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Surface roughness assessment with fluoride varnish application: An in vitro study

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ABSTRACT

Background: *The cause of cavities is initially due to roughness on the tooth surface, requiring fluoride varnish to prevent caries, as the varnish applies a fluoride compound to the tooth surface. Fluoride varnish reacts with the tooth enamel surface to form calcium fluoride and fluorapatite, thus making the enamel surface more resistant to demineralization and damage.* ***Purpose:*** *This study aims to compare the roughness of tooth enamel surfaces among three fluoride varnishes under acidic conditions.* ***Methods:*** *The research method uses three fluoride varnish materials: sodium fluoride 5% + tricalcium phosphate, calcium fluoride, and sodium fluoride 5% + casein phosphopeptide-amorphous calcium phosphate 2%. Samples of 81 teeth were divided into three groups (Group 1 without fluoride varnish application, Group 2 application of fluoride varnish with pH 3, and Group 3 application of fluoride varnish with pH 5). The teeth were tested before and after application of the varnishes using the scanning electron microscope and surface roughness tests.* ***Results:*** *The results showed a significant difference in the mean surface roughness of the enamel of the anterior deciduous teeth tested with fluoride varnish. The before and after comparisons in the pH 3 and pH 5 groups were very significant (p-value 0.000). The comparison results in each pH group after fluoride varnish administration showed no significant difference (pH 3 p-value 0.074 and pH 5 p-value 0.196). The tooth surfaces appear to be rougher after administration of an all-acid solution.* ***Conclusion:*** *There is a difference in surface roughness of primary teeth after being given fluoride varnish in low pH 3 immersion for 24 hours.*

Keywords: *anterior deciduous teeth; fluoride varnish; surface roughnes*
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INTRODUCTION

Dental caries is the most common health problem affecting about 60%–90% of children and adults in the world's population.[1] In the early stages of the caries process, bacterial fermentation of carbohydrates can lower the local pH level below its standard value (pH >5.5). Critical pH (<5.5) results in minerals dissolution on the enamel surface, in a process called demineralization.[2] Continued demineralization will result in cavities on the enamel surface.[3]

The success of topical fluoride in reducing the incidence of dental caries has been widely demonstrated. In recent years, studies have supported the idea that fluoride varnish, especially 5% sodium fluoride varnish, not only prevents caries but can also stop early caries lesions.[4] Five percent fluoride with added tricalcium phosphate (TCP) has a more significant remineralization increase than conventional 5% sodium fluoride.[5] Sodium fluoride 5% added with TCP is specially designed for pediatric use and has minimal toxicity.[6]

Fluoride varnish material such as calcium fluoride is one of the materials used in dentistry and can be found in fluoride varnish to prevent caries. Calcium fluoride is considered the best source of fluoride when compared to sodium fluoride (NaF) when the same concentration is used in the enamel with the gel form. When rinsed, calcium fluoride resulted in seven times greater fluoride deposition in healthy enamel when compared to NaF.[7] In addition, calcium fluoride has the advantage of demineralizing and remineralizing enamel

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and dentin. Calcium fluoride nanoparticles are thought to increase tooth strength, although further studies are needed. The release of fluoride ions in calcium fluoride prevents the process of demineralization of enamel and dentin, which in turn prevents secondary caries.[8]

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is an anticariogenic agent that can remineralize lesions on enamel and dentin surfaces. Routine administration of CPP-ACP can replace calcium lost due to demineralization.[9] The insoluble nature of CPP-ACP, its crystal structure at neutral pH, can hold calcium and phosphate that can enter under the surface of tooth enamel. High concentrations of calcium and phosphate ions in plaque can reduce demineralization and promote remineralization.[10]

Surface roughness is a test method used to evaluate changes in the surface of the hard tissues of the teeth. This is important to know because a rough surface is a medium that facilitates the attachment of bacteria and debris. The increase of surface roughness shows demineralization of enamel, and it can allow decay on the tooth.[11] Research studies have shown that demineralization, the degradation of changes in surface roughness of the enamel surface of primary teeth, can be evaluated through a scanning electron microscope (SEM).[12] A SEM has the advantage of an objective magnification reaching ten nanometers. The value of the surface roughness of the enamel can be measured using the surface roughness tester. The purpose of this study was to determine the effect before and after the application of various fluoride varnishes on the surface roughness of primary tooth enamel under acidic conditions.

MATERIALS AND METHODS

Eighty-one maxillary or mandibular anterior primary teeth have been cleaned and cut where the primary teeth have no caries or restorations, and no anatomical deformities. The materials used in the research are fluoride varnish (sodium fluoride 5% + tricalcium phosphate [3M™ Clinpro™ White Varnish], calcium fluoride [For-lux] and CPP-ACP [MI from GC Corporation, Japan] [sodium fluoride 5% w/w and CPP-ACP 2% w/w]). Distillation solution, pH 3 and pH 5 were used as a solution for soaking. A solution of pH 3 was obtained by adding 142.5 mL of acetic acid to 107.5 mL of water, while a solution of pH 5 was obtained by adding 15 grams of sodium acetate to 500 mL of water and adding 500 mL of 0.1 M acetic acid. The pH solution was measured using pH meter paper, and 25 ml of each solution was put into a different plastic vial.

Table 1. Distribution of control group and fluoride varnish group

Group	n
Control	
1a. Distillate water	9
1b. pH 3	9
1c. pH 5	9
pH 3	
2a. Sodium fluoride 5% + tricalcium phosphate	9
2b. Calcium fluoride	9
2c. NaF 5% + CPP - ACP	9
pH 5	
3a. Sodium fluoride 5% + tricalcium phosphate	9
3b. Calcium fluoride	9
3c. NaF 5% + CPP - ACP	9
Total	81

The tool used to measure roughness was the surface roughness test carried out at the Dental Material Testing & Center of Research Laboratory, Faculty of Dentistry, Trisakti University, Jakarta. The SEM test was carried out at the Faculty of Mathematics and Natural Sciences at Institut Teknologi Bandung.

This research methodology is in vitro experimental with pre- and post-test research design. A total of 81 anterior primary teeth were cleaned and polished to remove debris. Each tooth sample was embedded in 2x2x1 cm acrylic resin, with the labial surface facing upward to stabilize the placement of the tooth sample during roughness measurement.

The sample was divided into 3 groups, each consisting of 27 samples. The first group was the control group which was not given any treatment; the second group was the group that was immersed in a pH 3 solution; the third group was the group which was immersed in a pH 5 solution. After 24 hours of soaking, the varnish layer was cleaned using a scalpel knife and cotton swab soaked in acetone (Table 1).

The surface roughness of the anterior deciduous teeth was measured using a surface roughness tester then samples from each group were evaluated for roughness using SEM with a scan area of 200 x 200 μm and a magnification of 1100X.[13,14] Two measurements were made for each sample. The data analysis method used in this study was a paired t-test to find the statistical differences in the increase in roughness of each group (Groups 1, 2, and 3). The significance level was set at ($p < 0.05$). The one-way ANOVA and Tukey analysis methods were also used to determine differences in enamel surface roughness between test groups.

RESULTS

Comparison of the results of enamel surface roughness measurements between control groups and the fluoride varnish application showed an increase in enamel surface roughness based on applied with different pH solution. The highest mean in the control group that was not given fluoride varnish with pH 3 immersion was 1.415 μm (1b); the same was also found in the control group, which was not given fluoride varnish with pH 5 immersion 1.0815 μm (1c). The treatment group with the best fluoride varnish was found in the calcium fluoride group with the lowest average enamel surface roughness of 0.9815 μm (2b) by

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immersion at pH 3, while the treatment group with the best CPP-ACP fluoride varnish with the lowest average enamel surface roughness was 0.8481 μm (3c) at immersion with pH 5 (Table 2).

The statistical test results with the t-test resulted in a significant difference ($p<0.005$) in the entire test group except for the distilled water sample (Table 3). The test results based on immersion in a pH 3 solution in the study by comparing the fluoride varnish used showed a significant difference in the enamel roughness of primary teeth before and after application ($p<0.000$). Additionally, immersion in a pH 5 solution also demonstrated significant results in this group (Table 4).

In contrast to the test results above, tests in Group 2 and Group 3, utilizing Tukey's test showed no significant difference between the three fluoride varnish materials used in the study. This result shows that the three varnish fluoride materials have similarities in the roughness of the primary tooth enamel that has been immersed in pH 3 and pH 5 (Table 5).

The results of observations using SEM in Groups 1, 2, and 3 demonstrated increased roughness of tooth enamel after the teeth were immersed in a low-pH solution. However, in contrast, the teeth immersed in distilled water did not show an increase in tooth enamel roughness (Figures 1–3).

Table 2. Average surface roughness of primary tooth enamel using fluoride varnish and control at pH 3 and pH 5

Group	Before		After	
	Mean (μm)	SD	Mean (μm)	SD
Control (Group 1)				
1a. Distillate water	0.3593	0.0846	0.3778	0.1106
1b. pH 3	0.4778	0.1236	1.415	0.487
1c. pH 5	0.4889	0.1213	1.0815	0.2887
pH 3 (Group 2)				
2a. Sodium fluoride 5% + tricalcium phosphate	0.3481	0.1094	1.374	0.1106
2b. Calcium fluoride	0.4593	0.1051	0.9815	0.2376
2c. NaF 5% + CPP - ACP	0.4778	0.1067	1.341	0.414
pH 5 (Group 3)				
3a. Sodium fluoride 5% + tricalcium phosphate	0.4481	0.1144	0.9074	0.2350
3b. Calcium fluoride	0.4852	0.1334	0.9370	0.2058
3c. NaF 5% + CPP - ACP	0.4481	0.0747	0.8481	0.1804

Table 3. Comparative test results of surface roughness of primary teeth before and after immersion by group

Group	n	gain	t-count	t-table	p-value
Control (Group 1)					
1a. Distillate water	9	0.019	-0.852	-2.306	0.416
1b. pH 3	9	0.937	-6.014	-2.306	0.000*
1c. pH 5	9	0.589	-6.011	-2.306	0.000*
pH 3 (Group 2)					
2a. Sodium fluoride 5% + tricalcium phosphate	9	0.893	-9.041	-2.306	0.000*
2b. Calcium fluoride	9	0.522	-9.695	-2.306	0.000*
2c. NaF 5% + CPP - ACP)	9	0.863	-6.388	-2.306	0.000*
pH 5 (Group 3)					
3a. Sodium fluoride 5% + tricalcium phosphate	9	0.459	-6.239	-2.306	0.000*
3b. Calcium fluoride	9	0.452	-6.180	-2.306	0.000*
3c. NaF 5% + CPP - ACP	9	0.400	-7.083	-2.306	0.000*

* $p<0.005$

Table 4. Result of comparison of surface roughness of primary teeth before and after immersion between groups

Group	n	SD	F-value	p-value
pH 3 (Group 2) + control	72	0.277493	25.29	0.000*
pH 5 (Group 3) + control	72	0.277493	18.87	0.000*

*$p<0.005$

Table 5. Comparison of surface roughness of primary teeth after application of fluoride varnish immersion pH 3 and pH 5

Group	n	F-value	p-value
pH 3 (Group 2) + control	36	2.54	0.074
pH 5 (Group 3) + control	36	1.66	0.196

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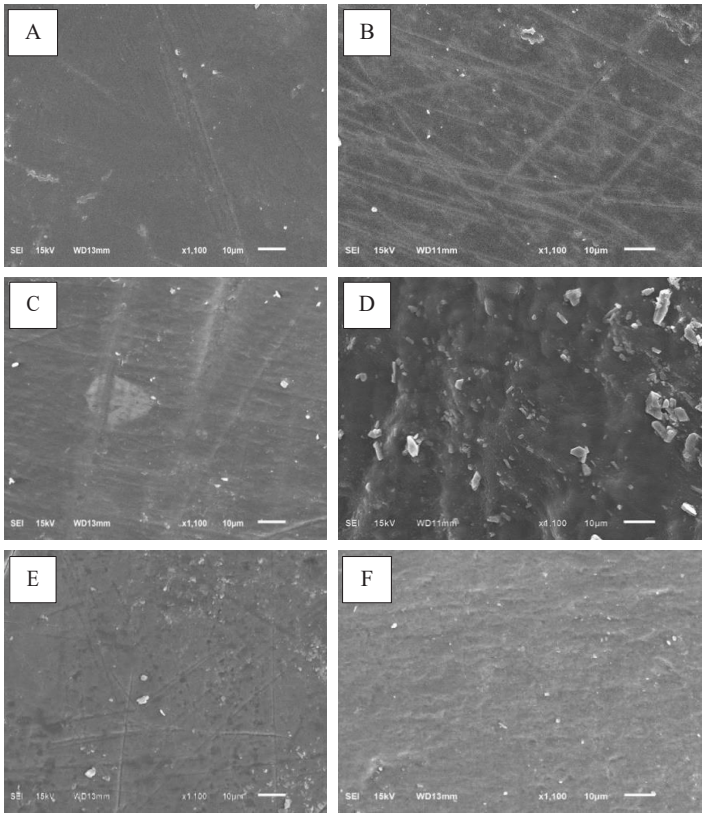

Figure 1. Overview of enamel roughness of primary teeth by SEM testing at 1100x magnification. Control group (Group 1) before and after immersion without fluoride varnish application. (a and b distilled water, c and d pH 3, e and f pH 5).

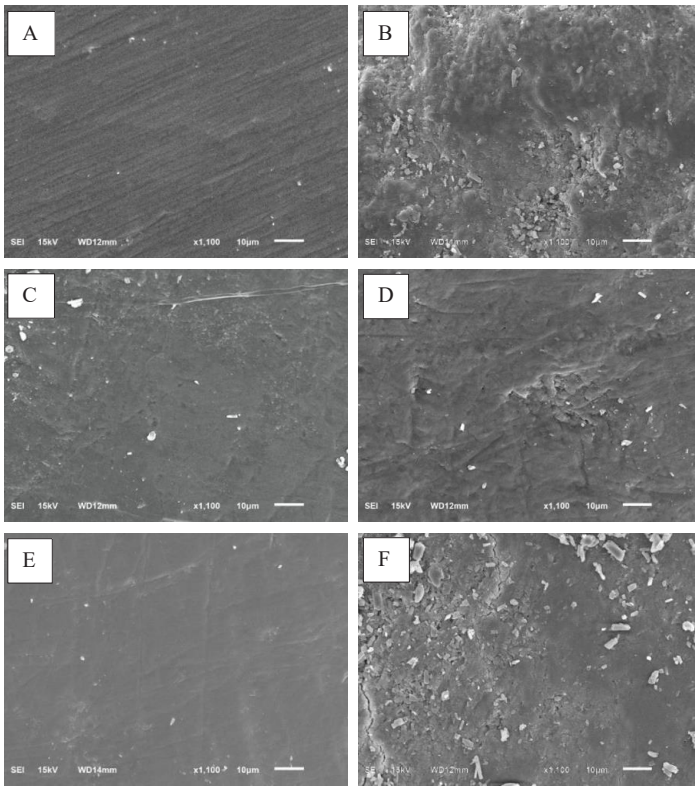

Figure 2. An overview of the enamel roughness of primary teeth by SEM testing at 1100x magnification. Group pH 3 (Group 2) before and after immersion in fluoride varnish application. (a and b sodium fluoride 5% + tricalcium phosphate (TCP), c and d calcium fluoride, e and f NaF 5% + CPP-ACP).

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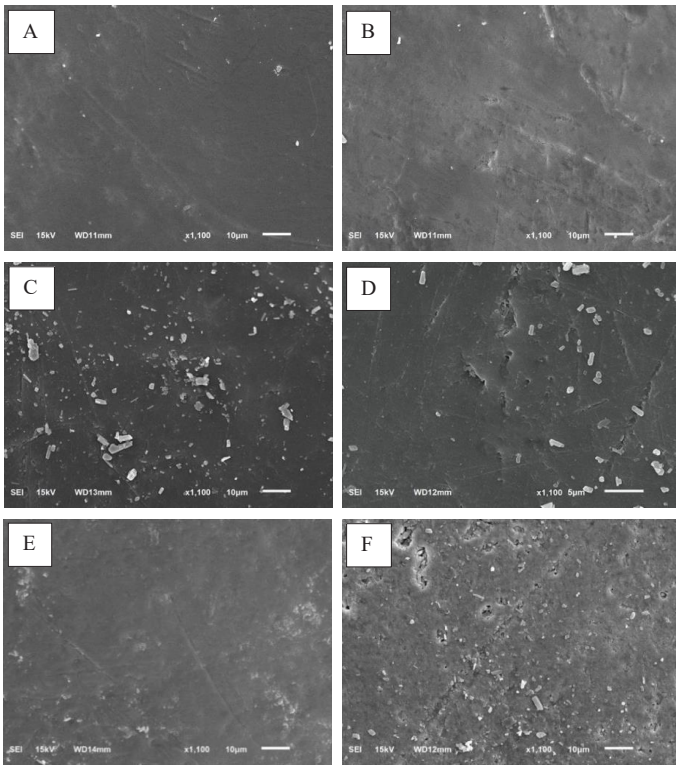

Figure 3. Overview of enamel roughness of primary teeth by SEM testing at 1100x magnification. pH 5 group (Group 3) before and after immersion in fluoride varnish application. (a and b sodium fluoride 5% + tricalcium phosphate (TCP), c and d calcium fluoride, e and f NaF 5% + CPP-ACP).

DISCUSSION

Tooth surface roughness is considered one of the determinants in describing the effect of remineralization and demineralization processes on the surface; continuous remineralization can reduce tooth decay.[13] Several studies have proven the potential of fluoride varnish as an effective anti-caries agent. When used correctly, fluoride varnish can reduce the incidence of caries by 40–56% and reduce enamel surface roughness.[14] Research that has been done previously found that fluoride varnish was able to reduce fissure caries by 36%, effected a reduction of 66% for non-carious fissure surfaces, as well as demonstrated a 51% reversal of tooth decalcification structure and a 35–21% reduction in enamel demineralization.[15]

In this study, there were differences in the surface roughness of the primary tooth enamel, which had been soaked in a solution of pH 3 and pH 5 accompanied by the application of fluoride varnish (sodium fluoride 5% + tricalcium phosphate group, calcium fluoride group and NaF 5% + casein phosphopeptide-amorphous calcium phosphate group) (Table 1). There was an increase in the surface roughness of the primary tooth enamel after immersion in a solution of pH 3 and pH 5 for 24 hours, even though fluoride varnish was applied; this was due to the high value of surface roughness in the pH 3 group due to low pH which could trigger the demineralization process in the teeth. What happens can cause the release of calcium ions in tooth enamel. The release of calcium ions in tooth enamel can cause microporosity resulting in roughness on the tooth surface. The lower the pH level in the oral cavity, the faster tooth decay occurs.[16,17] This is because a low pH will cause a continuous increase in hydrogen ions in the tooth enamel; these ions can damage hydroxyapatite in tooth enamel and dissolve enamel crystals, therefore the roughness of the teeth increases.[18]

In the groups of pH 3 and pH 5, the increase in surface roughness still occurred even though fluoride varnish was applied. According to the results of research conducted by Lippert F, it was stated that fluoride varnishes have a susceptibility to loss of fluoride ions at low pH, so the varnish cannot work optimally.[19]

However, when compared with the results of surface roughness in the control group or the group without fluoride varnish, the increase in roughness was higher, with a value of 0.937 at pH 3 and 0.589 at pH 5, meaning that the administration of fluoride varnish could increase the ability of primary teeth to withstand the demineralization process caused by the pH cycle and reduce the level of roughness on the tooth surface. These results can be found in a study conducted by Baothman and Assery.[4]

Immersion in pH solution was carried out for 24 hours in the treated group, simulating the consumption of sweet drinks or foods that can cause an acidic atmosphere for 4 minutes every day for 1 year (4 minutes x 30 days x 12 months = 1440 minutes = 24 hours).[20] The American Dental Association Council on Scientific Affairs concluded that fluoride varnish should be applied every six months

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because it is effective in reducing the prevalence of caries and also preventing caries in primary and permanent teeth in children and adolescents.[21]

The application of fluoride varnish in this study was only carried out once in 24 hours, which simulated the application of fluoride varnish, which is carried out once a year. However, the surface roughness of the enamel still experienced an increase in roughness after fluoride varnish was applied to the teeth compared to before immersion. This study showed that immersion of the teeth using pH 3 resulted in more roughness on the enamel surface compared to pH 5 and controls. This is also the same in all treatments using fluoride varnish. Surface roughness can be seen in the examination using SEM (Figures 1–3); from this, it can be seen that the application of fluoride varnish, which is only done once, is not enough and needs to be repeated. Fluoride varnish application should be repeated every three or six months per year.[22]

When the pH in the oral cavity is below a critical pH, demineralization can quickly occur. In this situation, the inorganic elements of the enamel become soluble. Fluoride varnish plays an essential role in protecting teeth, especially in preventing demineralization of tooth enamel. Fluoride varnish is believed to form an intraoral fluoride reservoir via calcium ions.[2]

Each fluoride varnish used in this study has different advantages and effectiveness. However, based on the results of research carried out and tested with statistical tests, there are significant differences before and after treatment with fluoride varnish application by immersing pH 3 and pH 5 (Table 4). When compared between fluoride varnishes, namely between the sodium fluoride 5% + tricalcium phosphate group, the calcium fluoride group, and the NaF 5% + casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) group, there was no significant difference in surface roughness of the primary tooth enamel (Table 5). The result is in line with the study conducted by Baothman and Assery, which stated that there was no difference in enamel roughness in primary teeth after applying fluoride varnish.[4]

From the research that has been done, it is evident that there are differences in the surface roughness of the primary teeth before and after fluoride varnish. The increase in surface roughness occurred in all study groups. However, in the pH 3 group where calcium fluoride varnish was applied, the increase in roughness was lower than without calcium fluoride varnish. In contrast, in the pH 5 group, the NaF 5% + CPP-ACP varnish was better, and the enamel surface roughness was minor compared to the group with another varnish. It proves fluoride varnish can reduce the demineralization process in primary teeth under acidic conditions to prevent early caries.

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