Clinical, Cosmetic and Investigational Dentistry

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ORIGINAL RESEARCH

Effect of Fluoride Varnish on Enamel Microhardness: An in vitro Study

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Background: Remineralization counteracts demineralization, which is the loss of mineral ions from dental enamel, by restoring the mineral. Remineralization itself could be enhanced by external factors such as fluoride contained in fluoride varnish. This study aimed to determine the difference in enamel microhardness before and after applying 5% sodium fluoride (NaF) fluoride varnish.

Materials and Methods: This experimental laboratory in vitro study used 40 premolar crowns as samples. All samples were demineralized in a pH 4 demineralizing solution for 6 hours. The samples were split into two groups, each containing 20 samples. Group I (Control) acted as a control and Group II (Fluoride Varnish) was treated with 5% NaF (fluoride varnish). Then all samples were immersed in artificial saliva for seven days. A Vickers microhardness tester measured the samples' pre-demineralization, post-demineralization, and post-remineralization microhardness. The dependent sample *t*-test analysis was used to determine the difference in microhardness in each group. The two sample *t*-test analysis was used to determine the difference in microhardness between groups.

Results: The findings indicated that fluoride varnish application increases the demineralized enamel microhardness in Group II (Fluoride Varnish) from 174.63 VHN (SD = 23.12; 95% CI: 163.81–185.44) to 270.58 VHN (SD = 26.52; 95% CI: 258.17–282.99) (p-value = 0.000). The increase in Group II (Fluoride Varnish) was higher than in Group I (Control) after remineralization of seven days (p-value = 0.002).

Conclusion: The findings show a significant increase in enamel microhardness following fluoride varnish application, measured on the seventh day (p-value 0.000). This demonstrates the effectiveness of fluoride varnish in increasing enamel hardness under these in vitro experimental conditions. This study can serve as a reference for clinicians selecting fluoride varnish as a caries prevention effort.

Keywords: enamel microhardness, fluoride varnish, remineralization, demineralization

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Introduction

Caries is a multifactorial disease that occurs if an imbalance between demineralization and remineralization processes leads to the local loss of hard tooth tissue. Recurrent demineralization may occur due to environmental pathology, such as enamel exposure to acid produced by bacteria, which causes the hard tooth tissue to dissolve and be destructed, clinically forming a carious lesion.[1–3]

Teeth demineralization is the primary mechanism of mineral loss in the enamel, which consists of 96% hydroxyapatite (HAP).[1,2,4,5] The mineral loss, initiated by exposure to acidic conditions, leads to erosion and carious lesions.[1–3,6]

Restoring them by remineralizing both enamel and dentin is possible to stop the loss of minerals. This process involves replacing the minerals lost from the enamel and dentin due to demineralization. Saliva ions naturally carry out remineralization and can be enhanced by external factors or elements such as fluoride.[2,6,7] Fluoride acts as a catalyst for the diffusion of calcium and phosphate ions into the tooth, remineralizing the lesion's crystal structure.[1,8,9] It also supports fluorapatite (FAP) mineral formation, which is more acid-resistant.[10] The rebuilt crystal surface consisting of HAP and FAP is more resistant to acid attack.[1,6,11]

Received: 20 March 2025
Accepted: 4 June 2025
Published: 12 June 2025

Fluoride varnish, a topical fluoride, is applied to the teeth to form a thin film that lasts long using sustained-release fluoride.[10,12,13] It usually comprises 5% sodium fluoride (NaF) with 22,600 ppm of fluoride ions or 1% difluorosilane (DFS) with 1000 ppm of fluoride ions.[12,14,15] The 5% sodium fluoride (NaF) is the most effective for preventing caries and as a bactericidal.[10] The application is easy to perform and inexpensive.[10] The American Dental Association (ADA) Council on Scientific Affairs supports the use of fluoride varnish as a caries prevention agent.[11]

Topical fluoride, including fluoride varnish, inhibits bacteria, forms FAP, reduces demineralization, and increases remineralization.[15] Fluoride targets bacterial acids, reducing their formation and preventing bacterial colonization of teeth. In vitro studies show that fluoride ion inhibits bacterial enzymes. Fluoride released from the reservoir would inhibit demineralization and increase remineralization rate.[15] The topical application of fluoride causes ion exchange between HAP and fluoride ions. It interacts with ions in HAP and replaces the hydroxyl group (OH^-) with fluoride ions (F^-), forming FAP ($Ca_{10}(PO_4)_6F_2$). FAP crystal has a more stable structure than HAP since it is more acid resistant and has lower solubility.[6,11,15] When fluoride varnish is applied, it creates calcium fluoride, which acts as a reserve of fluoride ions. If the pH level in the plaque drops, the reserve becomes a source of fluoride. By releasing fluoride, the enamel's loss of phosphates and calcium is stopped, and the fluoride diffuses with the acid from the plaque into the enamel.[11,15]

The mineral content of enamel is directly related to its microhardness value. Enamel microhardness, its mechanical resistance to indentation, measures changes in mineral levels caused by demineralization or remineralization.[2,12,16,17] The surface material's ability to resist penetration by a diamond point or steel ball at a particular load is used as a basis for several surface microhardness tests, including the Vickers, Knoop, Brinell, Barcol, Rockwell, and Shore tests.[12,17]

Previous in vitro studies have investigated several remineralizing agents' effects on enamel microhardness. For example, Hidayat et al (2022) reported that CCP-ACP increased enamel microhardness in demineralized teeth on the 21st day of application, while Irmaleny et al (2023) reported that fluoride varnish and CCP-ACP increased enamel microhardness in bleached teeth on the 7th day of application.[17,18] Meanwhile, Sulistianingsih et al (2017) reported that Theobroma cacao (cocoa bean extract) increased enamel microhardness in demineralized teeth on the 7th day of application.[19] However, these studies were done under different conditions hence making direct comparison more difficult. This leaves a gap in the information on fluoride varnish's ability to restore enamel microhardness in demineralized teeth. The Vickers microhardness test was chosen due to its precision and reproducibility. This method was also used across multiple studies regarding enamel microhardness, allowing direct comparisons between findings in this study with those of previous ones.[13,17–20]

This study aimed to determine the difference in enamel microhardness before and after applying 5% sodium fluoride (NaF) fluoride varnish. The null hypothesis for this study is that there is no significant difference in enamel microhardness before and after fluoride varnish application. The findings are intended to serve as a reference for clinicians in selecting appropriate remineralizing agents for patient care.

Materials and Methods

This study complies with the Declaration of Helsinki within the newest version (2024) and all the sample collected were given verbal and written consent also have been allowed by Universitas Padjadjaran Research Ethics Committee. This experimental laboratory in vitro study used 40 mandibular and maxillary premolar teeth. The sample size was determined according to the Central Limit Theorem. A sample size of 30 or greater is generally sufficient to approximate a normal distribution.[18] The samples are selected according to inclusion and exclusion criteria. The inclusion criteria were human premolars extracted for orthodontic reasons from patients aged 20–23 years. Each tooth was carefully inspected both visually and under transillumination to ensure eligibility. Teeth were excluded if they exhibited visible caries, restorations, hypocalcification, erosion, or cracks. All samples were stored in a 0.9% NaCl solution.

All samples were cut horizontally 1–2 mm below the CEJ (cemento-enamel junction) using a separating disc bur (Renfert Dynex Brillant Grinding Disc 0.8 x 20mm) to separate the crowns from the roots. The teeth crowns were positioned on the liquid epoxy resin in a mold with the buccal side facing up.[21–23] The resin is removed from the mold after hardening. The upper surface of the samples was leveled and smoothed using silicon carbide paper grit for accurate microhardness measurements. Each sample was cleaned with distilled water before being tested.[22,23]

The samples' pre-demineralization, post-demineralization, and post-remineralization microhardness were measured by a Vickers microhardness tester (DUROLINE M Micro-Vickers) with a 200-gram force applied three times to the buccal surface of each tooth for ten seconds.[18] All measurements in this study were performed by the same examiner to ensure intraexaminer reliability. All samples were demineralized using an isotonic drink (POCARI SWEAT ION WATER, licensed from Otsuka Pharmaceutical Co., Ltd.,) with a pH of 4 for six hours in an incubator with a temperature of 37°C. The samples were split into two groups, each containing 20 samples. The samples were randomly assigned to each group using the Google Random Number Generator to ensure unbiased allocation. The examiner was blinded to group assignments during measurements to minimize assessment bias.

Group I was the control without the remineralization material and Group II was treated with 5% NaF (Clinpro White Varnish: 3M ESPE, Lot 9332047 1) applied directly using a brush for one coat. Then all samples were immersed in artificial saliva (composed of 12 mm NaCl, 3.4 mm KSCN, 17.8 mm NaHCO3, 16 mm KCl, 22 mm Urea, 1.5 mm Na2HPO4, and 1.5 mm KH2PO4) for seven days.[17] Artificial saliva in the two groups was replaced every day. The 7-day period was selected to enable direct comparison with prior studies investigating remineralization agents. Those studies also have shown that a 7-day period was enough for noticing a statistically significant increase in enamel microhardness due to remineralization.

Statistical analyses were done using IBM SPSS Statistics Version 29 (IBM Corp.,). The dependent samples *t*-test analysis was used to determine the difference in microhardness in each group. The two-sample *t*-test analysis was used to determine the difference in microhardness between groups.

Results

The enamel microhardness of Group I (Control) decreased after demineralization from 279.15 VHN (SD = 37.87; 95% CI: 261.43–296.87) to 178.98 VHN (SD = 24.37; 95% CI: 167.58–190.39) but increased after remineralization to 234.75 VHN (SD = 38.66; 95% CI: 216.66–252.85). The enamel microhardness of Group II (Fluoride Varnish) decreased after demineralization from 261.92 VHN (SD = 33.81; 95% CI: 246.10–277.74) to 174.63 VHN (SD = 23.12; 95% CI: 163.81–185.44) but increased after remineralization to 270.58 VHN (SD = 26.52; 95% CI: 258.17–282.99), Figure 1.

There was a significant difference between the pre-demineralization and post-demineralization enamel microhardness values in both Group I (Control) and Group II (Fluoride Varnish), with a p-value 0.000. In both groups, the post-

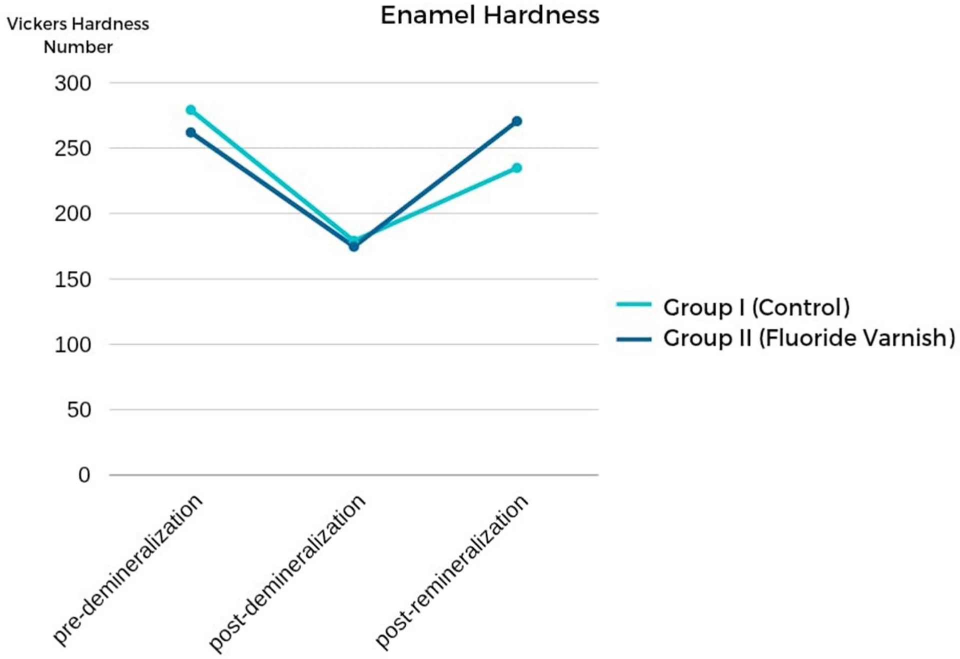

Figure 1 A graph showing the average enamel hardness values in Group I (Control) and Group II (Fluoride Varnish).

Table 1 Dependent t-Test Analysis Result Showing the Differences in the Microhardness of Group I (Control) and Group II (Fluoride Varnish)

	Group I (Control)		Group II (Fluoride Varnish)	
	Pre-Demineralization and Post-Demineralization	**Post-Demineralization and Post-Remineralization**	**Pre-Demineralization and Post-Demineralization**	**Post-Demineralization and Post-Remineralization**
Mean differences	100.17	−55.77	87.29	−95.96
p-value	0.000	0.000	0.000	0.000
Conclusion	Highly significant	Highly significant	Highly significant	Highly significant

Table 2 The Average Value of Microhardness and the Results of Independent t-Test Analysis of Group I (Control) and Group II (Fluoride Varnish). *significant Difference Between Groups (p < 0.05)

	Group I (Control)	Group II (Fluoride Varnish)	Mean Differences	p-value
Pre- demineralization	279.15 (37.87)	261.92 (33.80)	17.23	0.137
Post-demineralization	178.98 (24.37)	174.63 (23.11)	4.35	0.565
Post-remineralization	234.75 (38.66)	270.58 (26.51)*	−35.83	0.002

demineralization microhardness values were lower than the pre-demineralization microhardness values. There was a significant difference between the post-demineralization and post-remineralization enamel microhardness values in both Group I (Control) and Group II (Fluoride Varnish), with a p-value 0.000. In both groups, the post-remineralization microhardness value was higher than the post-demineralization microhardness value. The results of the dependent samples *t*-test analysis are shown in Table 1.

There was no significant difference in the microhardness of pre-demineralization enamel in Group I (Control) and Group II (Fluoride Varnish) (p-value = 0.137). Post-demineralization enamel microhardness in Group I (Control) and Group II (Fluoride Varnish) had no significant difference (p-value = 0.565). Post-remineralization enamel microhardness in Group I (Control) and Group II (Fluoride Varnish) had a significant difference (p-value = 0.002). The results of the two sample *t*-test analysis are shown in Table 2.

Discussion

This study showed a significant reduction in tooth enamel microhardness, indicating the demineralization that occurs. Demineralization in this study was carried out by immersing the sample for 6 hours in an isotonic drink, an acidic solution, in an incubator with a temperature of 37°C. This is in line with research conducted by Hidayat et al, which shows that isotonic drinks can demineralize tooth enamel because it has a pH of 4, which can dissolve HAP in tooth enamel.[17] Kim et al also demonstrated that beverages with a pH below 4.0, such as fruit juice, soft drinks, and other market-available drinks, can reduce tooth enamel microhardness.[21] The oral cavity can become acidic due to food or drink consumption and fermenting bacteria, which can dissolve HAP in tooth enamel if the pH value is lower than the critical pH value.[1–3] The critical pH value for enamel varies depending on multiple factors, but generally, it is around 5.5.[1,2,4]

The process of demineralization can be reversed, and the lesions that form can be repaired as long as cavitation has not been formed and cariogenic bacteria have been reduced.[15] Calcium and phosphate ions move from tooth enamel to saliva during demineralization and vice versa during remineralization.[15] The remineralization process will replace the minerals that were lost due to demineralization.[2,6] However, it depends on pathological factors that support demineralization (cariogenic bacteria, fermentable carbohydrates, and salivary disorders) and protective factors that promote remineralization (antibacterial agents, adequate saliva, and remineralizing ions). Remineralization can occur naturally or can be facilitated with the use of remineralizing agents.[1,9]

https://doi.org/10.2147/CCIDE.S526038

In Group I (Control), there was a noticeable improvement in tooth enamel microhardness, which suggests that remineralization occurred. Specifically, after seven days of remineralization in artificial saliva, Group I (Control) experienced an increase in enamel microhardness. The pH of the artificial saliva used during this study is neutral, at 7, higher than the critical pH value of 5.5. Typically, saliva has a pH range of 6.2–7.6, and its buffer capacity plays a crucial role in controlling demineralization and providing primary protection against tooth erosion.[22,23] Saliva contains ions that aid natural remineralization by depositing calcium and phosphate ions.[6,15,23] The artificial saliva used in this experiment also contains phosphate ions that aid in the remineralization process—this natural improvement results in increased minerals in tooth enamel.[23]

The microhardness of tooth enamel significantly increased in Group II (Fluoride Varnish), indicating successful remineralization. This group experienced an increase in enamel microhardness after fluoride varnish application and immersion in artificial saliva. Remineralization was facilitated by the combination of artificial saliva and fluoride, present in the 3M Clinpro White Varnish used (with 22,600 ppm fluoride). These findings support previous studies by Irmaleny et al and Sulistianingsih et al, where enamel microhardness increased significantly after topical fluoride varnish application.[18–20] The high adhesion of fluoride varnish to the enamel surface enables it to remain in place for several days, allowing for more fluoride absorption on the teeth.[10] Most fluoride ions are released in the first three weeks following application.[13–15]

The study found that applying fluoride varnish resulted in a harder enamel surface due to calcium fluoride formation from high fluoride concentrations. Fluoride ions react with tooth enamel to form calcium fluoride, a fluoride ion reservoir. As a result, fluoride ions released from the reservoir react to prevent the mineral loss, become a remineralization catalyst, and promote the formation of a more stable mineral called FAP.[11,14,15,18]

Fluoride varnish in this experiment played a role in increasing natural remineralization and forming FAP in tooth enamel.[11,15] It acts as a catalyst for the diffusion of calcium and phosphate ions into teeth, increasing mineral content.[1,8,9] The experiment also showed that fluoride varnish plays a role in increasing remineralization by forming FAP. This mineral is more acid-resistant and is formed by substituting hydroxyl groups on HAP with fluoride ions. Fluoride can only replace 10% of the hydroxyl groups on the enamel surface. A hydroxyapatite-fluorapatite bond is created as a result of this partial replacement, and it is more durable, acid-resistant, and less soluble.[15]

Another remineralizing agent, such as CCP-ACP has also been proven to increase enamel microhardness. Hidayat et al and Irmaleny et al reported that the application of CCP-ACP on demineralized and bleached teeth respectively had increased the enamel microhardness. CCP-ACP works differently than fluoride varnish since it promotes enamel remineralization by delivering calcium and phosphate ions.[17,18]

The findings indicated that fluoride varnish application increases the demineralized enamel microhardness. The increase in microhardness was greater in Group II (Fluoride Varnish) than in Group I (Control) after seven days of remineralization. The study suggests that fluoride varnish supports remineralization hence the null hypothesis for this study is rejected.

This study aligned with a previous study by Kim et al which supports FV as an effective agent for enamel remineralization, demonstrated by the increase of enamel microhardness.[13] This study also aligned with the recommendation of the ADA Council of Science Affairs about the fluoride varnish application two to four times a year as a caries prevention method. The application of fluoride varnish is easy and affordable yet effective.[14]

The limitation of this study is that the enamel microhardness test after remineralization was only conducted on a single time point (only on the seventh day after remineralization) and in vitro condition. This study was also conducted using only a single test method and with a single agent. It may be beneficial to conduct several microhardness tests over a longer period, using different test methods, and multiple agents.

Conclusion

The findings show a significant increase in enamel microhardness following fluoride varnish application, measured on the seventh day (p-value 0.000). This demonstrates the effectiveness of fluoride varnish in increasing enamel hardness under these in vitro experimental conditions. This study can serve as a reference for clinicians selecting fluoride varnish as a caries prevention effort.

Acknowledgment

No financial support or research grants were received to conduct this study.

Disclosure

The authors report no conflicts of interest in this work.

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