# EFFECT OF FLUORIDE VARNISH ON SURFACE MICROHARDNESS OF WHITE SPOT LESIONS ON PRIMARY TEETH

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**Abstract**. The aim of this study was to compare the changes in surface microhardness (SMH) of white spot lesions (WSL) on primary teeth between those treated with three applications in one week of 5% sodium fluoride with amorphous calcium phosphate (ACP) and those treated with single application of the same sodium fluoride with ACP varnish. Thirty extracted sound primary anterior teeth were divided randomly into 3 groups: group 1: control group (no treatment); group 2: treated with a single application of fluoride varnish in one week; and group 3: treated with a three applications of fluoride varnish in one week. WSL were induced in all teeth by immersing them in demineralizing solution, then undergoing seven days of pH-cycling. Group 2 was treated with a single application of fluoride varnish on day 1 and group 3 was treated with three applications in one week on days 1, 3 and 5. The Vicker's microhardness number (VHN) was measured at baseline, after inducing the WSL and after pH-cycling. The percent change in surface microhardness (% SMH change) was calculated. The one-way ANOVA and Tukey's tests were used with 95% confidence levels to compare differences. The mean surface microhardness levels after WSL were formed were significantly lower than baseline in all groups. The mean surface microhardness levels after pH-cycling in groups 2 and 3 were significantly greater than their mean values after inducing WSL. There was no significant difference between the mean % SMH change values for groups 2 and 3. However, the mean surface microhardness of both the treatment groups were significantly higher than the mean surface microhardness of the control teeth after the treatment groups had applied the tooth varnish. We conclude under the study conditions, applying 5% NaF varnish with ACP three applications in one week resulted in no significant difference in the surface microhardness of WSL on primary teeth than a single application, suggesting a single application may be adequate. *In vivo* studies are needed to confirm these findings.

**Keywords:** fluoride varnish, primary teeth, surface microhardness, white spot lesion

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## INTRODUCTION

Caries can occur in the primary teeth of children and can be severe (Ismail *et al*, 1999). The earliest clinical signs of early childhood caries are white spot lesions (WSL), which are non-cavitated areas of subsurface demineralization (Fejerskov *et al*, 2003). Early stages of WSL can be arrested and potentially reversed (Featherstone, 1999). Without proper management, WSL can progress to cavities in the enamel surface (Thylstrup *et al*, 1994). Thus, it is important for the dentist to recognize and treat WSL. A goal of contemporary dentistry is to manage WSL non-invasively to prevent caries progression and preserve the integrity of the healthy tooth. WSL are managed non-invasively using remineralizing agents (Ismail *et al*, 2013). The best known remineralizing agent is fluoride. Fluoride is known to increase the resistance of tooth mineral to demineralization by plaque acids; however, it also plays a role in promoting remineralization and repair of WSL (Featherstone, 1999). Numerous studies support the effectiveness of professionally applied fluoride varnish in preventing incipient enamel caries lesions (Weintraub *et al*, 2006; Azarpazhooh *et al*, 2008; Weyant *et al*, 2013). The Council on Scientific Affairs of the American Dental Association (ADA) recommends applying 2.26% fluoride varnish at least twice per year for caries prevention and it is the only topical fluoride agent recommended for children younger than 6 years old (Weyant *et al*, 2013).

There have been several clinical trials evaluating caries prevention by fluoride varnish using different frequencies of application in both permanent and primary dentition. Petersson *et al* (1991) suggested three applications in one week conducted annually could be more effective than twice yearly. Weintraub *et al* (2006) reported although more frequent varnish applications were more beneficial, one application was preferable to none. Gugwad *et al* (2011) applied 5% sodium fluoride varnish (Cavity Shield) three times a week to molars and found it could prevent caries in primary teeth. Sköld *et al* (2005) and Weinstein *et al* (2009) found in randomized control trials there was no advantage to more intensive treatment. Another study reported, intensive fluoride application is recommended when treating young children and special needs populations (Adair, 2006). Thus, it is important to further clarify WSL treatment and prevention regimens for primary teeth since children are at higher caries risk.

In this study, the measurement of SMH was used as the basis for comparison. Featherstone (1999) reported a good correlation between enamel microhardness and mineral loss in caries lesions. Thus, mineral loss or gain in enamel can be measured as hardness change after demineralization or remineralization (Lata *et al*, 2010).

The aim of this study was to compare the changes in surface microhardness (SMH) of WSL on primary teeth between those treated with three applications in one week of 5% sodium fluoride with amorphous calcium phosphate (ACP) and those treated with a single application of the same sodium fluoride with ACP varnish.

## MATERIALS AND METHODS

**Specimen preparation**

Thirty human primary incisor extracted or naturally exfoliated teeth were collected and stored in 0.1% thymol solution at room temperature until use. The coronal portion of each tooth was embedded in self-curing acrylic resin blocks using a cylindrical plastic tube, leaving a 2×2 mm square window on the labial enamel surface exposed. The enamel surfaces were polished with 800, 1,000, 2,000 and 4,000 grit silicon carbide abrasive paper to obtain a flat, smooth surface. The specimens were kept in deionized water until use.

**White spot lesion formation**

Each specimen was immersed in 3 ml demineralizing solution (2.2mM $CaCl_2$ and $NaH_2PO_4$ and 0.05 M $CH_3COOH$, with the pH adjusted to 4 using 1.0 M KOH) (Thaveesangpanich *et al*, 2005) for one hour at 37°C in an incubator (Sheldon Manufacturing, model 1545, Cornelius, OR) to develop WSL. Each specimen was then rinsed with 15 ml deionized water and wiped dry.

**Grouping**

The specimens were randomly divided into three groups ($n$=10): group 1 (control group), treated with distilled water; group 2, treated with a single application of 5% sodium fluoride with ACP (Enamel Pro®) on day 1; group 3, treated with three applications of the same sodium fluoride with ACP varnish on days 1, 3 and 5. The fluoride with ACP varnish was applied to the 2×2 $mm^2$ window of the teeth in groups 2 and 3 using a microbrush according to the manufacturer's instructions. Before each new application, the previous varnish was carefully removed with a scalpel blade.

**pH- cycling process**

The pH-cycling process was performed for seven days (Buzalaf *et al*, 2010). During the seven-day period, the specimens were kept in demineralizing solution (2.2mM $CaCl_2$, 2.2 mM $NaH_2PO_4$, and 0.05 M $CH_3COOH$, with the pH adjusted to 4.7 using 1.0 M KOH) for 3 hours twice a day and with 2 hours in remineralizing solution (1.5 mM $CaCl_2$, 0.9 mM $NaH_2PO_4$, and 0.15 M KCl, with the pH adjusted to 7.0 using 1.0 M KOH) between the two demineralizations. Each tooth was washed in deionized water for 1 minute before and after each demineralization. The specimens were then kept in remineralizing solution overnight at 37°C in a controlled environment incubator shaker. The demineralizing and remineralizing solutions were freshly prepared for each pH-cycling process.

**Surface microhardness measurement**

SMH was measured with a Vickers diamond indenter (Antor Parr MHT- 10 Microhardness Tester). The SMH was recorded using the Vickers microhardness numbers (VHN). The measurements were made by assessing three indentations at a spacing of 100 microns under a 100-g load for 20 seconds. The SMH was measured at baseline, after WSL formation and after each pH-cycling process. The following equation was used to calculate the %SMH change: %SMHC= 100 ×(SMH after pH-cycling / SMH after WSL formation)/(SMH at baseline -SMH after WSL formation).

**Statistical analysis**

The means and standard deviations were calculated. The mean SMH at baseline, after WSL formation and after pH-cycling were tested for normal distribution using the Kolmogorov-Smirnov test. If the distribution was normal, differences in SMH between baseline and after WSL formation and after the pH-cycling process within the same group were compared using the paired samples $t$-test. The one-way ANOVA and Tukey's post hoc tests were used to compare the SMH values and the %SMH change among the 3 groups. A $p$-value $<0.05$ was considered statistically significant.

Table 1
Surface microhardness among control and treatment groups before and after application of 5% sodium fluoride with amorphous calcium phosphate varnish.

| Studied group | Mean tooth surface microhardness in VHN (±SD) | | | |
|---|---|---|---|---|
| | Baseline | After WSL formation | After pH-cycling | %SMHC |
| Control (*n*=10) | 349.86 (±18.54) | 272.24 (±31.40) | 260.80 (±41.11) | -27.27 (±68.27) |
| Single application of 5% NaF+ACP (*n*=10) | 331.86 (±10.94) | 264.91 (±42.18) | 381.04 (±32.86) | 258.20 (±226.47) |
| Three applications of 5% NaF+ACP in 1 week (*n*=10) | 341.38 (±21.96) | 239.86 (±55.19) | 463.29 (±29.74) | 288.49 (±167.25) |

SD, standard deviation; WSL, white spot lesion; NaF, sodium fluoride; ACP, amorphous calcium phosphate; %SMHC, percent change in tooth surface microhardness.

## RESULTS

The data were found to be normally distributed. Table 1 shows the baseline, after WSL formation and after pH-cycling VHN values and the %SMH change for each study group. The mean (± SD) SMH values at baseline were not significantly different among the groups ($p$=0.056). After WSL formation, all groups showed lower surface microhardness values compared with baseline; and there were no statistically significant differences among the groups ($p$=0.264).

The mean (± SD) SMH value after pH-cycling in the control group was not significantly different from after WSL formation ($p$=0.463). But the mean (± SD) SMH values after pH-cycling for the single application and three applications groups had a significant increase in SMH from baseline after WSL formation ($p$=0.000). There were significant differences among the groups of after pH-cycling ($p$=0.000).

Fig 1 shows the mean %SMHC values for the study specimens. The mean %SMHC for control group was -27.27 (±68.27), for single application group was 258.20 (±226.47), and for three applications group was 288.49 (±167.25). The Tukey's post hoc test for paired group comparison for the mean %SMHC in the control group was significantly different from single application group ($p$=0.002) and three applications group ($p$=0.001). However there was no significant difference between the single application group and three applications group ($p$=0.914).

## DISCUSSION

The noninvasive treatment of early caries lesions by remineralization has the potential to play a major role in clinical caries management (Reynolds, 2008).

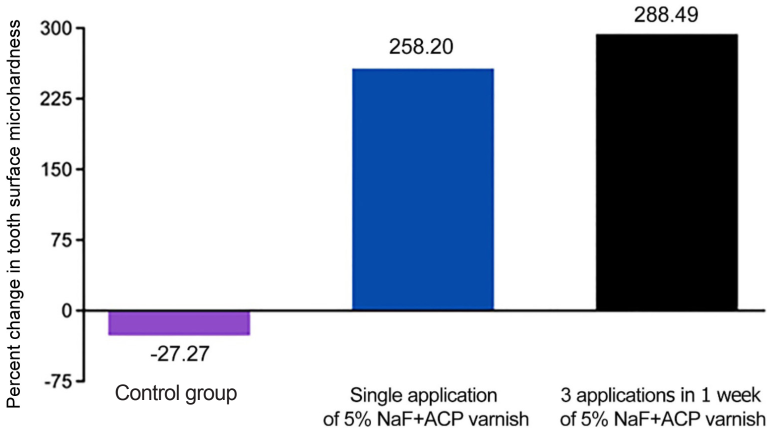


Fig 1–Percent change in mean surface microhardness of studied primary teeth. NaF, sodium fluoride; ACP, amorphous calcium phosphate.

Fluoride varnishes have been used to treat and prevent incipient enamel caries lesions due to their high fluoride concentration and adhesion capacity to tooth enamel (Azarpazhooh and Main, 2008). Fluoride varnish needs to be reapplied to maintain its caries-preventive effect. (Beltrán-Aguilar *et al*, 2000). The purpose of this study was to compare the changes in SMH of WSL on primary teeth between those treated with three applications in one week of 5% sodium fluoride with ACP and those treated with a single application of the same fluoride varnish.

In our study, after WSL formation, there was a decrease in the mean SMH values for all groups. After pH-cycling, which was treated by 5% NaF with ACP as a single application or three applications in one week, the mean SMH values of these two treatments groups increased significantly. However, there was no significant difference between the mean %SMHC value of these two treatments groups. Fluoride ions promote the formation of fluorapatite in enamel in the presence of calcium and phosphate ions produced during enamel demineralization (ten Cate, 1999). This fluorapatite formation can reduce the solubility of tooth mineral and inhibit demineralization during cariogenic challenges (Hicks *et al*, 2004). When fluoride varnish, which is highly concentrated in topical agents, is applied to the tooth surfaces, a calcium fluoride($CaF_2$)-like layer is formed; this $CaF_2$-like layer is relatively soluble and can act as a reservoir for fluoride ions during acid attacks (Lussi *et al*, 2012). It seems the fluoride uptake from the varnish increased the resistance of the enamel to cariogenic challenges. In our study, 5% NaF with ACP varnish was capable of remineralizing incipient caries lesions.

Adequate quantities of calcium and phosphate ions must be present in remineralizing solution for remineralization to occur; therefore, a calcium phosphate-based formula was added to the fluoride varnish in our study to enhance remineralization. Jablonowski *et al* (2012) compared the amount and rate of fluoride release from newer fluoride varnishes with older traditional fluoride varnishes and found Enamel Pro®, containing 5% NaF and ACP varnish, had the greatest cumulative fluoride release. Castillo and Milgrom (2004) suggested that multiple applications of fluoride varnish within a short time frame (three applications in one week) produced greater and longer release of fluoride. Despite the advantages of the 5% NaF and ACP varnish (Enamel Pro®) we used in this study and the intensive application, the mean %SMHC value for the single application group and three applications in one week group were not significantly different from each other.

In our study, the fluoride varnish was applied on the 1st, 3rd and 5th days during the pH-cycling for the three applications in one week; the first application of 5% NaF and ACP varnish may have created this superficial remineralized layer of fluorapatite which is much less soluble than hydroxyapatite (Featherstone, 1999). The deposition occurred primarily in the surface layer, leading to blocking of the surface layer pores, forming a subsurface lesion with a highly mineralized surface layer (ten Cate and Featherstone, 1999). When the teeth were exposed to a cariogenic challenge again through pH-cycling, the fluoridated region may have dissolved more slowly or not at all, causing not much mineral loss, causing the second and third applications to not significantly improve the enamel surface microhardness. Another explanation for the lack of difference between the single versus the three applications could be the triple applications were too close to each other in timing, resulting in no noticeable difference. The frequency of the applications did not seem to play a major role. This result is in agreement with Marinho *et al* (2013) who reviewed fluoride varnishes for preventing dental caries in children and adolescents and found no additional benefit to more frequent applications of varnish in caries-preventive effectiveness.

In conclusion, in our study conditions, application of 5% NaF and ACP varnish three times in one week gave no greater efficacy in treating WSL on primary teeth than a single application and represents a greater, unwarranted expense.

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