

The effect of topical fluoride products on surface microhardness of enamel of primary teeth (An in vitro study)

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ABSTRACT

Aim: To compare the effect of three types of topical fluoride products on the microhardness of enamel of primary teeth by using pH cycle in vitro.

Materials and Methods: 50 Fifty sound human primary mandibular central incisors teeth were used in this study and randomly divided into five groups 10 teeth sample in each group: deionized water (Control negative), pH cycling (Control positive) and the three remaining groups were treated by three different fluoride products then exposed to pH cycle. The surface microhardness of enamel were measured.

Result: The data analyzed with one way ANOVA test and Duncan's multiple range test ($P \leq 0.01$). The results showed that the mean surface microhardness in all groups increased significantly after treated by three different topical fluoride products.

Conclusion: All specimen groups treated with fluorides showed increase in enamel microhardness in compared to control group. The results of this study suggest that the use of fluoride varnish significantly improves the protective ability of the varnish on primary teeth in vitro.

Key words: Topical Fluorides, pH cycle, Surface Microhardness.

INTRODUCTION

Oral health is a fundamental component of overall health. All children should have access to preventive and treatment-based dental care.⁽¹⁾ Dental health can affect the functional, psychological and social dimensions of a child's well-being. Oral pain has devastating effects on children, including lost sleep, poor growth, behavioural problems and poor learning.⁽²⁾ Primary teeth, are the first set of teeth in the growth development of humans.⁽³⁾ The enamel of primary teeth is only half as thick as that of permanent teeth. Moreover, it has a lower mineral content and higher organic content, rendering it more susceptible to carious lesions than the enamel of permanent teeth.^(4, 5) Tooth decay is the most common chronic disease in childhood.^(6,7) Dental caries is an infectious micro biological disease that results in localized dissolution and destruction of the calcified tissue of teeth, Caries is a multifactorial disease; it is a complicated process depends on many factor.^(8,9) Dental caries is a dynamic diet microbial disease involving cycles of demineralization and remineralization. The early stages of this process are reversible by modifying or eliminating etiologic factors (such as plaque biofilm and diet) and increasing protective factors (such as fluoride exposure and salivary flow).⁽¹⁰⁾

Effective efforts to prevent caries and non-invasive treatment of initial caries lesions in young children are needed. fluoride is widely accepted as the most effective tool for caries prevention.⁽¹¹⁾ The discovery of the anti-cariogenic properties of fluorides is one of the most important landmarks in the history of dentistry.⁽¹²⁾ fluoride is the most commonly used remineralizing agent. The cariostatic effect of fluoride is primarily due to its ability to decrease the rate of demineralization by forming fluor-hydroxyapatite and enhancing the remineralization of incipient carious lesions.⁽¹³⁾ fluoride-containing compounds are used in topical and systemic fluoride therapy for preventing tooth decay. they are used for water fluoridation and in many products associated with oral hygiene.⁽¹⁴⁾

Fluoride incorporated into the enamel mineral during tooth development has little effect on the caries process. It is the fluoride that is incorporated post-eruptively during the caries challenge that plays an important role in caries prevention.⁽²¹⁾ topical fluorides are presented in different types such as tooth pastes, fluoride containing mouth washes, gels, foams and fluoride varnishes. Fluoride has two main mechanisms; namely, preventing demineralization of the normal enamel and improving enamel remineralization through incorporation of fluoride in enamel composition.⁽¹⁵⁾The use of fluoride gels in trays. This was followed by the introduction of fluoride foams used in trays, which are generally considered easier to use than gels and have less risk of ingestion of fluoride. Less total fluoride is applied with foam, and a lower volume of product is used. These factors reduce the risk of the patient gagging and swallowing fluoride and also the amount of fluoride that could be ingested as a result.

Fluoride varnish has been used for more than 30 years as a method of professional application of topical fluoride. NaF varnish was introduced for use as a desensitizing agent and cavity liner.⁽¹⁶⁾In the last two decades, application of fluoride varnish has been very much popular, due to its advantages. ease of manipulation, safety and simplicity of application procedural steps are considered as the reasons for its widespread acceptance.⁽⁷⁾Fluoride varnish requires less treatment time in comparison with fluoride gel, which needs tray and suction for its application. The exposure time between fluoride and the tooth surface increases when fluoride varnishes are used due to its adhesion to the tooth surface.⁽¹⁷⁾The foam forms of APF and NaF require a much smaller amount to fill the trays and thereby reduce the risk of ingestion.^(18, 19) Surfaced microhardness is a physical property which assesses the effect of chemical and physical agents on hard tissues of teeth, and a useful way to examine the resistance of fluoride treated enamel against caries.⁽²⁰⁾

MATERIALS AND METHODS

Study Design: The following is an experimental design using a microhardness tester to test the strength of the enamel after repeated cycles of demineralization and remineralization process.

Preparation of the Sample: Fifty (50), freshly extracted highly mobile, free of dental caries, human primary incisors teeth were collected from children between (7-9) years old. Any remaining soft tissues were hand scaled and the extracted teeth were washed thoroughly with tap water and stored in deionized water with (0.1%) thymol to avoid dehydration and further microbial growth. The coronal portion of the teeth embedded in self cure acrylic resin block using plastic tube. The labial surface was exposed to external surface and each enamel block was grounded wet using 220, 400, 1200, and 2000 grit silicon carbide paper to obtain flat and smooth surface.

Grouping the samples:

- 1. Group 1:** control negative group N=10, the sample was kept in deionized water only.
- 2. Group 2:** control positive group N=10, the sample was exposed to pH cycling.
- 3. Group 3:** N=10, the sample was exposed to fluoride varnish for 24 hours by a fine brush according to manufacturer's guide once a week.
- 4. Group 4:** N=10, the sample was exposed to fluoride foam for 1 minute by a fine brush according to manufacturer's guide once a week.
- 5. Group 5:** N=10, the sample was exposed to fluoride gel for 1 minute by a fine brush according to manufacturer's guide once a week.

Table 1: Formulation details for fluoride gel, varnish and foam tested in this study.

Products / pH-Value	Fluoride source Concentration(ppm)	Ingredients
1. Fluoride Varnish, pH-Value: (5.5 – 8).	NaF (5 %, corresponds to 2.26% fluoride ions).	Natural resin, ethanol, flavors and scents ,additives, Sodium fluoride, Free from aspartame ,gluten, saccharine, Xylitol, rosin.

2.Fluoride Foam pH-Value: (5.5 - 7).	NaF (2.72%, corresponds to 1.23% fluoride ions).	Water , Flavors and scents, additives, sodium fluoride, Free from aspartame ,gluten ,saccharine, CFC free, Xylitol.
3. Fluoride gel pH-Value: (3.5 – 8).	NaF (2.72 % corresponds to 1.23% fluoride ions).	Water ,O- phosphoric acid (<3%),flavors and scents, additives ,Sodium fluoride, Free from aspartame, gluten, saccharine, Xylitol.

pH Cycling and Simulation of Acid Challenge:

Teeth were submitted to the formation of artificial caries by pH cycling .⁽²²⁾ keeping the teeth in demineralizing solution (CaCl₂ 2.2mM, NaH₂PO₄ 2.2mM, and acetic acid 0.05M; pH of 4.5, adjusted with KOH 1M; 15mL per tooth) for 3 hours and in remineralizing solution (CaCl₂ 1.5mM, NaHPO₄ 0.9mM, and KCl 0.15mM; pH of 7.0; 15mL per tooth) for 20 hours. All the teeth were briefly washed in deionized water between solutions and placed in artificial saliva for 30 minutes at the end of the demineralization process and for 30 minutes at the end of the remineralization process (Sodium chloride NaCl, 0,400g, Potassium chloride KCl 0,400g, Calcium chloride dehydrate CaCl₂H₂O, 0,795g, Sodium dihydrogen phosphate NaH₂PO₄ 0,69g, Sodium hydrogen phosphate anhydrous Na₂S₉H₂O 0,005g, Urea 1,0g, distilled water 1000ml); pH of 7.0).⁽²³⁾ The duration of each cycle was one day (24 hours) and the teeth were subjected to a total of 10 cycles. The demineralizing-remineralizing solutions were changed daily, and the artificial saliva was changed at every treatment.⁽²⁴⁾ All of these stages were carried out in an incubator at 37 ± 2°C.⁽⁷⁾

Surface Microhardness Measurement

Surface microhardness was measured for all specimens using a Vickers microhardness testing machine (OLPERT, Germany), with applied load of 700g for 15 second via a Vickers diamond pyramid indenters, which have a square-based diamond indenter with a 136° angle and 600x magnification of microscope. The indentations were made for each specimen at three different locations, and then the average of three measurements were calculated and obtained as one reading.⁽²⁵⁾ Indentation result can be seen at projector screen in the form of shadow shaping rhomb, the diagonal length is measured with micrometer. After indentation was made on the enamel specimen surface, The Vickers hardness number (VHN) was determined from the mean values obtained from three indentations on the surface of each specimen using the formula: $HVN = 1.854 \times P / d^2$. Microhardness of enamel surface was measured at baseline, before and after pH-cycling regime in each tested group. $HVN = \text{Sample hardness (Kg/mm}^2\text{)}, D = \text{Measurement result (mm)}, P = \text{weight is given (kg)}$.⁽²⁶⁾

RESULTS

Descriptive statistics that include mean, standard deviation, minimum and maximum values among tested groups were listed in Tables (1),(2). One way analysis of variance demonstrated significant differences on the surface microhardness among tested groups as shown in Tables (3), (4). Duncan test revealed that microhardness MH for control negative group, control positive group, Varnish group, Foam group and Gel group significantly different from each other at $p \leq 0.01$. Varnish group had SMH significantly highest other groups then Foam group significantly higher than Gel, control negative, control positive groups, as shown in figures (1),(2).

DISCUSSION

The purpose of this in vitro demineralization / remineralization cycling study was to evaluate the ability of topical fluoride products to protect enamel surface against demineralization challenges. Simulation of the natural mouth environment forces the researchers to use pH-cycling techniques. Different modifications of this technique have been applied for investigating caries processes and effect of caries preventive agents. Therefore, pH-cycle creating models can be accepted as a good evaluating method of the caries process and also provide standard study

conditions.⁽²⁷⁾ Because of these reasons, the present research was designed on a pH cycle and determined the effects of different three topical fluoride products in comparison to non-fluoride application group (control group) on *in vitro* primary enamel.

In pediatric dentistry, topical application of fluoride is a vital routine preventive procedure. Therefore, it is important to identify more effective methods of fluoride application.⁽²⁸⁾ NaF is a preferred agent for caries investigations. Therefore, in this study the fluoride treatment were prepared with NaF.

Considering the importance of the surface layer in caries progression, the evaluation of changes in this region is relevant, thus SMH measurement is a suitable technique for studying de-remineralization process. Micro hardness measurement is inappropriate for a material having fine microstructure, brittle nature, non-homogenous or prone to cracking like enamel. SMH indentations provide a relatively simple, non-destructive and rapid method in demineralization and remineralization studies.^(29, 30) In the present study, VHN was adopted as the basis for investigation because the square shape of indent obtained in VHN is more accurate to measure. Even the minute changes in the square shape indent obtained after the test can be easily detected.^(31, 32) Mineral loss or gain in enamel can be measured as hardness change after demineralization or remineralization processes.

In an *in vitro* study reported that fluoride varnishes were more effective in remineralization of enamel than a repeatedly used less concentrated NaF solution.⁽³³⁾ In the present study, the values of surface microhardness indicate that demineralization resistance of enamel is more in samples of group (Varnish) followed by group (Foam) and group (Gel). Fluoride varnish (22600 ppm) had almost two fold higher concentration in comparison to that of fluoride gel (12300 ppm) and fluoride foam (12300 ppm) and it also had a longer contact duration with the teeth, and its protective effect against demineralization was higher to that of fluoride gel and fluoride foam. It has been mentioned that the concentration and exposure time of topical fluorides affect the properties of fluoride reaction on the tooth surface.^(6,7) In the primary dentition, varnish effectiveness (measured by percent of caries reduction) ranges from 30% to 63.2%⁽³⁴⁾ Seppa et al. in a three-year clinical trial reported that fluoride gel and varnish had equal effects on children's tooth decay⁽³⁵⁾; on the contrary, in a two-year study on children, Tewari et al. showed that fluoride varnish had a stronger effect on preventing tooth decay.⁽³⁶⁾ Hong et al. have evaluated the effect of Karigel-N gel (5000 ppm) and sodium fluoride varnish (22600 ppm) in preventing dentin and root caries *in vitro* in two separate studies.

The results showed that weekly use of fluoride varnish resulted in a higher prevention of dentin and root demineralization in comparison with weekly use of Karigel-N gel.^(37, 38) This come in agreement with; that the weekly use of fluoride varnish resulted in a higher prevention of enamel demineralization in comparison with weekly use of fluoride gel and fluoride foam. Research has also proven that APF forms more fluoride and is more efficient in reducing enamel demineralization when compared with neutral gel.⁽³⁹⁾ The amount of F formed in the enamel depends on the F concentration and the pH of the product applied and how long it remains in contact with the enamel. Thus, the better results observed for the group "3" may be related to a longer contact period of the varnish with the enamel and the neutral pH (5.5-8) presented by sodium fluoride. Microhardness after treatment with neutral foam is much higher than microhardness after treatment with APF foam and gel. Thus, this finding may be due to the higher concentration of fluoride in the neutral foam.⁽⁴⁰⁾ An important factor contributing to the overall activity of topical fluorides is the mechanism of fluoride retention in the mouth and its subsequent clearance. The results of the present study as well as those from available literature indicate that fluoride varnish are superior agent than fluoride foam and fluoride gel and hence must be more frequently used to combat dental caries. However, the present study is an *in vitro* one the results of which may be quite different from the dynamic process that occurs in the *in vivo* situation. Therefore, further *in vivo* studies are necessary on to validate the findings of the present study.

CONCLUSION

1. All the fluoride products tested in this *in vitro* study promoted a reduction in the demineralization of the artificial carious lesions in enamel of primary teeth. However, none of the products used here was able to completely prevent the formation of lesions. The highest cariostatic effect was achieved by the fluoride varnish and the lowest by the fluoride gel, Fluoride varnish, fluoride foam, and fluoride gel were significantly different in comparison with the control group regarding prevention of demineralization.

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Table (1) The descriptive statistics and the results of statistical analysis for comparison of microhardness values among the study groups before induction of caries lesion.

professional topical fluoride products	No.	Mean	Standard deviation	Minimum	Maximum
Control negative	10	265	3.40279	260	270
Gel	10	287	1.50787	285	289
Foam	10	299	1.28145	297	301
Varnish	10	322	1.23544	320	323

Table (2) The descriptive statistics and the results of statistical analysis for comparison of microhardness values among the study groups after induction of caries lesion.

professional topical fluoride products	No.	Mean	Standard deviation	Minimum	Maximum
Control positive	10	237	3.61284	230	240
Gel	10	279	0.51299	279	280
Foam	10	295	1.07115	293	296
Varnish	10	318	0.50262	317	318

Table (3) ANOVA test for comparison of microhardness values among the study groups before induction of caries lesion.

Source of variance	Sum of Square	df	Mean Square	F- value	P - value
Between groups	33269.350	3	11089.783	2.606	0.000*
Within groups	323.400	76	4.255		
Total	33592.750	79			

*a Significant difference existed at $p \leq 0.01$

Table (4): ANOVA test for comparison of microhardness values among the study groups after induction of caries lesion.

Source of variance	Sum of Square	df	Mean Square	F – value	P – value
Between groups	69397.200	3	23132.400	6.288	0.000*
Within groups	279.600	76	3.679		
Total	69676.800	79			

* a Significant difference existed at $p \leq 0.01$

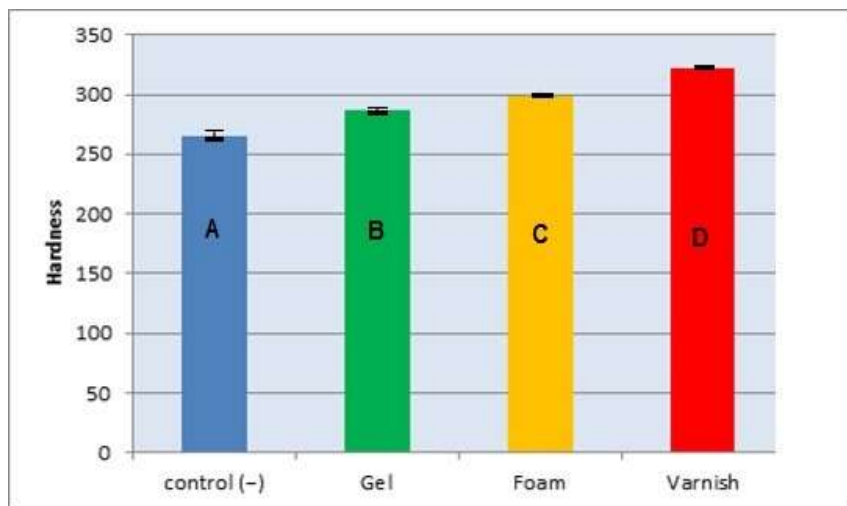


Figure (1): Histogram show the mean \pm SD of microhardness values among the study groups before induction of caries lesion, and Duncan's multiple range test results. Means with the different letters were statistically significant (at $P \leq 0.01$).

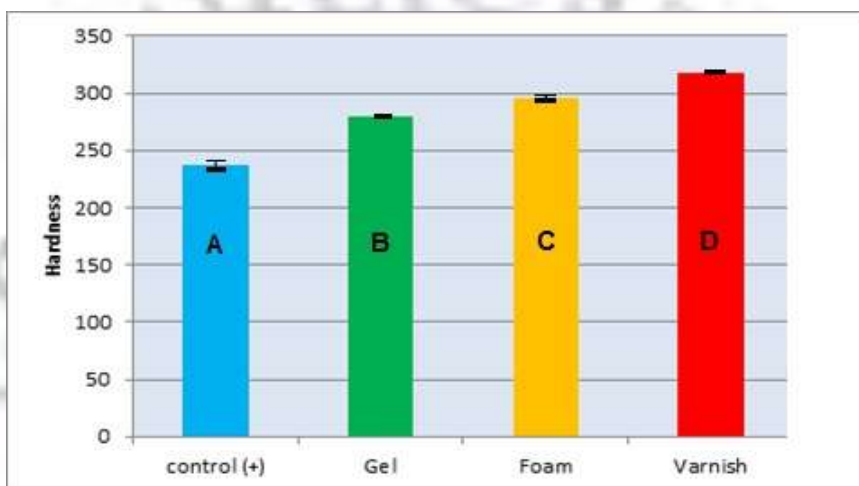


Figure (2): Histogram show the mean \pm SD of microhardness values among the study groups before induction of caries lesion, and Duncan's multiple range test results. Means with the different letters were statistically significant (at $P \leq 0.01$).