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A COMPARISON OF CONVENTIONAL SODIUM FLUORIDE VARNISH AND NANO SODIUM FLUORIDE GEL REGARDING FLUORIDE UPTAKE INTO ENAMEL OF DECIDUOUS TEETH: AN *IN-VITRO* STUDY WITH SEM-EDX ANALYSIS

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ABSTRACT: ***Objectives:*** **Dental caries is the most common chronic disease worldwide and different forms of fluoride are considered to be a helpful preventive tool. Producing substances in nanoscale can significantly improve their mechanical and chemical properties. Therefore, the aim of the present study was to assess nano NaF gel regarding fluoride uptake when applied on enamel of deciduous teeth.**

Method: **A total number of 40 enamel samples were obtained from caries-free primary canines. Samples were randomly assigned to four groups (G1=NaF5%, G2=NanoNaF 1%, G3=Nano NaF 5%, G4= control) (10 samples in each group). Each group was divided into two subgroups named sound (S) and demineralized (D) (5 samples in each subgroup). Half of the samples in each group were demineralized then enamel samples were treated with the fluoride type specified for each group. Finally, SEM-EDX microscope was used to determine fluoride uptake. Data were analyzed using the following statistical tests; one-way Anova, Kruskal Wallis-H, Tukey post hoc test and Dunn's post hoc test.**

Results: **Fluoride was not detected in G1 and G4 groups. However G2 and G3 groups revealed a significant increase regarding fluoride content. Fluoride content was significantly higher in samples with higher Ca/P ratio.**

Significance: **Considering the high fluoride uptake achieved by Nano NaF, it might be possible to decrease the dose of fluoride needed at each dental appointment meanwhile gain better results regarding possible caries prevention. Also, we may be able to increase the time intervals between fluoride therapy sessions, especially important in young children lacking enough cooperation.**

Key Words: Deciduous teeth; Nanotechnology; Pediatric dentistry; Topical fluoride.

INTRODUCTION

There has been a documented decrease in the incidence of dental caries through the last few decades. However dental caries is still stated to be the most common chronic

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disease worldwide, and affects 60 to 90% of school aged children and most of the adults (World Health Organization 2018).[1-3] Prevention of dental caries is especially important in children as dental caries can result in lost school time, restricted activity days, problems in eating, speaking, and learning as well as the common complications such as pain, infection, tooth loss, and reduced quality of life.[4] Topical fluoride has been confirmed to present anticariogenic properties,[5] especially during the post eruption enamel maturation period, defined as the first 2 years after tooth eruption in the oral cavity.[6] Applying fluoride on the tooth surface results in the precipitation of calcium fluoride deposits and formation of fluorapatite. The main preventive effect of professional fluoride application is due to the steady release of fluoride from the calcium fluoride deposits which serve as a long-lasting fluoride reservoir.[7]

Nanotechnology introduces the use of nanomaterials (ranging from 0.1 to 100 nm) which present unique properties regarding their increased specific surface area and the consequent increase in surface reactivity and quantum-related effects.[8,9] Nanotechnology has provided great advances in prevention, diagnosis, and treatment of oral diseases.[10] In this regard, some studies have been carried out in order to evaluate the effect of producing substances in nanoscale on remineralization of incipient caries and prevention of new carious lesions. Examples of these nanoparticles are nanohydroxyapatite, nano silver fluoride, Calcium fluoride nanoparticles, Calcium phosphate-based nanomaterials, amorphous calcium phosphate (ACP) nanoparticles and nano bioactive glass materials.[10] However, only one study was carried out in order to analyze the effects of nano sodium fluoride, in which the endurance of permanent teeth toward dental caries was assessed.[12]

It has been stated that excessive intake of fluoride can be related to many skeletal diseases, cardiovascular and neurological problems and imbalances in thyroid hormones.[13,14] While many consider consuming a reasonable range of fluoride is essential as it plays an important role in the maturation of bone and teeth,[15] others do not see fluoride as an essential element for human growth and development and is accordingly not necessary for the development of healthy teeth and bones.[16] Today, fluoride therapy has brought about major concerns regarding the potential of chronic toxicity, typically manifesting as dental fluorosis. This is due to repeated ingestion of small amounts of fluoride as they may not be capable of or may not understand the importance of expectorating topical fluoride agents.[17] If the effectiveness of fluoride therapy in enhancing enamel's resistance to caries progression is increased, apparently, we can reduce dental visits scheduled for fluoride application and perhaps obtain better results in dental caries prevention with fewer visits. Due to the proven increase in the effectiveness of many substances produced in nanoscale, this study was planned to evaluate effectiveness of nano NaF in remineralization of incipient caries and increasing enamel's resistance to caries progression in deciduous teeth. Maybe this could guide us on the path of producing nano NaF products with lower fluoride concentrations compared to the routine forms, meanwhile increasing the fluoride uptake by the tooth structure. Furthermore, by improving the cost effectiveness of fluoride therapy it may be possible to offer such a treatment to more community members. We hope that the result of this study be useful in enhancing the effects of fluoride therapy in children.

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METHOD AND MATERIALS

Sample Preparation

This *in vitro* study was carried out on children who referred to the Department of Pediatric Dentistry, University of Medical Sciences, Kerman, Iran, and their caries free primary canines needed to be extracted. The protocol of the study was approved by the Ethics Committee, Faculty of Dentistry, Kerman University of Medical Sciences, Kerman, Iran, under the code IR.KMU.REC.1398.675 and process No. 98000755. Samples were selected randomly, considering the following inclusion criteria; needing extraction of caries free primary canines for reasons such as orthodontic treatment, having not received any topical fluoride treatment in the past year, and no history of supplemental fluoride consumption for child and mother (during pregnancy). The enamel surface of teeth was checked with a magnifying glass and if a craze line was detected, that tooth was excluded from the study.

Forty sound primary canines were extracted and stored in 0.1% buffered thymol solution (pH 7.0, 4ºC) before initiating sample preparation. The enamel surfaces were ground flat with a silicon carbide disc (600 grades of Al 2 O 3 paper; Buehler Ltd.) for 20 seconds in order to eliminate the fluoride originally present in the outer layers of teeth and reduce inter-individual differences due to different oral environments. A 3×3 mm enamel sample was separated from the buccal surface of the crowns using a disk in a handpiece (Buehler Ltd., Lake Bluff, IL, USA). The thickness of the enamel samples was approximately 1 mm, thus consisting of enamel only.

Simple random sampling technique was performed in order to assign samples to the four defined different groups (G1, G2, G3, G4) (10 samples in each group). Furthermore, each group was divided into two subgroups named sound (S) and demineralized (D) (5 samples in each subgroup) (Table 1).

Table 1. Sample size in each group

Group	Surface treatment	No. of samples	Sub group	No. of samples
G1	NaF 5%	10	Sound	5
			Demineralized	5
G2	Nano NaF 1%	10	Sound	5
			Demineralized	5
G3	Nano NaF 5%	10	Sound	5
			Demineralized	5
G4	Control	10	Sound	5
			Demineralized	5

The sample size was determined by a statistics expert opinion, considering similar articles.[18] In order to carry out the simple randomizing technique, each time a canine was extracted, an individual who was blind to the treatment groups was asked to assign each tooth to one of the groups and subgroups numbered 1 to 8, until 5 specimen was assigned to each group.

Preparation of nano NaF gel

The nano fluoride gel was prepared in Laboratory of Nanomaterials and nanostructures, Pharmaceutical Research Center, Kerman School of Pharmacy, Iran. In order to make the gel, ethanol water was added to a carbomer powder or CMC (Carboxymethyl cellulose) along with 3 or 6% mint plant extract. The mixture was placed on a stirrer (Heidolph Company, Germany) for 45 minutes during which a few drops of 0.02 M NaOH solution and 0.01 g of chitosan (Merck Company, Germany) were added. The resulting solution was placed in the reflux system (Kimia Aghigh/ Iran) to perform growth and nucleation processes. After adding certain amounts of sodium fluoride in specific weight concentrations including 5%, 3%, 2% and 1% under magnetic synchronous conditions at 30ºC for 2 hours, the resulting gel was placed in the microwave (Samsung, Japan) at 300 watts for 6 minutes and each time was immediately placed in an ultrasonic bath (Elma-ultrasonic, Germany) at 60 watts for 5 minutes. To produce sodium fluoride nanostructures, reverse micelles use an aqueous solution of reactive raw materials that can be converted to insoluble nanoparticles. In this research, nanoparticles synthesized in micelles were obtained by various methods, including the hydrolysis of reactive raw materials using microwaves. Finally solvent removal and subsequent calcination lead to the production of the final product.

Surface treatment

Prior to surface treatment with any type of fluoride, samples in the demineralized subgroups were individually immersed in 30 mL of demineralizing solution for a period of 10 days, at 37ºC in order to produce a subsurface lesion with a lesion depth around 70–80 µm. In other words, half of the samples were demineralized using the following demineralizing solution: 3 mM $CaCl_2 \cdot 2H_2O$, 3 mM KH_2PO_4, 50 mM lactic acid, 6 µM tetraethyl methylhydroxydiphosphonate, and traces of thymol, pH 5.0 adjusted with 10 M KOH (Sigma-Aldrich, Germany). As for the sound subgroup, no demineralization was carried out prior to surface treatment.[17] PH meter (Metrohm company/ PL-700PV/ Indonesia) revealed a pH value of 7 for all fluoride forms.

Group 1: In this group, both subgroups (S & D) were treated with NaF 5% varnish (Clinpro White Varnish, 3M ESPE, USA). After drying the enamel samples, the varnish was applied on the enamel surfaces with a soft micro brush and the samples were then immersed individually in 30 mL of artificial saliva for 12 hr. The saliva was composed of the following reagents (v = 500 mL): 0.001 g ascorbic acid, 0.015 g glucose, 0.29 g NaCl, 0.085 g $CaCl_2 \cdot 2H_2O$, 0.08 g NH_4Cl, 0.635 g KCl, 0.08 g NaSCN, 0.165 g KH_2PO_4, 0.1 g carbamide, and 0.17 g Na_2PO_4 (Merck company/ Germany). Thereafter, the varnishes were removed from the surface with a scalpel and a flexible swab soaked in a 50% acetone solution (Merck company/ Germany).

After the treatment, samples were dried in a vacuum desiccator (Kimia Exir company, Iran) over a continuous period of 2 days.[13]

Groups 2 and 3: For the second and third groups, the steps mentioned above were carried out except that nano NaF 1% and nano NaF fluoride 5% were used respectively (instead of NaF 5%).

Group 4: No fluoride treatment was carried out on samples of the fourth group (control group).

Furthermore, specimens were covered with a layer of gold and analyzed using SEM-EDX microscope (TescanVega TS5130MM, Oxford Instruments, England).

Statistical analysis

The sample size formula for one way analysis of variances is as follows:

$$\text{Eq. (1):} \quad n = \frac{\varphi 7 u^2 k^2 \sigma^2}{(\mu_{max} - \mu_{min})^2}$$

The details of above formula are as follows:

$$\text{Eq. (2):} \quad \lambda = \frac{\sigma_m^2}{\sigma^2}$$

$$\text{Eq. (3):} \quad \sigma_m^2 = \sqrt{\frac{(\mu_i - \bar{\mu}_w)^2}{K}}$$

$$\text{Eq. (4):} \quad \varphi = \sqrt{\frac{\lambda}{k}}$$

In which the σ^2 ; is the variance of each group, σ_m^2 ;is between groups variance, K; is the number of groups, μ_{max} ;is the maximum mean between K groups, μ_{min} ;is the minimum mean between K groups.

.Descriptive analysis (mean and standard deviation) was reported. The comparison of means was conducted with one-way Anova, if each group had normal distribution and all groups have constant variances. The Kruskal Wallis-H was used instead of Anova, if above assumption was not met. If Anova analysis was significant, Tukey post hoc test was performed. The Dunn's post hoc test was performed instead of Tukey test for significant Kruskal Wallis-H test.

RESULTS

Table 2 shows the mean (± standard deviation) of the element content present on enamel samples based on each surface treatment. Fluoride was not detected in samples of NaF 5% and control group. However, when samples treated with nano

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NaF (1% and 5%) were assessed, a significant increase was noted regarding fluoride content. The amount of fluoride was higher on demineralized enamel samples treated with nano NaF compared to their sound counterpart. However, this was not reported to be significant according to Kruskal-Wallis test (p-value=0.095).

Table 2. Mean (± standard deviation) of the element content (%) present on enamel samples, according to each treatment. (S = sound, D = demineralized, F = fluoride, Ca = calcium, P = phosphorus, Ca/P = calcium/phosphorus ratio)

Group	Element							
	F		Ca		P		Ca/P	
	S	D	S	D	S	D	S	D
NaF 5%	0	0	45.55 ±14.81	64.34 ±4.39	17.86 ±4.79	25.05 ±2.17	2.54 ±0.35	2.57 ±0.10
Nano NaF1%	4.65 ±7.50	20.65 ±13.27	59.49 ±19.34	58.20 ±5.76	22.43 ±6.90	11.15 ±11.33	2.63 ±0.29	12.97 ±10.32
Nano NaF5%	18.89 ±10.80	21.20 ±11.61	62.96 ±4.90	55.39 ±6.20	18.90 ±9.48	13.85 ±8.29	4.54 ±3.19	5.94 ±4.62
Control	0	0	68.47 ±1.19	68.86 ±1.60	27.40 ±1.19	27.55 ±1.26	2.50 ±0.13	2.51 ±0.18

When the Ca/P ratio of enamel samples were assessed, Kruskal-Wallis test reported significant differences (p-value=0.009) between treatment groups.

When fluoride content was assessed regarding Ca/P ratio, results indicated that fluoride content is significantly higher in samples with a higher Ca/P ratio, which confirms the precipitation of fluoride in the form of CaF_2 in samples treated with nano NaF (Pearson correlation coefficient=0.705, p-value=0.002).

Representative SEM images of samples in the control group and NaF 5% group, showed no particular surface CaF_2 precipitation (Figures. 1 and 2). However, CaF_2 precipitates were obviously evident in the nano NaF 5% group (Figure 3) and as for the nano NaF 1% group, more indistinct but still dense precipitates were observed (Figure 4).

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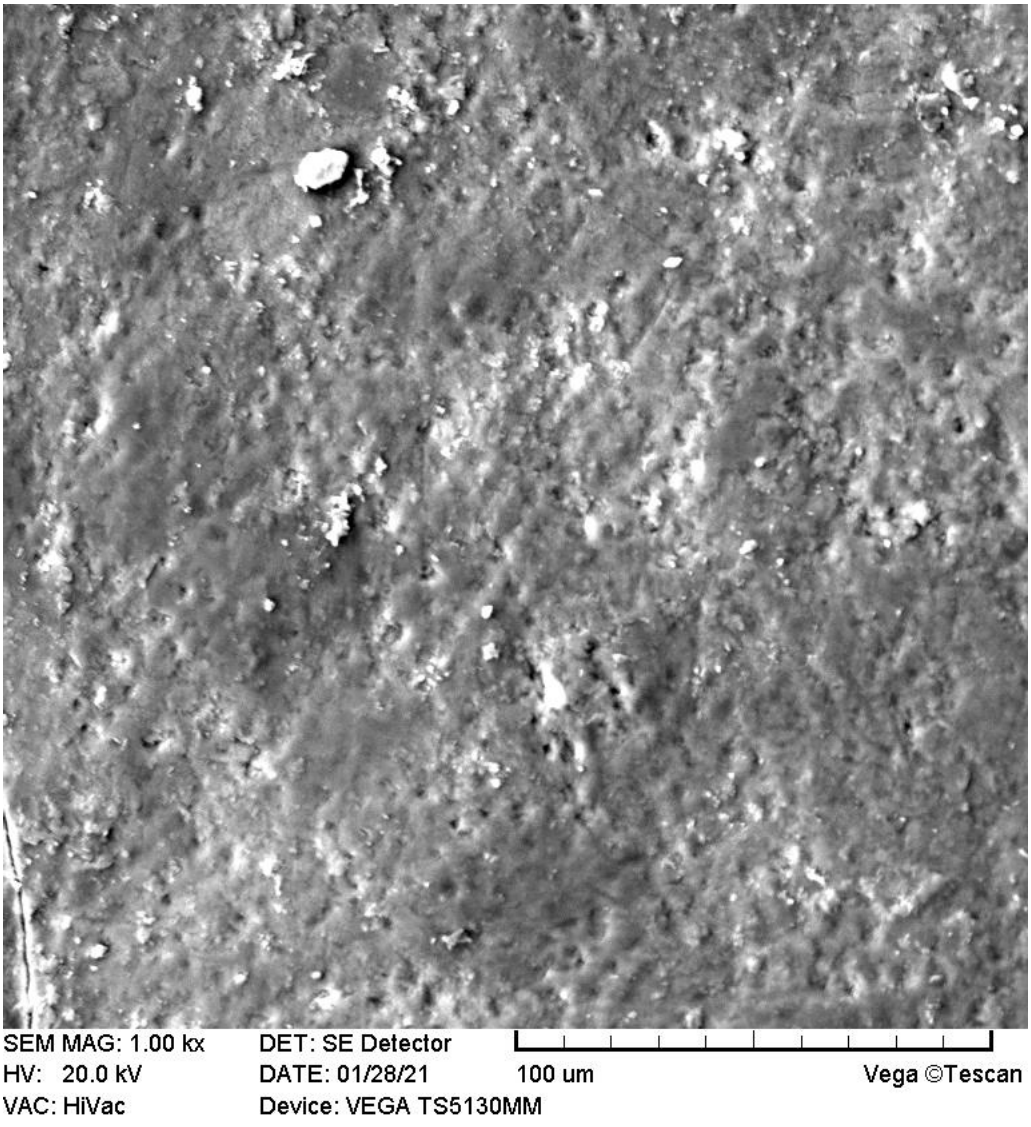

Figure 1. Representative scanning electron microscopy image of enamel sample in control group.

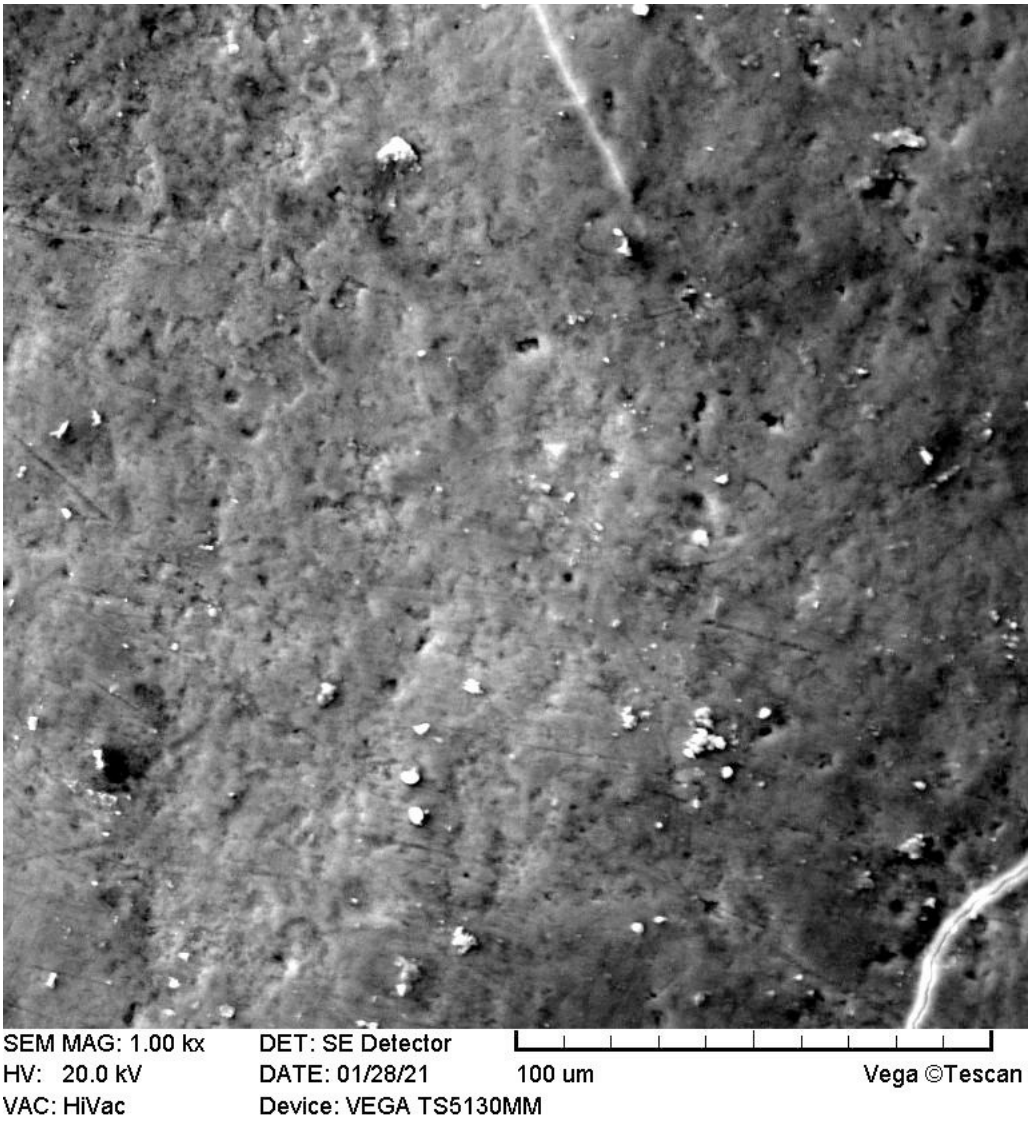

Figure 2. Representative scanning electron microscopy image of enamel sample treated with NaF 5% varnish.

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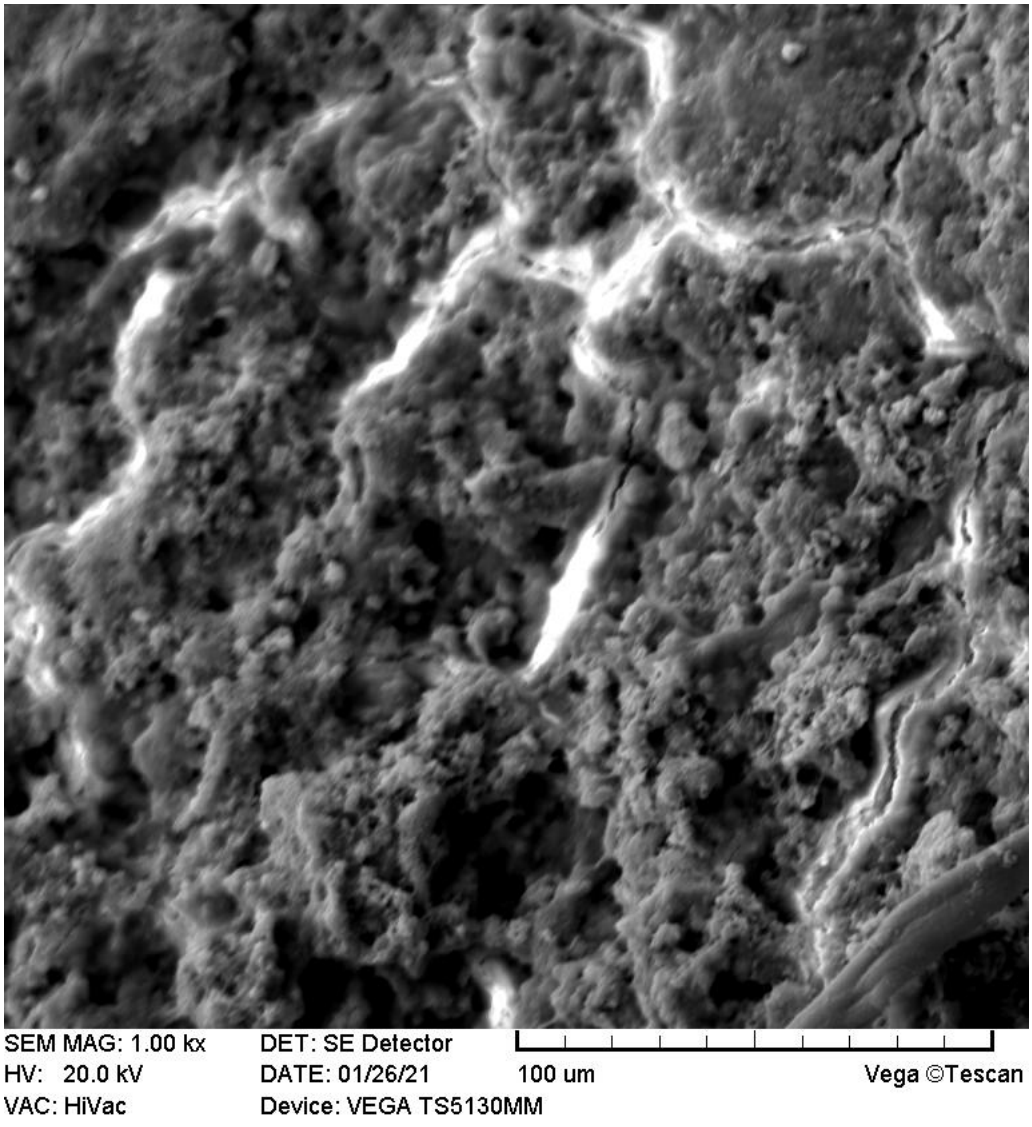

Figure 3. Representative scanning electron microscopy image of enamel sample treated with nano NaF 5% gel.

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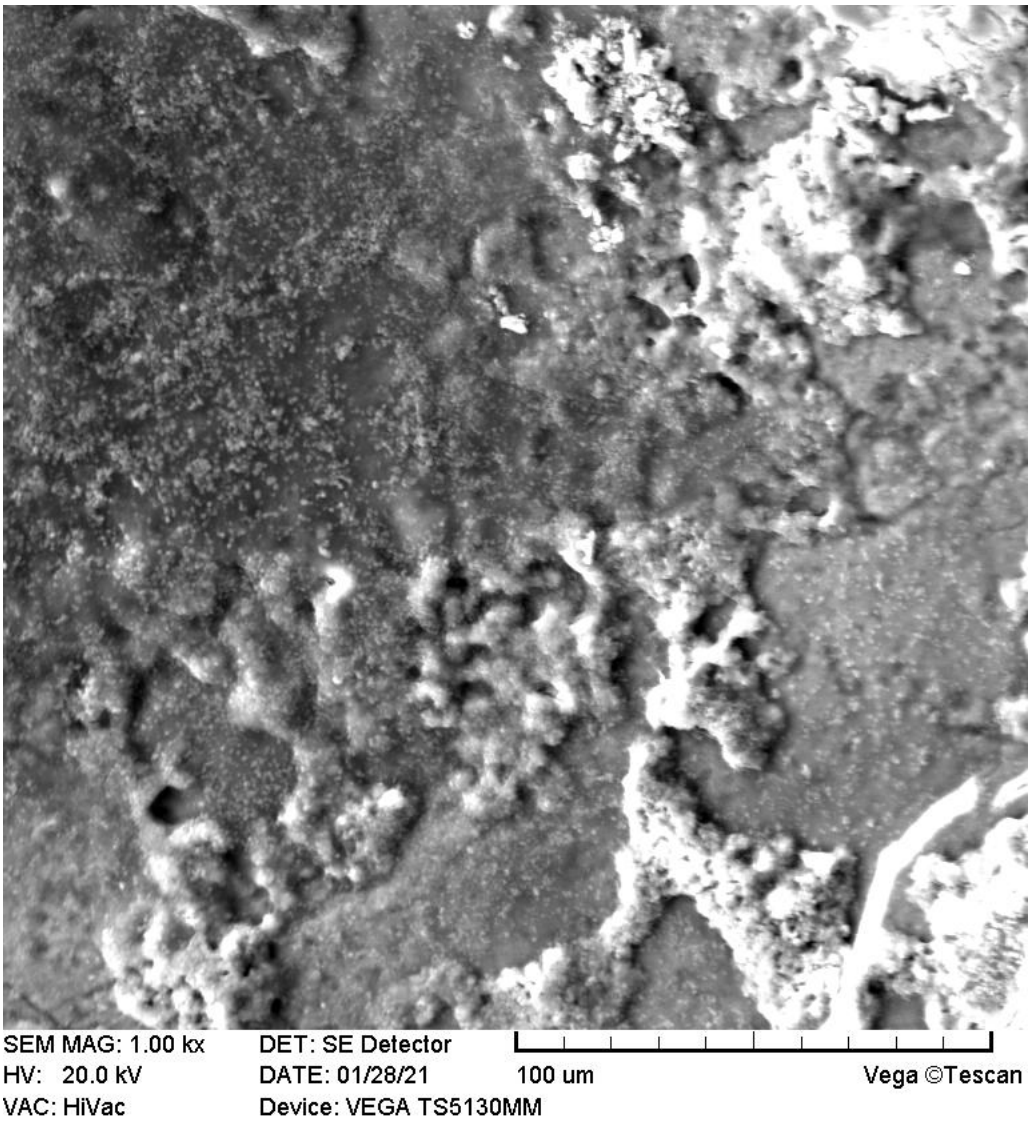

Figure 4. Representative scanning electron microscopy image of enamel sample treated with nano NaF 1% gel.

DISCUSSION

The present study showed higher fluoride content in enamel samples treated with fluoride gel in nano scale when compared with NaF and control group. This seems to be consistent with the results obtained by Fidaya et al. who examined the fluoride content of permanent enamel samples treated by NaF and nano NaF on which demineralization and remineralization cycles were carried out to assess the endurance of permanent teeth toward dental caries. They concluded that nano NaF application can increase the amount of fluoride (0.1289%) and fluorapatite (20.35%) when compared with NaF application.[12]

According to the present study, no fluoride element was detected in samples of NaF and control group which seems to be quite relevant with the results of a study carried out by Comar et al. in 2017.[18] The study design was similar to the present study and results revealed no fluoride content for enamel samples of deciduous teeth in control group (0.0 ± 0.0%). As for the NaF 5% group, fluoride content was stated to be 0.1 ±0.1% and 0.3± 0.2% in the sound and demineralized subgroups, respectively.[18] This can be attributed to the different pH values of fluoride varnishes used in the studies. It has been shown before that there is an intimate correlation between lower pH-values (pH<7) of fluoride agents and the amount of CaF_2-like precipitates on enamel.[19,20] Therefore, the lower pH value (5.0) of fluoride varnish used by Comar et al. (FGM Produtos Odontológicos, Joinville, Brazil) can increase the chance of fluoride element detection on enamel samples when compared to the fluoride varnish used in our study (Clinpro, 3M, America) with a pH value of 7.1. In this regard, Vicente et al. reported a mean weight percent of 0.71±0.30 for 65% of enamel samples treated with Clinpro varnish.[21] However, SEM images further showed relatively high degrees of demineralization. This was stated to be related to the high viscosity and low wettability of Clinpro, producing less contact between varnish and dental tissue. They considered the detected fluoride to be the remains of varnish which were not eliminated completely in spite of attempt to eliminate varnish by dental prophylaxis brush.[21] However, in the present study we used a scalpel and cotton swab soaked in 50% alcohol solution in order to remove fluoride which may be more effective than prophylaxis brush. This may be the reason that our study revealed no fluoride for the NaF 5% varnish group. Furthermore, the 0% fluoride element determined in samples of the control group can be attributed to the elimination of the outer layer of enamel, which is assumed to be the fluoride rich layer, by the process of disking samples prior to surface treatment. The results reported by Scholz et al. regarding fluoride uptake by enamel samples were in accordance with our study.[19] They revealed 0 atomic % regarding fluoride element in all control specimens without gel application. They also reported an atomic percent of 12.7 for specimen treated with acidic sodium fluoride gel (12500 ppm F^-, pH 4.75) and atomic percent of 0.1 for specimen treated with neutral sodium fluoride gel (12500 ppm F^-, pH 7.0).[19]

Different experimental fluoride solutions have been examined regarding fluoride uptake. For example, an *in vitro* study carried out by Hjortsjo et al. aimed to investigate fluoride uptake by enamel specimens treated with solutions of 0.2 and 0.4% HF (hydrogen fluoride) (pH 3.09 and 2.94), 1.74% SnF_2 (pH 2.9), 0.68% TiF_4 (pH 1.6) and 0.84% NaF (pH 4.5).[22] A maximum of 13.69 weight percent of fluoride was reported for 0.2% HF in the study.[22] Furthermore, an *in vitro* study by Comar et al. assessed fluoride element of enamel samples treated with NaF and TiF_3.[18] They reported a maximum level of 5.4% fluoride content in permanent enamel samples treated with TiF4 4.00%.[18] Promising results have been found by our research group with the use of NaF gel in nano scale regarding uptake of fluoride *in vitro*. Highest levels of fluoride element were detected in the nano NaF 5% group with a mean fluoride uptake of 21.20%.

The Ca/P ratio was also determined as this is an indicator of dental tissue mineralization.[21] Higher Ca/P-ratios following enamel treatment with nano sodium fluoride in the present study are indicative for CaF_2-precipitation.

Regarding fluoride uptake by demineralized and sound enamel samples, the present study revealed higher fluoride content in demineralized enamel samples which was stated to be insignificant. This was consistent with the results obtained by Comar et al. who reported a slightly higher fluoride element in demineralized enamel samples treated with NaF 5% when compared to sound enamel samples (0.1% and 0.3% respectively).[18]

In accordance to the results of Scholz et al. the representative SEM-EDX images of samples in the control group were similar to images of the NaF 5% group, showing no particular surface CaF_2 precipitation (Figures 1 and 2).[19] Comar et al. also reported no visible surface precipitation by the treatment of samples with different concentrations of NaF varnish. The samples treated with NaF varnishes presented a smooth surface similar to that of the control group.[18] This was further confirmed by the quantitative analysis of the specimen. However, CaF_2 precipitates were obviously evident in images obtained by the nano NaF 5% group (Figure 3) and as for the nano NaF 1% group, CaF_2 precipitates were observed but they were not so dense (Figure 4).

Results of the current study stated no significant difference regarding fluoride element in samples treated with different concentrations of nano NaF (1% and 5%). Maybe this could guide us on the path of producing nano NaF varnishes with very low fluoride concentrations which still present high fluoride elements when applied on the tooth structure. Thus, reducing the risk of potential toxicity related to the ingestion of fluoride during fluoride therapy appointments. Furthermore, we may be able to reduce dental visits scheduled for fluoride application but also obtain the intended results in dental caries prevention.[23] This may be particular helpful in the caries prevention of young children lacking enough cooperation for routine dental visits. Results also indicated no significant difference in fluoride content of sound or demineralized enamel samples in each group, which shows the effectiveness of fluoride therapy both before and after the initiation of incipient caries. However, in order to confirm these statements much more work needs to be carried out *in vitro* and especially *in vivo* to consider those variables that cannot be reproduced in the laboratory.

In the present study, the fluoride weight percentage was quantified by SEM-EDX microscope which has a detection limit of 2 microns from surface of enamel samples. It also has an element detection limit of 1000 ppm which makes it less reliable when detecting elements with low concentrations compared to more advanced techniques such as Wavelength Dispersive X-ray Spectroscopy (WDS). However, EDX is a well-established, easy to use, analytical technique which allows repetitive measurements on the same samples if it is required for any reason.[24]

There were several limitations to the present study. As this study was an *in vitro* study, it was difficult to precisely imitate the *in vivo* settings regarding the amount of fluoride which was applied on each enamel sample, also, moisture control which may be very difficult to achieve when working on uncooperative young children, was not addressed in the present study. Another limitation which can be mentioned when such *in vitro* studies are carried out is the inability to simulate oral functions such as swallowing and chewing. Therefore, variations between patients regarding fluoride uptake in clinical settings is not fully considered. Furthermore, using polished enamel

surfaces in an attempt to standardize samples creates different substrates on which fluoride is applied, compared to natural teeth. Finally, we suggest further *in vitro* and *in vivo* studies be carried out to assess different aspects of enamel endurance towards caries, after application of nano NaF.

CONCLUSION

Considering the high efficacy of nano NaF (1% and 5%), revealed in the present study, it might be possible to decrease the dose of fluoride needed at each dental appointment and at the same time, obtain better results in preventing caries progression. Another advantage we may gain is the possibility of increasing the time intervals in between fluoride therapy sessions as this is especially important in young children lacking enough cooperation. These measures may improve the cost effectiveness of a routine session of fluoride therapy and make it possible to offer such a treatment at community level.

REFERENCES

[1] Widita E, Pamardiningsih Y, Vega CA. Caries risk profiles amongst preschool aged children living in the Sleman District of Yogyakarta, Indonesia. Journal of Dentistry Indonesia 2017;24(1):1-6.

[2] de Sant'Anna GR, dos Santos EAP, Soares LES, do Espirito Santo AM, Martin AA, Duarte DA, et al. Dental enamel irradiated with infrared diode laser and photo-absorbing cream: Part 2—EDX Study. Photomedicine and Laser Surgery 2009;27(5):771-82.

[3] Urquhart O, Tampi M, Pilcher L, Slayton R, Araujo M, Fontana M, et al. Nonrestorative treatments for caries: systematic review and network meta-analysis. Journal of Dental Research 2019;98(1):14-26.

[4] Iheozor-Ejiofor Z, Worthington HV, Walsh T, O'Malley L, Clarkson JE, Macey R, et al. Water fluoridation for the prevention of dental caries. Cochrane Database of Systematic Reviews 2015;18(6).

[5] Mohammadi F, Horri A, Poureslami P, Poureslami H, Mahvi AH, Jalali F. Evaluation of fluoride uptake in the enamel of primary teeth after using different concentrations of a green tea extract: an *in-vitro* study. Fluoride 2020;53(2 Pt 1):268-75.

[6] Poreslami HR, Khazaeli P, Sajadi FS, Hasanzadeh R. Comparison of fluoride uptake into enamel from sodium fluoride gel 0.05% produced in Iran and stannous fluoride 0.4% gel. Journal of Kerman University of Medical Sciences 2012;19(2):140-8.

[7] Lee Y, Baek H, Choi Y, Jeong S, Park Y, Song K. Comparison of remineralization effect of three topical fluoride regimens on enamel initial carious lesions. Journal of Dentistry 2010;38(2):166-71.

[8] Calisir M. Nanotechnology in dentistry: Past, present, and future. In: Tekinay AB, editor. Nanomaterials for Regenerative Medicine [part of the series: Turksen K, series editor. Stem cell biology and regenerative medicine]. Cham, Switzerland: Humana Press, an imprint of Springer Nature Switzerland; 2019. pp. 197-216.

[9] Selvakumar J, Radhika B, Shamini S, Mahendra J, Preethi R, Shyma, MN, Poorani R. Emerging nanotechnologies in dentistry: A review. World Journal of Pharmaceutical Research 2019;8(13):599-620.

[10] Moothedath M, Moothedath M, Jairaj A, Harshitha B, Baba SM, Khateeb SU. Role of nanotechnology in dentistry: Systematic review. Journal of International Society of Preventive & Community Dentistry 2019;9(6):535.

[11] Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: a review of literature. International Journal of Clinical Pediatric Dentistry 2019;12(2):139.

[12] Fidya, Rachmawati R, Effendi MC, Dewi NKAF. The effect of NAF 5% and nano NaF to the permanent tooth endurance toward dental caries. Journal of International Dental and Medical Research 2015;8(2):34.

[13] Kheradpisheh Z, Mirzaei M, Mahvi AH, Mokhtari M, Azizi R, Fallahzadeh H, Ehrampoush MH. Impact of drinking water fluoride on human thyroid hormones: a case-control study. Scientific Reports 2018;8(1):1-7.

[14] Yousefi M, Yaseri M, Nabizadeh R, Hooshmand E, Jalilzadeh M, Mahvi AH, Mohammadi AA. Association of hypertension, body mass index, and waist circumference with fluoride intake; water drinking in residents of fluoride endemic areas, Iran. Biological Trace Element Research 2018;185(2):282-8.

[15] Mohammadi AA, Yousefi M, Yaseri M, Jalilzadeh M, Mahvi AH. Skeletal fluorosis in relation to drinking water in rural areas of West Azerbaijan, Iran. Scientific Reports 2017;7(1):1-7.

[16] Scientific Committee on Health and Environmental Risks (SCHER). Opinion of critical review of any new evidence on the hazard profile, health effects, and human exposure to fluoride and the fluoridating agents of drinking water. Brussels, Belgium: Directorate General for Health and Consumers, European Commission; 2011 May 16. pp. 2-4.

[17] Nowak A, Christensen JR, Mabry TR, Townsend JA, Wells MH. Pediatric dentistry-E-book: Infancy through adolescence: Philadelphia, USA: Saunders, an imprint of Elsevier Health Sciences; 2018. 673 pages.

[18] Comar LP, Souza BM, Al-Ahj LP, Martins J, Grizzo LT, Piasentim IS, et al. Mechanism of action of TiF4 on dental enamel surface: SEM/EDX, KOH-Soluble F, and X-Ray diffraction analysis. Caries Research 2017;51(6):554-67.

[19] Scholz KJ, Federlin M, Hiller K-A, Ebensberger H, Ferstl G, Buchalla W. EDX-analysis of fluoride precipitation on human enamel. Scientific Reports 2019;9(1):1-11.

[20] Harris NO, Garcia-Godoy F. Primary preventive dentistry. Upper Saddle River, NJ: Pearson Education; 2004.249 pages.

[21] Vicente A, Ortiz Ruiz AJ, González Paz BM, García López J, Bravo-González L-A. Efficacy of fluoride varnishes for preventing enamel demineralization after interproximal enamel reduction. Qualitative and quantitative evaluation. PloS One 2017;12(4):e0176389.

[22] Hjortsjö C, Young A, Kiesow A, Cismak A, Berthold L, Petzold M. A descriptive *in vitro* electron microscopic study of acidic fluoride-treated enamel: potential anti-erosion effects. Caries Research 2015;49(6):618-25.

[23] Jahanimoghadam F, Poureslami H, Shamsaddin H, Horri A, Khazaeli P, Mahvi AH. Effect of ER: Yag laser on sodium fluoride varnish uptake by primary tooth enamel: An *in-vitro* study. Fluoride 2016;49(4 Pt 2):538-48.

[24] Sorozini M, Dos Santos R, Silva E, Dos Anjos M, Perez C. Assessment of Ca and P content variation in enamel during an eight-week bleaching protocol using energy dispersive X-ray fluorescence. Spectrochimica Acta Part B: Atomic Spectroscopy 2017; 131:93-8.