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ORIGINAL RESEARCH ARTICLE

In Vitro Evaluation of the Effect of Fluoride Varnish Application Frequency on Initial Enamel Lesions

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Abstract

Purpose: The aim of this study was the in vitro evaluation of the effect of fluoride varnish application at different frequencies on initial enamel lesions by surface microhardness (SMH) measurements.
Materials and Methods: This in vitro experimental study was performed on 80 extracted unerupted third molar teeth. The teeth were divided randomly into 4 groups and Duraphat Varnish (Colgate Company, Australia) was applied. Group 1: Fluoride varnish application once a year, Group 2: Fluoride varnish application 4 times at one week intervals, Group 3: Fluoride varnish application 4 times a year with an interval of 3 months, Group 4: Application of fluoride varnish twice a year at 6-month intervals. Artificial enamel carious lesions were created by inserting the specimens in demineralization solution for 96 hours. The Vicker's microhardness number (VHN) was measured at baseline, after demineralization and after fluor varnish application and 1 year after fluoride treatments of each group. The percent change in surface microhardness (% SMH change) was also calculated. Anova and The Kruskall-Wallis H test, t-test and/or Wilcoxon sign test were used with 95% confidence levels to compare differences.
Results: The surface microhardness values and recovery of surface hardness values of the groups were found as follows: Group 3> Group 2>Group 4> Group 1. VHN of Group 3 was statistically significantly higher than other groups ($p<0.05$).
Conclusions: The hardness values of the enamel increase as a result of all application frequencies, but this effect may be longer with frequent applications.

Key words: Demineralization; Fluoride varnish; Remineralization; Surface microhardness

Introduction

The first visible sign of a carious lesion is known as a white spot lesion. White spot lesion limited to the enamel tissue is demineralized areas with a superficial structure more porous than healthy enamel and no cavity formation is observed. This image is also called early caries lesion, new-onset caries lesion, initial caries lesion, initial enamel lesion.[1–3] White spot lesion is the stage where reversal of the lesion is possible. Remineralization is the treatment that aims to reverse the active initial caries lesion that has not cavitated or to arrest its progression to the cavitation stage. Studies on the remineralization of enamel have been carried out for nearly 100 years and it has been proposed that "non-invasive treatment of initial caries lesions with remineralization has the potential to be the most important advantage in the clinical management of the disease."[4,5]

Remineralization can be defined as the delivery and deposition into the caries lesion of the mineral elements, mostly calcium and phosphate, lost through demineralization of the tooth tissue. Providing a good oral hygiene, regulation of the diet and the use of fluoride and non-fluoride remineralizing agents are essential in remineralization of white spot lesions.[6]

Fluoride is the most commonly used remineralizing agent. Fluor increases remineralization and creates a low-solubility surface on the remineralized surface, similar to the acid-stable mineral fluoropatite (FAP). When saliva comes in contact with plaque on the tooth surface, it raises the pH of the plaque, neutralizing plaque acid, stopping demineralization and starting to reverse it. Partially demineralized crystal surfaces in the lesion act as nuclei and a new structure is formed on the crystal structure. This period induces remineralization, and mineral displacement begins in the partially demineralized areas of the carious lesion in the enamel and dentin structure. Fluor is adsorbed by the crystal surface and increases remineralization with the effect of calcium and phosphate ions, and new mineral formation begins. This newly formed surface does not contain carbonate and resembles a structure between hydroxyapatite and fluoropatite. Fluorapatite contains approximately 30,000 ppm of fluor and has a very low acid solubility. This new remineralized crystalline surface behaves like very low-resolution fluoropatite compared to the original high-resolution carbonate apatite surface.[7,8]

The effect of fluoride varnish, which is the most commonly used agent for remineralization has become an important research topic and has been stated as the 'gold standard' in remineralization.[9,10] In the literature, there are various studies on the frequency of use of fluoride agents in individuals in various risk groups in order to prevent caries formation and recommendations in international guidelines.[11–16] Although there are recommendations regarding the frequency of application of fluoride varnish, for caries prophylaxis, according to caries risk level, there is no recommendation on how often fluoride varnish should be applied for remineralization of white spot lesions, and no study has been found comparing the frequency of application. Therefore, the aim of this study was the in vitro evaluation of the effect of fluoride varnish application at different frequencies on initial enamel lesions by surface microhardness (SMH) measurements. The null hypothesis tested was that there was no difference in enamel microhardness between different fluoride application frequencies.

Material and Methods

This study was approved by the Institutional Ethics Committee (17.10.2017/36290600/103). Before starting the study, a 'power analysis' was carried out to determine the sample size. In this study, it was planned to work with 95% confidence level, 0.45 sensitivity and 82% power, and it was decided to work on 60 samples, 15 in each group. 5 more tooth samples were prepared as spares in each group, and the experiments were carried out with 20 samples in each group.

Impacted permanent third molar teeth with an indication for extraction as a result of clinical and radiological examination were used. In order to provide standardization, teeth with 2/3 of the apex formation completed were preferred. Informed consents were taken from the patients before extraction. Care was taken to ensure that the teeth were intact, not damaged during extraction, and that there was no structural defect in the tooth enamel. The absence of visible structural defects such as caries, discoloration, demineralization, hypomineralization, fractures or cracks on the tooth surfaces was determined by examining them with a Stereomicroscope (Leica MZ12, Meyer Instruments, Houston, TX, USA) at x10 and x25 magnification, and teeth with these structural defects were excluded from the study. Soft tissue residues remaining on the teeth after extraction were removed by brushing under running water. Teeth were kept in a 0.1% thymol solution at room temperature till used.

Before the study, tooth roots were removed from the crown-root junction under water cooling. Then, the crowns of the teeth were embedded in acrylic molds with the buccal enamel surfaces exposed. The enamel surfaces were ground flat using 600- 900-1200 grit silicon carbide papers. The buccal surface was cover with 2 coats of acid-resistant nail varnish, leaving 4x4 mm of window. Enamel samples were randomly divided into 4 groups according to the frequency of fluor varnish application (Duraphat Varnish, 22,600 ppm, (Colgate Company, Australia)

Group 1: Fluoride varnish application once a year
Group 2: Fluoride varnish application 4 times at 1 week intervals
Group 3: Fluoride varnish application 4 times a year with an interval of 3 months
Group 4: Application of fluoride varnish twice a year at 6-month intervals.

The first 1x4 mm area of each sample was determined as the control group and no treatment was applied to this area. Microhardness levels of the intact enamel surface were measured from this area and covered with 2 coats of acid-resistant nail polish. The Vicker's hardness number (VHN) was determined by using digital hardness tester (Shimadzu Micro Hardness Tester HMV-2, Shimadzu Corporation, Kyoto, Japan). Three indentations were conducted in different regions of each specimen randomly. Then the average value was calculated.

After the initial microhardness values were measured and the areas determined as the control group were covered with acid-resistant nail polish , each sample was kept in the demineralization solution (2,2 mM $CaCl_2$, 2,2 mM NaH_2PO_4, 0,05 M acetic acid, pH was adjusted to 4.4 using 1M KOH) for 96 hours in containers containing 10 ml of demineralization in order to create initial enamel lesions on the enamel surfaces.[17,18] After the demineralization of the enamel samples, microhardness was measured from the second 1x4 mm area and covered with nail polish.

After the microhardness measurements were completed on the demineralized enamel surfaces, they were covered with nail polish and Duraphat Varnish (Colgate, Palmolive Co., New York, USA) was applied to the exposed enamel surfaces in accordance with the manufacturer's instructions. It was applied as a thin layer and each of the samples was taken into containers containing 10 ml of artificial saliva solution,[19] which were renewed every 24 hours. 6 hours after the fluoride varnish was applied to each sample, the samples were taken from the artificial saliva solution, the fluoride varnish residues on the surface were cleaned with the help of a surgical scalpel and cotton pellets, and left back to the storage environment.

At the end of the last fluoride varnish application of each group (Group 1; at the end of the 1st application; Groups 2 and 3; at the end of the 4th application; Group 4; at the end of the 2nd application), microhardness measurements were made from the third 1x4 mm area and covered with 2 layers of nail varnish. One year after the first fluoride application of all samples, microhardness was measured from the fourth 1x4 mm area.

After all the measurements were completed, the percentage of surface hardness recovery (%SHR) was compared with the formula given below[20]: %SHR = (SH2 − SH1) / (SH0 − SH1) X 100 SH0: surface hardness at the baseline SH1: Surface hardness after demineralization SH2: Surface hardness after fluoride treatment

Statistical analysis

The data were analyzed with the SPSS 22 package program. One-way Anova was used for comparisons with more than two groups in case the obtained data showed normal distribution, and The Kruskall-Wallis H test was used if it did not show normal distribution. For group comparisons Scheffe test and for in-group comparisons, t-test and/or Wilcoxon sign test were used in paired groups. 0.05 was used as the significance level.

Results

The mean (±SD) SMH values of the surfaces of all groups at the beginning, after demineralization and after remineralization are presented in Tables 1, 2 and 3. There was no significant difference between the groups in terms of microhardness values of the initial enamel surface ($p>0.05$). Additionally, after demineralization, the values between the groups were not significantly different($p>0.05$). The microhardness value of the Group 1, which fluoride varnish was applied once a year, was significantly lower than the other groups ($p<0.05$). The microhardness value of the Group 2 (fluoride varnish was applied 4 times with an interval of 1 week,) was significantly lower than Group 3(fluoride varnish was applied 4 times a year with an interval of 3 months) and was significantly higher than Group 4 (fluoride varnish was applied twice a year with an interval of 6 months) ($p<0,05$). The order of the experimental groups according to their microhardness values was; Group 3> Group 2>Group 4> Group 1.

The microhardness values obtained from the enamel surfaces in all groups at the end of one year are shown in Table 4. The results obtained because of the comparison of the microhardness values

Table 1. Minimum , maximum and mean microhardness values (VHN) and standard deviation (SD) values of the sound enamel surface at baseline (p>0.05)

		Groups						Anova		
		n	Mean	Min	Med	Max	SD	F	p	Scheffe test
Baseline	Group 1	20	352.75	338.33	354.67	362.67	6.62	.491	.689	-
	Group 2	20	350.60	328.00	353.50	365.33	9.66			
	Group 3	20	350.58	333.67	351.33	364.33	8.97			
	Group 4	20	353.05	336.67	356.67	362.00	8.61			
	Total	80	351.75	328.00	354.50	365.33	8.46			

Table 2. Minimum, maximum and mean microhardness (VHN) values of the demineralized enamel surface after demineralization (DM) (p>0.05)

		Groups						Anova		
		n	Mean	Med	Min	Max	SD	F	p	Scheffe test
After DM	Group 1	20	72.42	72.83	61.33	85.67	6.47	.690	.561	-
	Group 2	20	70.28	69.17	60.00	79.67	5.92			
	Group 3	20	72.50	73.00	60.33	81.00	5.76			
	Group 4	20	70.70	71.00	61.00	83.33	6.54			
	Total	80	71.48	71.33	60.00	85.67	6.15			

Table 3. Minimum, maximum and mean microhardness (VHN) values of remineralized enamel surfaces and standard deviation values after remineralization. (RM) (p<0.05)

		Group						Anova		
		n	Mean	Med	Min	Max	SD	F	p	Scheffe testi
After RM	Group 1	20	110.90	109.33	105.33	121.67	5.01	168.676	.000	1-2
	Group 2	20	141.42	140.33	129.33	155.67	6.80			1-3
	Group 3	20	149.08	149.67	132.67	158.33	7.02			1-4
	Group 4	20	122.92	122.17	115.00	131.67	4.74			2-3
	Total	80	131.08	130.33	105.33	158.33	16.23			2-4
										3-4

Table 4. Minimum, maximum and average microhardness (VHN) values and standard deviation values obtained from remineralized enamel surfaces at the end of 1 year. (p<0.05)

		Groups						Anova		
		n	Mean	Med	Min	Max	ss	F	p	Scheffe test
1 year	Group 1	20	115.97	116.83	105.67	127.00	6.24	157.627	.000	1-2
	Group 2	20	137.48	136.67	128.33	149.67	6.01			1-3
	Group 3	20	152.05	151.67	142.00	162.00	5.84			1-4
	Group 4	20	123.07	121.83	117.33	133.33	4.57			2-3
	Total	80	132.14	129.67	105.67	162.00	15.03			2-4
										3-4

Table 5. Minimum, maximum and mean values of surface hardness recovery (SHR)(%) values and standard deviation values of the groups (p<0.05)

		Groups						Anova		
		n	Mean	Med	Min	Max	ss	F	p	Scheffe test
SHR (%)	Group 1	20	13.725	13.084	10.340	20.457	2.159	96.402	.000	1-2
	Group 2	20	25.376	26.002	18.959	30.314	3.237			1-3
	Group 3	20	27.553	28.203	18.735	32.864	3.129			1-4
	Group 4	20	18.474	18.267	13.626	24.437	2.926			2-3
	Total	80	21.282	21.294	10.340	32.864	6.222			2-4
										3-4

obtained from the measurements made at the end of 1 year between the groups were found to be the same as the results of the end-of-treatment values. When the mean values at the end of the treatment and the mean values at the end of 1 year were compared within each group separately, no significant difference was observed (p>0.05).

Percentage surface hardness recovery (%SHR) values are shown in Table 5. It was found that the SHR value of Group 1 was significantly lower than the other groups (p<0.05). The SHR value of Group 2 was significantly lower than Group 3 and significantly higher than Group 4 (p<0.05). Group 3 SHR value was found to be significantly higher than Group 4 (p<0.05).

Discussion

Although there are many studies on the remineralizing effect of fluor varnish, no study has been found in the literature in which the frequency of application is recommended or compared. Therefore, the aim of this study was to evaluate the effect of fluoride varnish application frequency on remineralization in invitro conditions in initial enamel lesions. The findings revealed that fluoride varnish application four times a year with an interval of three months was more effective.

In this study, the effect of fluoride varnish application at different frequencies on enamel remineralization was evaluated using the SMH analysis. The concept of remineralization has gained importance with the clear demonstration that the caries lesion progresses reasonably slowly in the early stages.[21,22] Fluoride varnish, which is the most commonly used agent in the prevention of caries and in the remineralization treatment of early enamel caries, is considered as the 'gold standard'.9 Considering the frequency of use of Duraphat varnish in various studies on the prevention of caries formation in individuals with different caries risk, the frequency of application of fluoride varnish in our study was determined as once a year, 4 times with an interval of 1 week, 4 times a year with an interval of 3 months, and twice with an interval of 6 months.[23–25] Surface microhardness is a physical property which assesses the effect of chemical and physical agents on hard tissues of teeth. This is a useful way to examine the resistance of fluoride treated enamel.[26] The advantage of this method is high accuracy and quantitative measurement capability.[27]

The values of surface microhardness obtained in the present study were in the range of 328- 365,3 VHN before demineralization which is near to the range of reported microhardness for normal tissue of enamel.[28–31] The surface mean micro hardness values for each group of the enamel specimens reduced to 60,0 - 85,67 VHN after the demineralization process for 96 h. There was no significant difference between groups. The microhardness values of the enamel we obtained after demineralization are compatible with other studies.[32,33]

Microhardness increased in all groups after fluoride applications. However, the microhardness values of the group that fluoride varnish was applied once a year were found to be significantly lower than the other groups. The microhardness values of the group that fluoride varnish was applied 4 times a year with an interval of 3 months were found to be higher in the other groups. Similar to the results obtained in this study, Cardaso et al. reported the microhardness value after remineralization as 93.6±28.4 where Duraphat was applied once.[34] In the study in which different remineralization agents were compared, the hardness value was measured as 119.00±12.50, similar to our study.[28] In studies evaluating the recovery of surface hardness on enamel surfaces after remineralization the percentage of surface hardness recovery (%SHR) values were found to be high in fluoride application groups.[20,32,34,35] In our study, as a result of the application of Duraphat varnish at different frequencies, the recovery values in the enamel surface hardness were found to be approximately the same as in other studies, and it was observed that the highest recovery was in the group that applied fluorine varnish 4 times a year at 3-month intervals. When the mean values at the end of the treatment and the mean values at the end of 1 year were compared within each group separately, no significant difference was observed (p>0.05). The surface hardness of the enamel remained the same as no factors changed for one year after the remineralization treatment. This shows us that if the factors that increase the activity of the caries lesion (poor oral hygiene, sugar-containing diet, lack of regular dental check-up, etc.) are avoided after the treatment, the enamel surface will maintain its treated state.

There are differing opinions on how often to apply fluoride varnish. Seppa and Tolonen[36], in their 2-year randomized study, concluded that applying fluoride varnish twice or four times a year did not make a significant difference in caries incidence. As a result of the same study, it was concluded that more frequent use of fluoride in children in the high caries risk group did not provide more benefits. Contrary to this, Modéer et al. stated that the incidence of caries decreased significantly as a result of a 3-year study by applying Duraphat fluor varnish 4 times a year in proximal caries of premolar and molar teeth.[24] Newbrun[37] and Ripa[38] in their studies suggested that in children in the high-risk group fluoride varnish should be applied 4 times a year. Arends and Schuthof showed by micro-hardness analysis and microradiography that 24-hour contact of fluoride varnish was sufficient to completely inhibit demineralization.[39] Oliveira stated that fluoride varnish at a concentration higher than 23,000 ppm of fluoride in weekly applications over 4 consecutive weeks favors the remineralization of carious lesions in both surface and sub-surface regions.[29]

In our study, as in similar studies, SMH, which decreased after demineralization, increased in all application frequencies after fluoride varnish application. However, the initial enamel hardness value was not reached after any application, and values obtained were far below this value. However, even at these values, it is reported to prevent enamel demineralization. Therefore, it can be said that even a single application will be sufficient.[36–39]

By applying demineralization again at the end of 1 year after fluoride varnish applications, the effectiveness of the obtained enamel surface hardness in preventing demineralization could be better determined. This is the limitation of this study.

Conclusion

In the light of the findings of this study, it was concluded that the frequency of fluoride varnish application 4 times a year and once every 3 months is more effective in the remineralization treatment of initial enamel lesions. However, since the hardness values at the end of the treatment remain the same after 1 year, we believe that less frequent fluoride varnish applications may be sufficient in the presence of good oral hygiene, regular oral and dental care and regular dental examination.

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Author Contributions

E.S.: Literature reading, performing experiments, collecting data, writing. Z.O.: Consulting, writing the article.

Conflict of Interest

Authors declare that they have no conflict of interest.

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