Effect of different fluoride concentrations on remineralization of demineralized enamel: an in vitro pH-cycling study

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Summary

Objectives. The purpose of this study was to determine the effects of three different fluoride mouth rinses (226, 450 and 900 ppm) in comparison to non-fluoride application group (control group) on demineralized enamel under in vitro pH-cycling conditions.
Methods. Initial demineralization was obtained by acetic acid for 24 hours. After remineralization for 11.5 h, pH-cyclus was as follows: demineralization with acid solution for 30 min., application of NaF (control (0), 226, 450 and 900 ppm F^-) for 2 min. and remineralization for 11.5 h. This procedure was applied twice. This 24-hour cycling application was repeated for 28 days. Vickers microhardness measurements were conducted at the beginning, after the initial demineralization and after 3, 7, 14 and 28 days pH-cycling applications.
Results. Remineralization begins after 14 days in all groups (Wilcoxon, $p > 0.05$). Only the group with 226 ppm fluoride reached the beginning microhardness ($p > 0.05$).
Conclusions. It was concluded that regular daily use of fluoride solutions with 226 ppm F^- enhanced remineralization in the pH-cyclus environment and reached the beginning microhardness. Demineralization did not continue in any fluoride treatment group, even in the control group.
Key words: fluoride, demineralization, remineralization, pH-cycling, microhardness.

Introduction

Enamel is consistently exposed to de-/remineralization in oral conditions. There is a delicate balance between demineralization and remineralization [1, 2]. The interruption of this balance results in caries, where fluoride is the most commonly used agent for „healing“ of the initial process. The presence of fluoride in saliva and plaque, during a cariogenic challenge, can inhibit the dissolution of enamel crystals and subsequently enhance remineralization. But additional fluoride applications are mostly recommended. Mouth rinses, gels or varnishes are preferred to enhance the remineralization and reduce the demineralization [1, 3, 4]. Enamel de-/remineralization processes were studied previously in vitro [3, 5, 6] and in vivo [7-9]. De-/remineralization processes have been tested by polarized light microscopy (PLM) [10], electron microscopy [11], quantitative microradiography (MRG) [6, 10, 12, 13], surface microhardness (Knoop, Vickers) [12, 14, 15], iodide permeability (Ip) [15] and calcium and fluoride analysis [13].

Previous studies have evaluated the fluoride efficacy of oral hygienic products, such as toothpastes or [10,16-20] mouth rinses [21-24].

The purpose of this study was to determine the effects of three different fluoride mouth rinses (226, 450 and 900 ppm) in comparison to non-fluoride application group (control group) on in vitro enamel demineralization under pH-cycling conditions.

Materials and Methods

Preparation of the tooth slabs: 28 caries free premolars extracted for orthodontic reasons were used in this study. Teeth were sectioned into two enamel slabs in the mesio-distal direction. The

vestibular side of each tooth was embedded in epoxy resin with the enamel surface parallel to the resin block surface. Enamel slabs were grinded with 320, 600 and 1200 grade silicon carbide discs and polished with aluminum paste. A 4 mm x 3 mm test area was obtained in the center of the specimen. These samples were randomly assigned into four groups (group 1, 2, 3 and control group) (n = 7).

Experimental design employed in this study is shown in *Figure 1.*

Experimental solutions:

- demineralization solution; it contained 2.2 mM/L $CaCl_2$, 2.2 mM/L KH_2PO_4 and 50 mM/L acetic acid, and the pH was adjusted to 4 with KOH. This solution was used to form the initial enamel lesion and was also applied for 30 minutes for daily demineralization [6].

- remineralization solution; it contained 1.5 mM/L $CaCl_2$, 0.9 mM/L KH_2PO_4 and 130 mM/L KCl, with pH adjusted at 7 [6].

- fluoride solutions contained NaF with concentrations of 226 ppm F^- (Group 1), 450

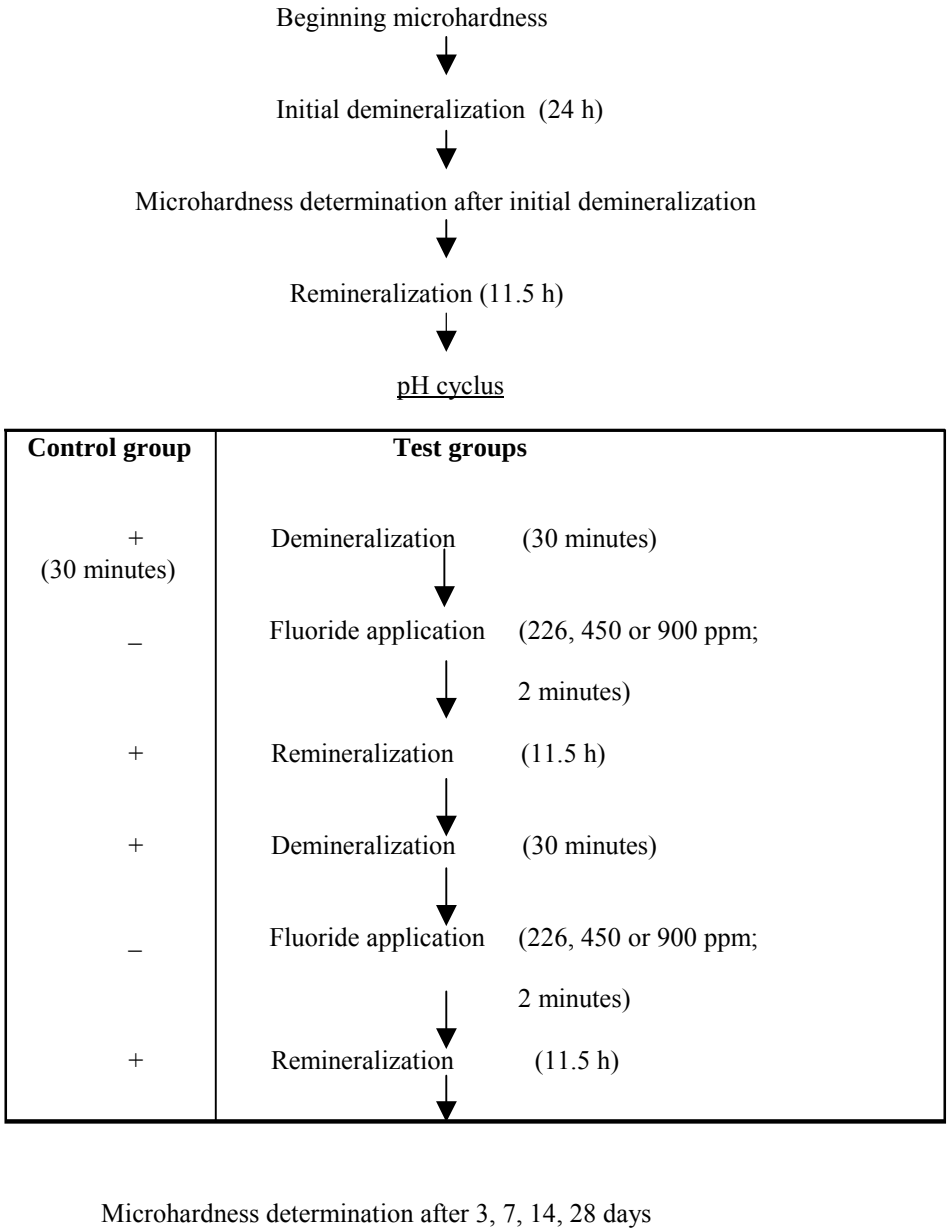

Figure 1. Experimental design

ppm F^- (Group 2) and 900 ppm F^- (Group 3). All solutions were freshly prepared every day.

Experimental process: *(Figure 1)*
For initial enamel demineralization, enamel slabs were stored in demineralization solution for 24 h and remineralized for 11.5 h. The pH-cyclus model started with a demineralization for 30 minutes. The experimental samples were treated with fluoride solutions for 2 minutes followed by remineralization for 11.5 h. This procedure was repeated twice. This cyclus was repeated for 28 days.

Microhardness testing:
Enamel microhardness was tested by a microhardness tester (Japan) with a Vickers diamond indenter loaded with 200 gr and applied for 10 seconds. The mean of five hardness measurements made at 35 μm intervals was used as representative Vickers Hardness Number (VHN). The diagonal length of the indentation was measured and converted to VHN.

Microhardness measurements were performed at the beginning, after the 24-h initial demineralization, and after the 3rd, 7th, 14th, and 28th day.

Statistical analysis:
Friedman and Wilcoxon tests were used to compare the significance of differences within the groups. Comparison between the groups was analyzed using Kruskal-Wallis test.

Results

Table 1 shows the mean and standard deviation of VHN values for group 1, 2, 3 and the control group. *Table 2* shows statistical analysis of the differences in microhardness values at various stages of the experiment (3rd, 7th, 14th and 28th day).

Microhardness values of the sound and the demineralized enamel:

No significant differences were observed among the microhardness values for all groups at the beginning. Microhardness values of enamel slabs after demineralization did not show any difference among the groups. Important differences were noted for microhardness values between the beginning and after initial demineralization in all groups ($p < 0.05$). Microhardness values after initial demineralization were not significantly different, which is important for the standardization of the study.

Microhardness measurements at the 3rd and 7th days:

No significant increase in hardness was observed in fluoride and control groups after the 3rd and the 7th days ($p > 0.05$) *(Table 2).*

Table 1. The mean and standard deviation (SD) of Vickers microhardness (VHN) value in the groups

Groups		VHN at the beginning	VHN after initial demineralization	VHN after 3rd day	VHN after 7th day	VHN after 14th day	VHN after 28th day
control	mean	368.54	305.20	319.42	319.52	323.22	31.94
(0 ppm)	SD	11.08	23.94	18.12	8.32	21.01	16.26
group 1	mean	366.51	305.11	320.20	328.14	340.11	354.00
(226 ppm F^-)	SD	10.351	25.18	31.93	33.31	26.36	28.19
group 2	mean	368.40	304.85	309.05	315.94	325.42	340.08
(450 ppm F^-)	SD	8.42	21.15	17.09	17.20	11.30	15.03
group 3	mean	365.80	302.48	310.57	319.65	327.34	342.54
(900 ppm F^-)	SD	9.70	22.08	17.54	15.16	19.93	19.93

Table 2. Statistically analyses of differences within groups

	Friedman		Demin 3rd day	Demin 7th day	Demin 14th day	Demin. 28th day	Beginning 28th day
	X^2	p	p	p	p	p	p
control	23.19	0.0005	0.093	0.116	0.028*	0.018*	0.018*
group 1	27.00	0.0005	0.091	0.063	0.018*	0.018*	0.173
group 2	27.54	0.0005	0.499	0.176	0.028*	0.018*	0.018*
group 3	25.36	0.0005	0.735	0.091	0.018*	0.018*	0.028*

**: significant according to Wilcoxon test (α: 0.05)*

Microhardness measurements at the 14th day:

The microhardness values in the fluoride and control groups were significantly different in comparison to the demineralized enamel after the 14th day ($p < 0.05$). All groups were remineralized after 14 days *(Table 2),* but did not reach the beginning microhardness.

Microhardness measurements at the 28th day:

The remineralization of all groups continued until the 28th day ($p < 0.05$). The obtained results at the 28th day showed that only group 1 (226 ppm F^-) reached beginning microhardness measurements ($p > 0.05$, *Table 2).* Group 2 and 3 did not show any differences related to the control group ($p > 0.05$).

Discussion

The present study was designed to determine the period of the expected remineralization under continuous pH conditions.

Simulation of the natural mouth environment forces the researchers to use pH-cycling techniques [25]. Different modifications of this technique have been applied for investigating caries processes and effect of caries preventive agents [10, 16, 17, 26, 27]. Therefore, pH-cyclus creating models can be accepted as a good evaluating method of the caries process and also provide standard study conditions. Because of these reasons, the present research was designed on a pH cycle and determined the effects of three different fluoride mouth rinses in comparison to non-fluoride application group (control group) on in vitro demineralized enamel. In this study, the experimental set-up was arranged in such a way that it simulated an oral environment subjected to acid and remineralization twice a day. In order to accomplish this, cariogenic acid, flouride and remineralization solutions were applied on the initially demineralized sample surfaces.

NaF is a preferred agent for caries investigations [3, 17, 18]. Therefore, in this study the fluoride treatment solutions were prepared with NaF. Fluoride applications (2 minutes) were used twice daily. The fluoride concentrations used in our study (226 ppm, 450 ppm and 900 ppm) are identical to the concentrations of fluoride rinses, which are clinically recommended (0.05%, 0.1% and 0.2% NaF solutions).

It was reported that microhardness profiles could be used for comparative measurements of hardness changes of dental hard tissue [12, 14, 15, 17, 21, 24]. A microhardness evaluation was fulfilled in this study.

Kodaka et al. evaluated the correlation between microhardness and mineral content in sound human enamel [28]. The study concluded that microhardness values do not reflect small differences in the mineral and organic contents of sound enamel, but are indications of gross changes, as observed in enamel caries.

Zero et al. indicated that both Ip test and surface microhardness (SMH) test had sufficient sensitivity to detect the very early stages of enamel demineralization [15]. The coating of the enamel pores with calcium fluoride layer can affect Ip test whereas SMH test is not affected by it.

Many authors have investigated fluoride concentration and efficacy of fluoride application.

White reported that there was an increase in remineralization and in the resistance of enamel against acid when toothpastes with sodium fluoride (0.243% F^-) and amine fluoride (0.34% F^-) were used [5].

Featherstone et al. showed that the maximum remineralization efficacy of fluoride was at 550-600 ppm F^- [29].

Damato et al. searched for the effects of NaF solutions on the artificially carious enamel using different concentrations [30]. Their results have shown that remineralization was high in the 500 ppm F^- group, but there was no additional remineralization when higher fluoride concentrations were applied.

Lammers et al. studied the effect of remineralizing solutions with or without 2 ppm F^- on the remineralization of bovine enamel with artificial subsurface lesions. However, these investigators did not use a pH-cycling model in their in vitro study. The group applied with fluoridated solution showed less remineralization in comparison to the nonfluoride group. They explained this finding by the inhibitory effect of fluoride at certain concentrations on the crystal growth [31].

Tagaki et al. reported that an in vitro pH-cycling model was used to evaluate the potential anti-caries effects of 13.2 and 52.6 mmol/l NaF and 3 mmol/l F^- two-component rinses. They observed that two-component rinses with 3 mmol/l F^- provided a degree of demineralization protec-

tion equal to a 13.2 mmol/l NaF (250 ppm F^-) rinse [32].

The importance of the frequency and period of application of fluoride rinses have also been investigated.

Kirkham et al. suggested that the degree of de-/remineralization increased with frequency of acid challenge [33].

Stephen reported that the frequency of fluoride rinsing is more important than the concentration of fluoride [18].

In our study, three different fluoride concentrations were used on the initially demineralized enamel and pH changes were simulated for 28 days. It was also determined that the frequency is more important. There was no significant increase in the microhardness values after 7 days. The remineralization of initially demineralized enamel needs more than 7 days, under continuous demineralization conditions (periods of 30 minutes, twice daily). The 14th day measurements showed that remineralization occurred in all groups. After the end of the cyclus only the group with 226 ppm reached the beginning microhardness. Due to the methodological differences between the pH-cycling studies, other researches cannot be compared or related directly with our study.

The present study showed that 226 ppm fluoride application is sufficient for remineralization, and there is no need to increase the concentration.

Remineralization is observed clinically as the disappearance of white spot lesions. It was reported that remineralization occurs during caries development [34]. The application of low concentrated fluoride products have been recommended to the individuals who have a high risk of tooth decay, white spot or initial enamel erosion lesion [2, 4].

High concentrated fluoride solutions form a calcium fluoride or calcium fluoride-like substance. These substances may act as a reservoir of fluoride in pH changes in oral conditions [1, 3, 35]. Another approach is that CaF_2 blocks the diffusion of ions into the enamel and fluoride cannot reach the subsurface lesion [10]. In toothpastes, due to the high fluoride concentration (1000-1500 ppm), a CaF_2 like substance may occur in plaque, on mucosal surface, on enamel surface or inside the caries-like lesions [1-3]. Studies show that remineralization of deep lesions is experimentally possible in both enamel and the underlying dentin [36]. Under in vivo conditions, the presence of precipitation inhibitors (e.g. salivary proteins, pyrophosphates or diphosphanates) in saliva might affect diffusion and precipitation through inhibition of crystal growth [34, 36]. The CaF_2 or CaF_2-like layer may be the explanation why additional remineralization did not occur with higher fluoride concentration (450 or 900 ppm) in our study.

In the present study, remineralization was observed in all groups, even in the control group, in spite of the demineralization periods. These findings are in agreement with Meyerowitz et al. intra-oral appliance model [21].

Clinically, continuous low pH, frequency of the cariogenic diet, saliva composition, saliva flow rate, salivary clearance, salivary buffering capacity, oral health habits, effectiveness of tooth brushing and the periodontal condition are important factors in caries activity. Additional fluoride application, such as mouth rinses, fluoridated chewing gums, professional fluoride applications should be recommended individually after detailed history and determination of the oral condition through caries activity test, saliva pH and other individual criteria. Recalls and carious activity tests can modify the recommendations for oral care.

According to the findings obtained, no relationship exists between the fluoride concentration and remineralization enhancement. The 226 ppm fluoride treatment group showed the best „healing“ in this study. The regular daily use of the fluoride mouth rinses with low fluoride concentration might enhance remineralization and play a role for reaching the beginning microhardness under continuous demineralization conditions.

Conclusions

It was concluded that the 226 ppm fluoride application is sufficient for the remineralization, and there is no need to increase the concentration under pH-cycling conditions. The regular daily use of 226 ppm F^- enhances the remineralization under pH-cyclus environment and reached the beginning microhardness after 28 days. Demineralization did not continue in any fluoride treatment group, even in the control group.

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