (Fuji Ortho-Novamin).* ***Results:*** *The results represent significant differences in the average levels of calcium (Ca) and phosphorus (P) among the treatment groups ($p \leq 0.05$). The changes to the enamel surface indicated the opening of enamel prisms, pits, and grooves. The levels of Ca and P increased after applying Novamin, especially in groups II and III, indicating remineralization on the enamel surface. When Novamin reacts with saliva, it starts to remineralize, and sodium ions are released. The sodium ions exchange with hydrogen cations, releasing Ca and P ions from Novamin particles.* ***Conclusion:*** *Novamin presented the capability to stimulate remineralization after orthodontic bracket debonding through altering the Ca and P levels on the enamel surface.*

Keywords: *acid etching; calcium level; enamel surface; Novamin; phosphorus level; medicine*
Article history: *Received 5 April 2023; Revised 6 May 2024; Accepted 7 May 2024; Online 15 March 2025*

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INTRODUCTION

Orthodontics is a dental rehabilitation treatment for malocclusion correction. The appliances consist of brackets, molar tubes, arch wires, and auxiliary components that attach directly to the enamel.[1] The function of the bracket as the power conductor is produced by arch wire and auxiliary components. Moreover, the brackets require durable adhesive material to attach to the enamel.[2] Damage to enamel tissue may occur if the shear bond strength of the adhesive material is greater than that of the enamel, and some enamel may be lost during bracket removal.[3]

Attaching the brackets involves multiple stages, including cleaning, conditioning, and finally, enamel surface bonding. Before the attachment process, 37% orthophosphoric acid, as the etching material, must be applied to the enamel. The primary purpose of the etching application is to remove contaminants and smear layers, which represent organic and inorganic layers, due to dental preparation. In addition, strong acid etching can enhance the interface bonding between the enamel and the composite resin (adhesive material), and it provides microporosity on the prismatic enamel surface as the bracket's retention site. This allows the resin to flow and penetrate to porosity, forming a mechanical interlocking system after the polymerization process.[4]

Hard tissues, such as enamel, can lose mineral ions of hydroxyapatite, referred to as the demineralization process.[5] Furthermore, demineralization caused by orthodontic treatment relates to obstacles in cleaning plaque around the orthodontic bracket site, so it provides a bacterial attachment on orthodontic bonding materials and the enamel surface during orthodontic treatment.[6] Demineralization is caused by the acidity of the etching solution and the duration of etching usage.[7]

DOI: 10.20473/j.djmkg.v58.i2.p157–162

In addition to the acid etching process, the bracket removal process is another orthodontic treatment that causes damage to the enamel.[8] This step evokes roughness on the enamel surface, as the etching process uses multi-fluted carbide burs, especially at the end of the cleaning process, and it induces 20–30 µm porosity of the enamel surface.[9]

Considering the impact of fixed orthodontic treatment, orthodontic patients require a remineralization agent to manage demineralization complications during orthodontic treatment. The remineralization agents might allow hydroxyapatite crystal reformation in the enamel through Ca and P deposition. The mineral accumulation will counter demineralization activity caused by orthodontic procedures and bacterial activity.[10] Remineralization occurs when Ca and P ions in the oral cavity are deposited into enamel crystals.[11]

The application of several materials can affect the enamel surface. Irianti's research found that the hardness of the enamel of a tooth that was submerged in a soy formula for 10 minutes was higher than that of a tooth that was soaked in a soy formula for five minutes.[12] A fluoride varnish application has also been proven to affect the surface roughness of primary teeth. Apriani's research showed that a fluoride varnish application can prevent early caries by reducing the demineralization process of primary teeth under acidic conditions.[13]

The results of a study by Adiba et al.[14] showed that teeth without a fluoride varnish had deeper erosion compared to teeth with a fluoride varnish. Another study indicated that teeth with a theobromine cocoa rind extract treatment had a harder enamel surface than those with a fluoride treatment.[15]

In this study, one of the materials used was Novamin, which is a component of bioactive glass particulates with a size of less than 20 microns. Novamin has been proven to be effective in helping to remineralize damaged tooth structures and prevent enamel breakage due to loss of precious minerals. However, there is limited information on the enamel content and microstructure surface after bracket removal and Novamin treatment. The reaction of minerals contained in Novamin and saliva stimulates Novamin activity. This reaction forms the hydroxyl-carbonate apatite (HCA) layer, the same as the enamel and dentine structure, indicating a remineralization process.[16] Therefore, the objective of this study is to determine the effect of Novamin on enamel content and microstructure surface after bracket removal using a scanning electron microscope (SEM).

MATERIALS AND METHODS

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medicine and Health Sciences, Muhammadiyah University of Yogyakarta, Indonesia, number 234/EP-FKIK-UMY/IV/2018. This research comprised a laboratory-based experiment that was carried out in March–April 2018 in the dental and oral skills lab room at Yogyakarta Muhammadiyah University Hospital. The experiment used samples of extracted premolars to which Novamin and orthodontic adhesives were applied. Immersion using artificial saliva was carried out in the biochemistry laboratory of Yogyakarta Muhammadiyah University. Observation of elemental content using an SEM was carried out at the Gunung Kidul Natural Materials Technology Research Institute.

A total of 25 extracted premolars was used in this study based on the sample size formula. The samples were categorized into five groups: a control group (I) that did not receive any treatment; Biofix, which contains inorganic filler (41.52%), bisphenol A-glycidyl methacrylate (Bis-GMA) (34.78%), dimethacrylate urethane ethylene, titanium dioxide, sodium fluoride, and a catalyst (II); Biofix-Novamin (III); Fuji Ortho (containing resin-modified glass ionomer cement) (IV); and Fuji Ortho-Novamin (V). The Novamin used was from commercial toothpaste that contains 5% Novamin (Sensodyne Repair & Protect Whitening).

The premolar teeth were obtained from the orthodontic clinic from patients undergoing orthodontic treatment that required the removal of premolars to gain space. The extracted teeth were soaked in NaCl solution at room temperature. The saline solution was periodically replaced every two days until the research was carried out to keep it moist.

In groups II and III, the enamel surfaces were cleaned and treated with acid etching using 37% phosphoric acid for 30 seconds, then rinsed and dried completely (Figure 1A). The brackets were then attached to the teeth using Biofix and polymerized with an LED light cure for 20 seconds. Groups IV and V underwent similar procedures to the previous two groups (enamel surface cleaning, 37% phosphoric acid etching, rinsing, and drying completely). After that, the brackets were applied using Fuji Ortho LC resin and polymerized with an LED light cure for 20 seconds (Figure 1B).

The samples were soaked in artificial saliva for seven days, simulating the conditions in the oral cavity. After seven days, the brackets were removed from the enamel surfaces with removal pliers, and any adhesive remnants were cleaned using orthodontic pliers (Figure 2). The enamel surfaces were then polished using a debonding bur. The Novamin (Sensodyne Repair & Protect Whitening) was applied to groups III and V with toothbrushes for three minutes. The application of Novamin was applied every 12 hours for 10 days.

After 20 days, the samples were observed using the energy-dispersive spectroscopy (EDS) technique of the SEM analysis to evaluate the enamel composition (Figure 3). The sample was coated with a gold–palladium alloy, and an SEM was used to observe the difference in the enamel surface. The levels of Ca and P were measured using EDS, which is a part of the SEM that can see the composition on the surface of the specimen. Energetic

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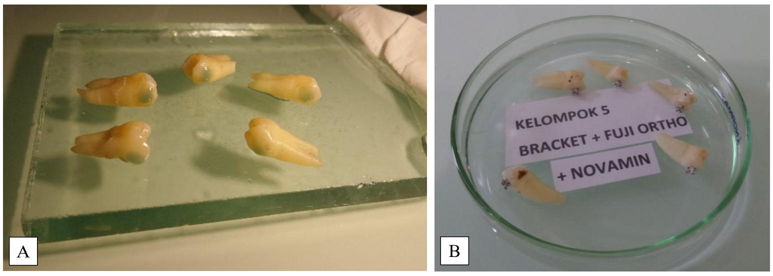

Figure 1. The immersion process of the samples. A. Acid etching process on the premolars' enamel surface, B. Mounting brackets, and immersion of all samples in artificial saliva.

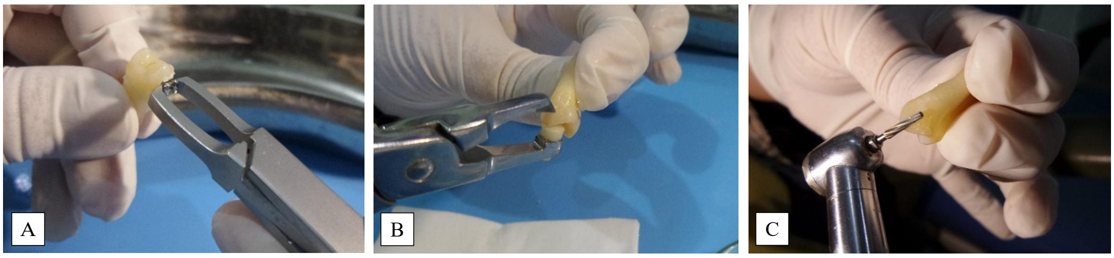

Figure 2. The bracket removal process. A. Bracket removal, B. Cleaning of the adhesive remnants using orthodontics pliers, C. Smoothing the enamel surface and polishing using a debonding bur.

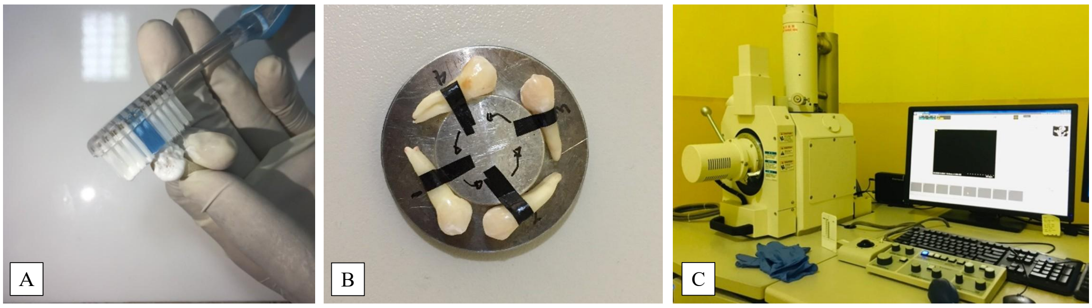

Figure 3. The application of the material and the analysis processes. A. Novamin application on groups III and V, B. SEM observation, C. The application of SEM with energy-dispersive x-ray spectroscopy (EDX) analysis.

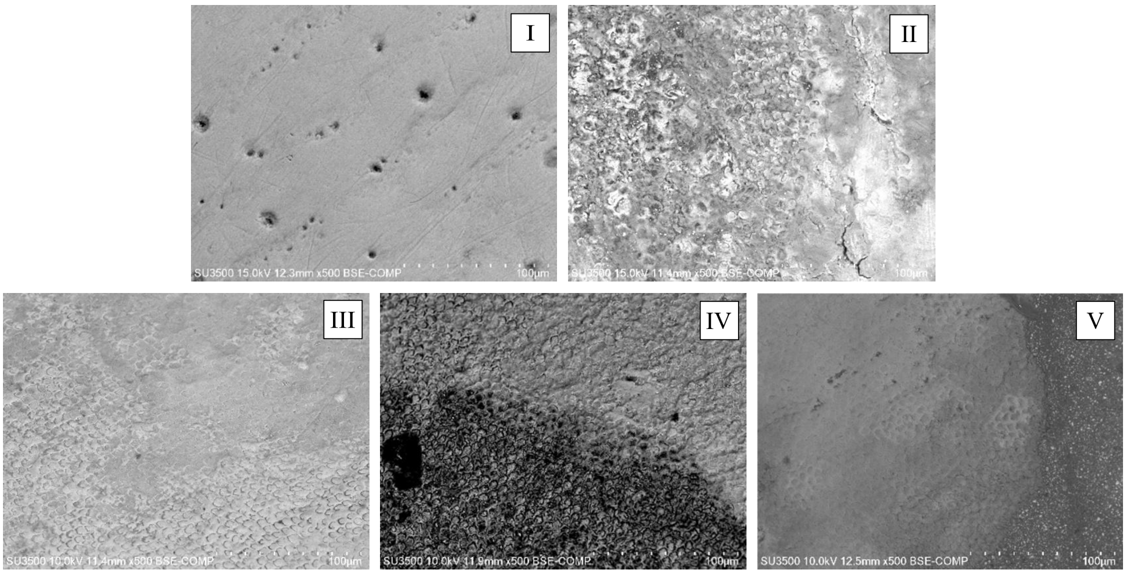

Figure 4. Enamel surfaces' SEM analysis following orthodontic bracket debonding (500 magnification); I (control), II (Biofix), III (Biofix-Novamin), IV (Fuji Ortho) and V (Fuji Ortho-Novamin).

DOI: 10.20473/j.djmkg.v58.i2.p157–162

electrons that illuminate the surface will produce X-ray photons, which will detect the composition contained in the specimen.

The normality of the data was assessed using the Shapiro–Wilk test. Subsequently, a one-way analysis of variance (ANOVA) was performed to evaluate significant differences among the groups. A p-value of < 0.05 was considered statistically significant. All analyses were conducted using the Statistical Package for the Social Science (SPSS) software program (IBM corporation, Chicago, Illinois).

RESULTS

An SEM electron microscope was used to observe and investigate the enamel surface characteristics (Figure 4). The results show significant differences in characteristics between the control group (I) and the treatment groups (II–V). Grooves and dentinal tubule openings were observed in the control group. The SEM observation showed an irregular enamel surface, and the edge of the enamel prism could not be identified in the Biofix group (II). In the Biofix-Novamin group (III), a smooth surface was present on the enamel topography after remineralization. Visible mineral deposits spread to the former surface of the enamel that exhibited porosities. In the Fuji Ortho group (IV), a cobblestone-like appearance was present, with a disappeared prism line on the enamel surface. The Fuji Ortho group (V) presented a smooth enamel surface and closure of the dentinal tubules.

In addition to analyzing the enamel surface topography, the SEM analyzes the elements and compounds of solid objects quantitatively, such as Ca, P, and O. Table 1 shows the levels of Ca, O, and P on the enamel surface of the orthodontic bracket debonding after Novamin application. The control group presented the highest levels of Ca and P (37.9 ± 4.3 and 16.3 ± 1.2, respectively) and group II presented the lowest levels (26.6 ± 2.4 and 11.7 ± 1.1, respectively). Group II indicated the highest level of oxygen (41.7 ± 2.0), and group V demonstrated the lowest level (39.5 ± 4.7). The groups with Novamin application (III and V) showed higher mineral and lower oxygen levels than the groups without Novamin application (II and IV).

All results except oxygen levels showed statistically significant differences ($p < 0.05$). Treatment-wise, group II was significantly different from group III regarding Ca levels but did not significantly differ from group IV (Table 1). The ANOVA test results indicated that the Ca and P levels significantly differed between groups II and III ($p < 0.05$).

DISCUSSION

SEM with EDX is a suitable method for analyzing tooth enamel deposits. This study revealed the topography of the enamel surface that each group presented based on the SEM-EDX analysis. After debonding and bracket removal, the enamel topography presented erosive and rough surfaces. The fixed orthodontic treatment procedures probably caused demineralization and erosive on the enamel surface. Previous studies revealed that fixed orthodontic treatment procedures cause demineralization, leading to the formation of white spots and dental caries.[17] This study revealed that the enamel surface in the groups with Novamin application was smoother than those of the other groups. The mineral compound in Novamin may cover the porosity to refine the enamel surface. Novamin, a mineral-rich material, remineralizes the enamel surface. The remineralization process occurs when the Ca and P contained in Novamin is provided adequately in the oral environment. Moreover, it enhances the pH of the oral environment, leading to ion precipitation to form the HCA layer.[18] Novamin contains CPP-ACP and nanocomplexes, including carbonate-hydroxyapatite nanocrystals and calcium sodium phosphosilicate. Hsu et al. revealed that silica-containing Novamin releases crystalline HCA, which has the same structure as enamel.[16] Novamin also contains 45% SiO_2, 24.5% CaO, 24.5% Na_2O, and 6% P_2O_5 that allow tissue integration by generating HCA.[19]

Moreover, the SEM analysis indicated that the surface of the control group's enamel crystal was homogeneous with pits and grooves. Therefore, the treatment groups produced several types of patterns on the enamel surface due to the etching procedure. Next, the acid etching process resulted in the dissolution of hydroxyapatite in the enamel, which provided microporosity mechanical bonding of the resin material. Pithon classified acid etching patterns as the following: Type 1, the enamel surface tends to be rough due to the process of acid etching and produces hollow areas, with transparent edges of the enamel prism; Type 2, the

Table 1. Mineral and ion levels after Novamin application in orthodontic bracket debonding (n = 25)

Group	N	Ca (%)	O (%)	P (%)
Control (I)	5	$37.9^{b} \pm 4.3$	41.4 ± 3.6	$16.3^{c} \pm 1.2$
Biofix (II)	5	$26.6^{a} \pm 2.4$	41.7 ± 2.0	$11.7^{a} \pm 1.1$
Biofix-Novamin (III)	5	$36.3^{b} \pm 3.6$	40.5 ± 3.9	$15.1^{bc} \pm 1.3$
Fuji Ortho (IV)	5	$33.1^{ab} \pm 5.7$	40.5 ± 5.5	$13.8^{ab} \pm 1.6$
Fuji Ortho-Novamin (V)	5	$35.8^{b} \pm 3.6$	39.5 ± 4.7	$15.2^{c} \pm 1.1$
Sig.		0.003	0.929	0.000

Data is represented in mean ± standard deviation and was analyzed using a one-way ANOVA. Different letters indicate significant statistical differences ($p < 0.05$). Ca = calcium, O = oxygen, P = phosphorus.

DOI: 10.20473/j.djmkg.v58.i2.p157–162

edge of the enamel prism starts to disappear and leaves only the prism core of the enamel (the enamel surface in Type 2 is the opposite of the Type 1 enamel surface); and Type 3, the enamel surface is irregular, and the enamel prism pattern cannot be defined. The enamel surface in Type 3 is a combination of the Type 1 and Type 2 images.[20]

Groups III and V showed the effect of Novamin in the remineralization of the enamel surfaces (Figure 4). The remineralization process was revealed by the level of Ca and P enhancement, especially between groups II and III. However, the Ca and P levels did not show a significant difference between groups IV and V. The results showed significant differences in the Ca and P levels between the groups, particularly group II. Groups III and V (treatment with Novamin) presented high Ca and P levels. The enamel surface became more homogeneous due to the deposition of minerals from the remineralization process, which closes the dentinal tubules. The rough edges of the enamel prism after acid etching begin to thicken and look clearer.[20]

The 37% orthophosphoric acid etching procedure might reduce the level of Ca and cause mineral compound loss on enamel surfaces. Abreu et al.[21] stated that acid etching damages and demineralizes the enamel surface. Additionally, the etching process opened enamel prisms, which caused a loss of acquired pellicles on the enamel surface. Demineralization is the process of losing the hydroxyapatite crystal ion $[Ca_{10}(PO_4)_6(OH)_2]$ contained in enamel, dentine, cementum, and bone.[22] The pH level and the amount of calcium phosphate ions in saliva influence enamel demineralization.[23] Calcium phosphate is one of the hydroxyapatite components, an essential mineral for bone and tooth formation. Calcium plays a vital role in the elastic modulus's primary determinant in the bone matrix.[24] Meanwhile, the levels of O showed an insignificant difference (p-value > 0.05). This aligns with previous research conducted by Sabel et al.,[25] which showed significant differences in C (carbon), N (nitrogen), Ca, and P content in lesioned enamel compared to healthy enamel. The Ca content was found to be lower in lesioned enamel. More significant amounts of C and N and lesser amounts of Ca and P were observed in lesioned enamel compared with healthy enamel.[25] Regarding the levels of O, no significant differences were observed between lesioned and healthy enamel. Ca and P strongly influence the remineralization process, causing differences in Ca and P levels but not affecting O levels.

Due to the rich mineral compositions, this study used adhesive materials from Fuji Ortho LC and Biofix. Fuji Ortho LC is an orthodontic bonding material based on a resin-modified glass ionomer. This material can release fluoride by 9.47 ppm on the first day.[16–19] Biofix is a composite resin-based orthodontic bonding material that has a petroleum load (41.52%), Bis-GMA (34.78%), urethane dimethacrylate ethylene, dioxide titanium, sodium fluoride, and a catalyst. The polymerization of Biofix uses a curing light, which releases fluoride that demineralizes enamel. Research conducted by Pithon et al.[26] indicated that Biofix materials release fluoride at about 10.58 ppm on the first day of observation.

This study revealed that the Ca and P enhanced with Novamin treatment, especially in group III, were significantly different from group II. A previous study by Mohanty et al.[10] found that Novamin indicated a remineralization ability in lesions on the enamel surface around orthodontic brackets. In that study, the Ca/P ratios were evaluated by EDX analysis in a different period after Novamin treatment. The results showed a significant difference in Ca/P ratios after 10 days of treatment. Remineralization is the process of returning lost minerals by calcium phosphate ions assisted by fluoride, and this process involves the reforming of surfaces in areas that have undergone demineralization.[24]

Novamin activity will start remineralizing when reacting with saliva. After that, sodium ions are released and exchanged with hydrogen cations. This ion exchange releases Novamin's calcium and phosphate ions. Moreover, the release of sodium ions increases the pH and precipitates the released calcium and phosphate ions, causing them to settle and form a HCA layer, eventually closing dentinal tubules.[27]

This study observed that Novamin application produced a smooth enamel surface, indicating remineralization on the enamel surface after debonding fixed orthodontic treatment. Moreover, the levels of Ca and P increased on the enamel surface after Novamin application, especially with the treatment of Biofix-Novamin. Therefore, Novamin is an effective agent for repairing enamel damage caused by orthodontic brackets. However, this research only analyzed the surface of the enamel. Future research could use pure Novamin, measure the depth of the enamel microporosity, and be a marker of success of the remineralization process after the application of Novamin.

ACKNOWLEDGEMENT

This research was supported by funding from Muhammadiyah University of Yogyakarta. We thank our university for providing financial support, facilities, and infrastructure that greatly assisted to this research.

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DOI: 10.20473/j.djmkg.v58.i2.p157–162

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DOI: 10.20473/j.djmkg.v58.i2.p157–162