The protective effect of topical fluoride treatments in dentine lesions

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Chapter 6

The effect of equal fluoride concentrations as SDF and KF on demineralized dentine during pH-cycling:

Microradiography
Abstract

The present study aims to compare the efficacy of equal fluoride concentrations of either SDF or KF on demineralized dentine lesions in a non-microbial pH-cycling model. Demineralized dentine specimens were treated once by three different concentrations of either SDF or KF (0.26%, 1.025%, 4.1% F⁻) or no treatment (control). Subsequently, the specimens were subjected to 21-day of pH-cycling with daily six cycles of 0.5 h in the demineralization buffer and 2.5 h in the remineralization buffer, followed by a night period of 6 h in the remineralization buffer; in the weekends: continuous remineralization for 48 h. Mineral distribution and content among the experimental groups were examined using transversal microradiography (TMR). The surface morphology of the lesion surfaces and the cross sections (of either pH-cycled or not) were also examined using scanning electron microscopy (SEM). The mineral distribution profiles showed mineral deposition throughout the dentine lesions treated with SDF or KF. The amount of the deposition increased with the increase in fluoride concentration, showing a high mineral deposition in the 4.1%F⁻ groups, with a gradual reduction in this deposition at the lower fluoride concentrations. The quantitative measurements of the changes in the mineral content (ΔIML) showed demineralization in the 0.26%F⁻ groups with no significant difference from the control group, while significant remineralization was observed in the dentine lesions treated with 4.1%F⁻ of either SDF or KF followed by 1.025%F⁻ groups. SEM images observed distinct deposits of silver on the surface of dentine lesions treated with SDF and not pH-cycled. These deposits of silver were also observed in the cross sections of the dentine lesions treated with SDF at a depth of 150 μm and 300 μm. In conclusion, the present study revealed no differences in the amount of mineral deposition and in inhibiting demineralization or enhancing remineralization between the SDF and KF. The presence of silver on the surfaces and the cross sections of the dentin lesions treated with SDF had not enhanced the remineralization compared to the KF treated specimens. Under the chosen conditions, the current experiment revealed that the efficacy of SDF is mainly related to its high fluoride concentration and not to the presence of silver.
Introduction

The traditional restorative dental treatment comprised of complete caries removal, where all demineralized dentine was removed, leaving only healthy dental tissues [Innes et al., 2016]. This approach is often considered to be an overtreatment that might harm the pulp and cause unnecessary pulpal exposure [Kerkhove et al., 1967; Bjørndal et al., 2019]. For that reason, new approaches with non- to minimally-invasive treatments were applied to treat the carious lesions. Among these new approaches, non-restorative cavity control (NRCC) is an approach that may be applied to cavitated dentine lesions in the primary dentition and dentine lesions in smooth or root surfaces in the permanent dentition [Innes et al., 2016]. The NRCC approach aims to improve the oral hygiene for the entire dentition and maintain cavitated teeth functional, by daily brushing of specific cavities either by the patients or their parents/caregivers using fluoridated toothpaste. For this, it might be necessary to expose the cavitated dentine lesion to make it cleansable. This strategy may also be supported by the application of topical fluoride treatments to inhibit the caries progression by enhancing the remineralization of the carious lesions [Innes et al., 2016; van Strijp and van Loveren, 2018].

Recently, silver diamine fluoride solution (SDF) is advised as a supportive fluoride treatment in the NRCC approach [Slayton et al., 2018]. Clinical studies showed that SDF is more effective in dentine caries management than other fluoride treatments such as Duraphat varnish [Chu et al., 2002; Duangthip et al., 2015]. This is perhaps attributed to several qualities that explain the superiority of the SDF treatment. First, SDF contains the highest fluoride concentration (38% SDF contains 44,800 ppm F) among the fluorides for professional application, which is almost twice as high as the commonly used NaF varnish (5% NaF contains 22,800 ppm F) [Crystal and Niederman, 2016]. Secondly, the application of SDF to the tooth surface enhances the interaction between fluoride and silver ions with calcium and phosphate ions in the demineralized dentine to form a layer of calcium fluoride (CaF₂) and an impermeable layer of silver phosphate (Ag₃PO₄) on the treated surfaces [Yamaga et al., 1972]. The insolubility of CaF₂ deposits in neutral pH refers to the surface covering by phosphate and/or protein. However, the solubility of these deposits increases at low pH as a result of the protonation of the covering phosphate, causing calcium and fluoride release into the oral environment [ten Cate, 1997; Rošin-Grget, 2013]. Subsequently, the released fluoride is integrated into the hydroxyapatite by the alternating reactions of the dissolution and reprecipitation and thus forming the less soluble hydroxyapatite crystals (fluoridated hydroxyapatite). As a result of the high stability of these crystals, fluoridated hydroxyapatite can resist the demineralization better than hydroxyapatite of the tooth mineral and consequently protect against lesion progression [Yamaga et al., 1972]. As well
as the deposits of Ag$_3$PO$_4$ that form on the surface and also in deeper layers of the dentinal tubules are thought to inhibit diffusion and to provide phosphate to favoring the de- and remineralization balance [Willershausen et al., 2015; Li et al., 2019]. In addition to these qualities, SDF is also reported to have an antimicrobial effect. Recent studies, however, using sequencing techniques did not confirm changes in plaque composition overlying dentine surfaces treated with SDF [Milgrom et al., 2018; Mitwalli et al., 2019]. There are other alleged qualities of this product that could contribute to its superior effect as well. In vitro studies have shown that SDF penetrates the enamel to a depth of 25 μm and maintained around 2-3 times as much fluoride as NaF-PO$_4$, NaF, or SnF$_2$ [Suzuki et al., 1974]. This indicates that the SDF effect is higher than that of NaF or SnF$_2$ [Rosenblatt et al., 2009]. The penetration depth of fluoride into dentine can reach 50-100 μm, while silver can penetrate the dentine tissues deep, up to the pulp chamber (100-744 μm) [Shimooka, 1972; Yamaga et al., 1972; Willershausen et al., 2015]. These findings indicated that the fluoride and silver ions in SDF could penetrate the dentine tissue and perhaps aid in the process of mineral deposition in the deeper layers of the dentine lesions. However, the mechanism of penetration and how does it influence the mineral deposition within the lesion is still not clear.

Previous research conducted by the current authors compared equal fluoride concentrations of SDF and KF (0.26%, 1.025%, 4.1% F$^-$) in a non-microbial pH-cycling model [Alhothali et al., submitted]. The chemical data of that experiment showed no difference in inhibiting dentine demineralization or enhancing dentine remineralization between SDF and KF, with a net dose-response relationship between the fluoride concentrations and the amount of de- and remineralization of the dentine lesions. This conclusion disclosed that the SDF treatment has no extra protective effect over KF. However, the penetration of SDF and KF and their interaction with the mineral in the different layers of the dentine lesions are still unclear. Also, the role of silver ions in the different layers of dentine lesions after the SDF application is still not fully understood and whether it plays a role in the mineral deposition compared to KF.

Therefore, it is of interest to investigate the amount and the pattern of mineral deposition throughout the depth of the dentine lesions after treatment with SDF or another fluoride compound with the same fluoride concentration. In the current study, we compare the effects of 0.26%, 1.025%, 4.1% F$^-$ either as SDF or KF. As an evaluation method, we choose transversal microradiography (TMR) to evaluate the mineral content and distribution, besides scanning electron microscopy (SEM) to examine the surface morphology and the cross sections of the dentine lesions.
Materials and methods

Specimens preparation

Eighty bovine incisors were obtained from the slaughterhouse and prepared immediately. The crowns and roots were separated by a water-cooled diamond blade (Buehler Isomet, IL, USA). Standardized cylindrical coronal enamel-dentine specimens were prepared using a water-cooled hollow diamond burr (Diamant Boart, Vianen, The Netherlands; 6 mm diameter). Subsequently, specimens of 3 mm thickness were cut just below the dentine-enamel junction and above the pulp chamber using a diamond-coated wire saw of 220 μm thick (Well type 3242; Ebner-Mannheim, Germany). Next, the specimens were embedded in methylmethacrylate (Vertex, Dentimex, Zeist, The Netherlands) and ground flat by a silicon carbide abrasive grit 240 to 600 and stored in 4°C distilled water.

Lesion Formation

Specimens were attached to the bottom of a glass tray by nail varnish (8-10 specimens/tray size; 9.5x 7.5x 6 cm). Two-layer system of 8% methylcellulose gel and 150 ml of 0.1 M lactic acid (pH 5.0) was used for lesion formation at 37°C for 17 days [ten Cate et al., 2006].

Fluoride treatments preparation

A commercially available solution of silver diamine fluoride (38% SDF, ARGENATE, VladMiVa, Russia) was diluted (1:100 dilution factor in Milli-Q water) and analyzed with gas chromatography (GC-2010, Shimadzu, Japan) for fluoride concentration [van Loveren et al., 2005; Alhothali et al, submitted]. The concentration of fluoride in SDF was 4.1%. A 4.1% fluoride solution of potassium fluoride (MERCK AG, Darmstadt, Germany) was subsequently prepared. Both SDF and KF solutions have been diluted into three different concentrations of fluoride; 4.1%, 1.025%, 0.26%.

pH-cycling conditions

Specimens were pre-cycled for three days to determine the baseline values of calcium loss and uptake. After the pre-cycling period, the specimens were matched based on the net calcium loss values (0.99 ± 0.10 μmoles/cm²), while the remaining specimens were outliers and thus discarded. The matched forty-eight specimens were assigned to eight experimental groups (n=6) and treated once with SDF or KF (0.26%, 1.025%, 4.1% F); no treatment (control) and baseline group (no pH-cycling). Thirty microliters of either SDF or KF were pipetted to the surfaces of the dentine specimens and left for 2 min, then the specimens were rinsed with distilled water for 15 s. Subsequently, the specimens were placed in a 15-day pH-cycling robot [ten Cate et al., 1995]. The baseline group was kept in
a moist environment at 4°C. The conditions for pH-cycling were standardized on a six-day cycling schedule; each cycle included 0.5 h demineralization and 2.5 h remineralization. In between the de- and remineralization cycles, specimens were placed for 10 s in a 3 ml rinsing solution of 1.5 mmol / L CaCl₂, 0.9 mmol / L KH₂PO₄. After completion of the 6 cycles, specimens were placed in remineralization buffer at night for 6 h and during the weekends for 48 h. Remineralization buffer of 1.5 mmol / L CaCl₂, 0.9 mmol / L KH₂PO₄, 130 mmol / L KCl and 20 mmol / L Hepes, adjusted to pH 7.2 was used. Demineralization buffer of 1.5 mmol / L CaCl₂, 0.9 mmol / L KH₂PO₄ and 50 mmol / L acetic acid adjusted to pH 5.0 was used. Each specimen was cycled in 3 ml aliquots in these buffers. Buffers were refreshed daily.

Transversal microradiography (TMR)
At the end of the pH-cycling period, sections were cut in the middle of each specimen with the diamond-coated wire saw (Ebner-Mannheim, Germany). Two sections were obtained from each specimen and subsequently subjected to TMR analysis. The sections were placed on a Perspex holder and a droplet of water was placed on the surface to avoid dentine shrinkage [van Strijp et al., 1995]. The holder was then covered with a thin sheet of polyester. The sections were radiographed together with a 12-step aluminum stepwedge for 8 min using an x-ray generator (Philips, Eindhoven, The Netherlands). A microscope video camera micro-computer set-up [Lagerweij et al., 1994] and dedicated software (TMR 1.25e; Inspektor Research Systems, Amsterdam, The Netherlands) were used to analyze the radiographic images of the sections and the aluminum stepwedge. Data were expressed as mineral content profiles and integrated mineral loss (IML). Four scans were made per section and then averaged to represent the IML of that section. The average of two sections represented the IML of one specimen. Next, the average of six specimens represented the IML of one group.

Scanning electron microscopy (SEM)
Three additional specimens of six groups; no treatment control group with and without pH-cycling, 4.1%F KF group with and without pH-cycling, 4.1%F SDF group with and without pH-cycling, were fractured longitudinally using a sharp object and hammer. The outer surface and the cross section surface were examined using Scanning Electron Microscopy (SEM; Zeiss EVO LS15, Cambridge, UK). First, the specimens were gold sputtered and then examined at 10,000 magnification. The cross sections were examined at two different depths, starting from the surface of the caries lesion up to 150 μm and 300 μm.
Energy dispersive X-ray spectroscopy (EDS)

In addition to the SEM images, dentine specimens treated with SDF were subjected to additional examination using energy dispersive X-ray spectroscopy (EDS; Bruker XFlash*6|30, Karlsruhe, Germany) to analyze the chemical composition of the deposits on the outer surfaces and cross sections of the dentine lesions treated with SDF.

Statistical analysis

One-way ANOVA with Tukey’s HSD post hoc test (IBM SPSS software; version 25.0) was conducted at a significance level of $\alpha=0.05$ to compare the changes in mineral loss between the fluoride treatments and the control group.

Results

The average mineral content profiles of the baseline group, control group, and dentine lesions treated with 0.26%, 1.025% or 4.1%F$^-$ of either SDF or KF are presented in Fig. 1. The same content and pattern of mineral deposition were found throughout the depth of dentine lesions treated with 0.26%F$^-$ of either SDF or KF. Remarkably, the mineral content of the 0.26%F$^-$ treatments was equal to the baseline and control groups (Fig. 1A). A higher amount of mineral content was found throughout the depth of dentine lesions treated with 1.025%F$^-$ of either SDF or KF compared to baseline and control groups, while both SDF and KF showed the same content and pattern of mineral deposition throughout the lesion depth (Fig. 1B). Again, the mineral content profiles of the dentine lesions treated with 4.1%F$^-$ of either SDF or KF were significantly higher than the baseline and control groups, while both SDF and KF showed the same content and pattern of mineral deposition throughout the lesion depth (Fig. 1C). A dose response relationship was founded between the experimental groups (Fig. 1), the higher the fluoride concentration, the higher the mineral content throughout the lesion depth. As well as the differences between the fluoride groups and the baseline and the control groups increased with the increase in fluoride concentrations. Baseline and control groups showed approximately the same pattern of mineral deposition.

The quantitative measurements of the changes in mineral content of the dentine lesions are expressed as $\Delta$IML. Fig. 2 presents the $\Delta$IML of the experimental groups after the pH-cycling period. Significant differences were found in the amount of mineral content between the experimental groups (one-way ANOVA, $p<0.0001$). Although 4.1%F$^-$ as SDF disclosed the highest efficacy among the experimental groups, it showed no significant difference compared to 4.1%F$^-$ as KF, followed by 1.025%F$: KF = SDF$ and then 0.26%F$: KF
Figure 1. The average mineral distribution profiles of baseline lesion, control group and SDF or KF (A) 0.26%F; (B) 1.025%F; (C) 4.1%F treated dentine lesions. Y-axis represents the mineral content (vol%), and X-axis represents the depth throughout the dentine lesion (μm).
The effect of equal fluoride concentrations as SDF and KF on demineralized dentine during pH-cycling: Microradiography

SDF. SDF and KF (0.26%F⁻) revealed an equal amount of demineralization as the control group. These findings also reported the dose response relationship between the fluoride concentrations and the amount of de- and remineralization.

**Figure 2.** Changes in IML (ΔIML) of the control group and the dentine lesions treated with SDF or KF; 0.26%F⁻; 1.025%F⁻; 4.1%F⁻. Remineralization of the dentine lesions expressed as positive (+), and demineralization of the dentine lesions expressed as negative (-). The different characters indicated statistically significant differences between the experimental groups (ANOVA; p<0.05).

Fig. 3 shows the SEM observation of the surfaces of dentine lesions. Small distinct deposits were observed on the entire surface of the dentine lesions treated only with SDF (no pH-cycling). These deposits were located in the inter-tubular area and almost covered the dentinal tubules (Fig. 3E). These deposits were not observed on the surfaces of the other experimental groups. Fig. 4 presents the cross sections of the dentine lesions at 150 μm depth. Crystal-like deposits were found in the dentine lesions treated with SDF, but not in the other experimental groups. The amount of these crystals was higher after the pH-cycling period than the non pH-cycled SDF treated dentine lesions. Fig. 5 presents the cross sections of the dentine lesions at 300 μm depth. The same crystal-like deposits were again found only in the dentine lesions treated with SDF. However, the amount of these deposits declined with depth. EDS analysis revealed that these deposits composed of a high atomic ratio of silver.
Figure 3. SEM images of the surface of (A) control group; (B) control group with pH-cycling; (C) dentine lesions treated with KF (4.1%F); (D) dentine lesions treated with KF (4.1%F) and pH-cycled; (E) dentine lesions treated with SDF (4.1%F); (F) dentine lesions treated with SDF (4.1%F) and pH-cycled. Images were taken at 10,000 magnification. Arrow indicates small distinct deposits on the surface of the dentine lesion treated only with SDF (4.1%F).
Figure 4. SEM images of the cross sections at 150 μm depth of (A) control group; (B) control group with pH-cycling; (C) dentine lesions treated with KF (4.1%F-); (D) dentine lesions treated with KF (4.1%F-) and pH-cycled; (E) dentine lesions treated with SDF (4.1%F-); (F) dentine lesions treated with SDF (4.1%F-) and pH-cycled. Images were taken at 10,000 magnification. Arrows indicate deposits in the dentinal tubes of the dentine lesions treated with SDF (4.1%F-) with or without pH-cycling.
Figure 5. SEM images of the cross sections at 300 μm depth of (A) control group; (B) control group with pH-cycling; (C) dentine lesions treated with KF (4.1%F-); (D) dentine lesions treated with KF (4.1%F-) and pH-cycled; (E) dentine lesions treated with SDF (4.1%F-); (F) dentine lesions treated with SDF (4.1%F-) and pH-cycled. Images were taken at 10,000 magnification. Arrows indicate deposits in the dentinal tubes of the dentine lesions treated with SDF (4.1%F) with or without pH-cycling.
Discussion

Despite the numerous advances in dental care, dental caries remains a global health problem. As the philosophy of caries management has shifted from a surgical to a medical approach, dental caries is considered as a disease rather than a tooth cavity [Gao et al., 2016]. Thus, a range of fluoride agents has been proposed to arrest dentine caries and add extra benefits to the non-invasive treatment and so to the non-restorative cavity control (NRCC) [Innes et al., 2016; Slayton et al., 2018; van Strijp and van Loveren, 2018]. SDF is a treatment of choice used in treating dentine caries in the NRCC approach, which has a higher fluoride concentration than the most commonly used NaF varnish (5% NaF; 22,600 ppm F) [Crystal and Niederman, 2016]. However, the high fluoride concentration in the SDF product has been previously assessed only to a very limited extent. In vitro studies compared the anti-demineralizing effect of the 3.5%F- SDF solution and 2.2%F- NaF varnish when each of these products was applied to artificially created dentine lesions and then pH-cycled [Wierichs et al., 2018; Alhothali et al., submitted]. These studies disclosed a higher anti-demineralizing effect in the SDF group compared to NaF, indicating that the higher the fluoride concentration, the higher the anti-demineralizing effect. Another in vitro study compared the equal fluoride concentration of 4.5%F- of either SDF or KF on sound dentine that was subjected subsequently to 5 days of demineralization [Thanatvarakorn et al., 2016]. In that study, no significant difference in the anti-demineralizing effect between SDF and KF was revealed. These findings indicated that the superior effect of SDF is probably due to its high fluoride concentration. However, the anti-demineralizing effect of SDF and another fluoride product with the same fluoride concentration on artificially demineralized dentine has yet to be investigated. Therefore, this study aimed to compare the equal fluoride concentrations of SDF and KF (0.26%, 1.025%, 4.1% F-) on demineralized dentine in a non-microbial pH-cycling model.

It was of interest to investigate the pattern of mineral deposition and the amount of mineral content in the deeper layers of the dentine lesions after application of 0.26%, 1.025%, or 4.1% F of either SDF or KF. In addition, the penetration and the deposition of silver ions in the deeper layers of the dentine lesions after the application of SDF were also examined. This will help in determining the role of silver ions inside the dentine lesions and whether these ions could play a role in the mineral deposition compared to KF.

Regardless of the type of either fluoride treatment, the low fluoride concentrations (0.26%F-) were not sufficient to enhance more mineral deposition compared to baseline lesion and control group. Differently, the high fluoride concentrations of 1.025% and 4.1% of either SDF and KF showed more mineral deposition throughout the lesion depth compared to the baseline lesion and control groups. Despite the type of fluoride treatment, the current
findings indicated a dose response relationship between the fluoride concentration and the mineral deposition throughout the lesion depth. The mineral profiles of the baseline lesions and the control groups were similar. This observation differed from a previous experiment that showed different mineral content profiles between the baseline and control groups [Alhothali et al., submitted], which is possibly attributed to the changes in pH of de- and remineralization buffers from 4.8 and 7.1 to 5.0 and 7.2, respectively. As a result of the changes in pH, we assumed that the alternating process between de- and remineralization in the control group resulted in no mineral gain or loss throughout the lesion depth. The ΔIML revealed that the low fluoride concentrations of both treatments (0.26%F-) were not sufficient to influence the process of remineralization or even protect against demineralization. In contrast, the higher fluoride concentrations of 1.025% and 4.1% enhanced the remineralization of dentine lesions, irrespective of the type of treatment. These findings indicated that the superior efficacy of SDF is probably due to its high fluoride concentration, which is the highest among all topically applied fluoride in the dental office [Crystal and Niederman, 2016].

The current findings also disclosed that the non-fluoride component of SDF (silver ions) did not change the pattern of mineral profiles or the content of the mineral deposition in the SDF groups compared to KF groups. Since the TMR data showed no additional role of the silver ions in the mineral content of the SDF groups, SEM images were performed to observe the silver in the SDF groups. An enormous amount of small deposits were detected on the surfaces of the dentine lesions treated only with SDF, but not on the surfaces of the pH-cycled SDF treated dentine lesions. This seems to depend on the washing process during the pH-cycling that might either rinse off the deposits from the surface or encourage their penetration and the deposition in the dentinal tubules throughout the lesion depth. The SEM observations of the cross sections of the SDF groups confirmed the above mentioned allegation about the ability of the pH-cycling to encourage the penetration and deposition of the deposits in the dentinal tubules. However, the amount of these deposits declined with depth. Taking into account that the SEM images observed the deposits at two different depths of 150 and 300 μm. The 300 μm depth was selected to represent the sound dentine level since the average depth of the baseline lesion at the start point was 237 ± 51 μm. The depth of 150 μm was chosen to show the deposition at half distance of the 300 μm. It was clear that most of the deposits were accumulated at a depth of 150 μm in the pH-cycled dentine lesions. In contrast, few deposits were detected at the sound dentine level of the SDF treated groups. These findings were consistent with research showing that the penetration of the deposits is correlated to the extent of the lesions in the dental hard tissues [Li et al., 2019]. The EDS analysis revealed that the detected deposits on the surface and in the dentinal tubules of the dentine lesions treated with SDF contained a high atomic ratio of silver. This finding was also reported in a previous study that found that calcium,
phosphate, and silver were the significant components that were continuously present in the carious lesions treated with SDF [Li et al., 2019]. Despite the enormous amount of silver deposits on the surface or in the dentinal tubules of the dentine lesions treated with SDF, we observed no difference in the pattern of mineral profiles and the content of mineral deposition between SDF and KF treated samples. This indicates that the silver did not contribute to enhancing the remineralization of the dentine lesions throughout the depth. Further studies are needed to clinically examine the role of silver in the anti-demineralizing and anti-microbial activities of SDF.

Conclusion

The equal fluoride concentrations of either SDF or KF revealed an equal amount of mineral deposition and content throughout the depth of dentine lesions. Also, equal efficacy in inhibiting demineralization or enhancing remineralization was found in the dentine lesions treated with SDF or KF. Under the chosen conditions, the presence of silver in the SDF did not contribute to the process of de- and remineralization of the dentine lesions, showing no extra effect in the SDF group compared to KF. These findings indicated that the beneficial effect of SDF is perhaps attributed mainly to its high fluoride concentration and not to the presence of silver in the product.
References


