ORIGINAL ARTICLE

The effect of equal fluoride concentrations in silver diamine fluoride and potassium fluoride on demineralized dentin during pH-cycling: chemical data

Marwa Alhothali[1,2] | **Rob Exterkate**[3] | **Maxim Lagerweij**[1] | **Mark Buijs**[3] | **Cor van Loveren**[1,3] | **Guus van Strijp**[1]

[1]Department of Cariology, The Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

[2]Department of Dentistry, Comprehensive Specialized Clinics and Hospital of Security Forces, Mecca City, Saudi Arabia

[3]Department of Preventive Dentistry, The Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Correspondence
Marwa Alhothali, Department of Dentistry, Comprehensive Specialized Clinics and Hospital of Security Forces, 6818, Mecca City 24247, Saudi Arabia.
Email: m.alhothali@moimsd.gov.sa

Funding information
This study was funded by the Comprehensive Specialized Clinics and Hospital of Security Forces, Mecca City, Saudi Arabia.

Abstract

The actual contribution of silver in silver diamine fluoride (SDF) towards the anti-demineralizing effect is unclear. This study compared the effects of single applications of three concentrations of fluoride (4.1%, 1.025%, 0.26% F^-) in the form of SDF and potassium fluoride (KF) on demineralized dentin in a 15-day non-microbial pH-cycling model. Calcium loss and uptake in de- and remineralization buffers were analyzed daily. Fluoride release in both buffers was analyzed on days 1, 2, 3, and 8. The net calcium results of de- and remineralization cycles revealed dose-response protection without significant differences between equal fluoride concentrations of SDF and KF. In the demineralization cycles, all fluoride treatments, except KF 0.26% F^-, significantly inhibited demineralization, with KF 4.1% F^- being the most effective. In the remineralization cycles, remineralization was enhanced in all fluoride concentration groups in a dose-response manner with no difference between similar fluoride concentrations of SDF and KF. Daily fluoride effects were constant throughout the experiment. Fluoride analysis revealed statistically significant differences in fluoride release between the treatments on day 1 that diminished on days 2 and 3. The non-microbial model showed no differences between SDF and KF in inhibiting demineralization and enhancing remineralization of dentin lesions.

KEYWORDS
demineralization, fluorides, in vitro, remineralization

INTRODUCTION

Non-restorative cavity control has recently gained interest as a treatment for cavitated dentin lesions in both the pediatric and geriatric populations [1,2]. Non-restorative cavity control is a 3-stage approach that aims to involve the patient in the treatment by improving their oral hygiene and removing dental plaque from the cavitated lesions (stage 1). For this, the removal of the overhanging enamel may be necessary to make the dentin cavities accessible for brushing (stage 2). Further, applying topical fluoride products to the dentin cavities helps arrest the progression of the lesion (stage 3) [2,3]. Among the fluoride products,

silver diamine fluoride (SDF) has been used to treat dentin caries and can arrest approximately 68% of caries [4]. Therefore, the use of non-restorative cavity control with SDF application is considered a promising alternative to traditional therapy in treating cavitated dentin lesions.

The idea of SDF originated from the traditional use of silver nitrate ($AgNO_3$) and sodium fluoride (NaF) in preventing dental caries. When SDF is applied to a dentin lesion, the fluoride ions interact with the free calcium ions in the hydroxyapatite to form calcium fluoride (CaF_2) and fluorapatite ($Ca_{10}(PO_4)_6OH_{2-x}F_x$); simultaneously, silver ions interact with the free phosphate to form a layer of silver phosphate (Ag_3PO_4) deposits on the treated surface [5]. These deposits have also been observed in deeper layers of the dentinal tubules [4]. Both surface and dentinal tubule deposits are suggested to limit acid diffusion and to release phosphate to influence the de- and remineralization processes [6]. In addition, it is thought that silver contributes significantly to anti-microbial activity, thus reducing the demineralization activity of the dental plaque [7,8]. However, the actual contribution of silver in SDF towards the anti-demineralizing effect is still unclear and questionable [9,10].

Three different concentrations of SDF are available in the market (12%, 30%, and 38%) [11]. The 38% SDF contains the highest fluoride concentration among all topically applied fluoride products (equivalent to 4.5% F^-). This concentration is approximately twice as high as that in the commonly used NaF varnish (5% NaF; equivalent to 2.2% F^-) [12]. Wierichs et al. [13] suggested that the high fluoride concentration in SDF is the reason for its higher effect in re-mineralizing dentin lesions compared to NaF. They compared the SDF solution and NaF varnish with fluoride concentrations of 3.5% and 2.2%, respectively.[13] Another in vitro study applied an equal fluoride concentration (4.5% F^-) of either SDF or potassium fluoride (KF) once on sound dentin and then demineralized the specimens for 5 days; no significant difference was observed in the anti-demineralizing effect of SDF and KF [14]. These findings indicate that the effect of the SDF treatments in the process of de- and remineralization is associated with its high fluoride concentration and not the additional silver. However, the above studies were limited either due to their comparison of SDF to a product with a deviant fluoride concentration in a pH-cycling model [13] or to a product with the same fluoride concentration in a demineralization model only [14]. There is a lack of studies that examine in detail the effect of SDF when it is compared to another fluoride product with the same fluoride concentration in a pH-cycling model. Additionally, there is a need to understand if the alleged superiority of SDF is related to its high fluoride concentration or is brought about by the presence of silver. Therefore, the current study aimed to examine the effect of equal concentrations of fluoride (4.1%, 1.025%, and 0.26% F^-) in the form of either SDF or KF on demineralized dentin in a non-microbial pH-cycling model.

MATERIAL AND METHODS

Specimen preparation

Eighty bovine incisors were obtained from a local slaughterhouse and prepared immediately. A water-cooled diamond blade (Buehler Isomet) was used to separate the crowns and the roots. A water-cooled hollow diamond burr (6 mm diameter; Diamant Boart) was used to obtain standardized cylindrical coronal buccal specimens. A 220 µm diamond-coated wire saw (Well type 3242; Ebner-Mannheim) was used to cut just below the dentin-enamel junction and above the pulp chamber to form a 3 mm thick dentin specimen. Specimens were embedded in methyl methacrylate (Vertex; Dentimex) and subsequently ground flat using 240- to 600-grit water-cooled silicon carbide abrasive paper (Buehler CarbiMet PSA) and then stored at 4°C.

Dentin lesion formation

Nail varnish was used to attach the dentin specimens to the bottom of a glass tray (size 9.5 × 7.5 × 6 cm; 8–10 specimens/tray). The specimens were then covered with 150 ml of 8% methylcellulose gel. After one day, 150 ml of 0.1 M lactic acid (Sigma-Aldrich) at pH 5.0 was poured over a filter paper and the gel [15]. The specimens were demineralized for 17 days at 37°C.

Fluoride solution preparations

A commercially available silver diamine fluoride solution (38% SDF; pH 10.0; ARGENATE, VladMiVa) containing a complex salt fluoride of diamine silver was used in this experiment. This solution was diluted (1:100 dilution factor in Milli-Q water) and analyzed for its fluoride concentration using gas chromatography (GC-2010; Shimadzu). The fluoride concentration in the SDF was found to be 4.1%. Subsequently, a 4.1% fluoride solution of potassium fluoride (KF; Merck) was prepared by dissolving potassium fluoride powder in Milli-Q water. The pH of the final solution was 7.0. The SDF and KF solutions were subsequently diluted to obtain three different fluoride concentrations: 4.1%, 1.025%, and 0.26%.

pH-cycling conditions

A pH-cycling robot was used to automatically transfer samples between the demineralization buffer, a rinse, and the remineralization buffer [16]. Specimens were subjected to a 3-day cycling regime to determine the baseline values of

calcium loss and uptake. Following this, 42 specimens with similar values of net calcium loss (mean 0.99, SD 0.10 μmol/cm^2) were selected to form groups. The 42 specimens were then randomly assigned to one of six experimental groups (n = 6 in each group): 4.1% F$^-$, 1.025% F$^-$, or 0.26% F$^-$ of SDF and KF solution each and one control (no treatment) group. Thirty milliliters of each fluoride solution was applied once to the surface of the dentin specimens and left undisturbed for 2 min and then rinsed off with an excess of demineralized water for 15 s. The specimens were subsequently placed in the pH-cycling robot for 15 days [15]. During the intervening weekends of the experiment, the specimens remained in the remineralization buffer, from Friday (days 5, 10, and 15) until Monday morning. The pH-cycling conditions were standardized in six daily cycles; each cycle included demineralization for 0.5 h and remineralization for 2.5 h. In between, the specimens were placed in 3 ml of rinsing solution for 10 s. After completion of six cycles, the specimens were placed in the remineralization buffer for 6 h during the night and for 48 h during weekends. The remineralization buffer consisted of 1.5 mmol/L CaCl$_2$ (Sigma-Aldrich), 0.9 mmol/L KH$_2$PO$_4$ (Sigma-Aldrich), 130 mmol/L KCl (Merck), and 20 mmol/L Hepes (Sigma-Aldrich) adjusted to a pH of 7.2. The demineralization buffer consisted of 1.5 mmol/L CaCl$_2$, 0.9 mmol/L KH$_2$PO$_4$, and 50 mmol/L acetic acid (Sigma-Aldrich) adjusted to a pH of 5.0. The rinsing buffer consisted of 1.5 mmol/L CaCl$_2$ and 0.9 mmol/L KH$_2$PO$_4$ [16]. Each specimen was cycled in 3 ml aliquots of each buffer. The buffers were changed daily, except on weekends. Three positions in the pH-cycling robot were left without specimens to determine the baseline calcium content.

Calcium analysis

Samples of 200 μl were collected from the de- and remineralization buffers and diluted to 3 ml using 36 mmol/L La(NO$_3$)$_3$ to analyze the calcium content using atomic absorption spectroscopy (PerkinElmer Analyst 100; PerkinElmer).[16] Daily calcium loss and calcium uptake (caveat: data from days 5, 10, and 15 included the weekend in the remineralization cycle) were determined by calculating the difference from the baseline calcium value. The daily calcium loss and calcium uptake were cumulated over the experimental period to obtain cumulative calcium loss, cumulative calcium uptake, and cumulative net calcium change (the difference between calcium loss and calcium uptake).

Fluoride analysis

The collected samples of de- and remineralization buffers from days 1, 2, 3, and 8 were also analyzed for fluoride

content. On each day (1, 2, 3, and 8), duplicate samples were analyzed in batches. One milliliter of the samples and standard fluoride solutions were vigorously mixed with 200 μl of 1 M nitric acid (HNO$_3$) and 400 μl toluene reagent [17] in 1.8 ml autosampler vials (Brown Chromatography).

Gas chromatography was operated in split mode and was equipped with a cooled auto-injector (AOC-20i; Shimadzu), a backflow system, and a flame ionization detector. The separation took place in two RTX-1 columns (15 and 30 m, 0.32 mm internal diameter, 0.5 μm film thickness) at a gradient temperature of 35 to 50°C (with an increase of 2°C/min). Calibration curves were used to determine the fluoride concentration in the de- and remineralization buffers based on the internal standard method.

Statistical analyses

Data were analyzed using spss version 25.0 (IBM). Data are presented as means and standard deviations. One-way analysis of variance (ANOVA) was used to analyze the cumulative calcium data in the de- and remineralization buffers and the net calcium change in the experimental groups at the end of the experiment. The post-hoc Tukey's (HSD) test was used to examine the significance of the difference among all groups. Differences in the daily outcomes for calcium loss, calcium uptake, and net calcium change, as well as the differences in the fluoride released into the de- and remineralization buffers on days 1, 2, 3, and 8, were tested using multivariate analysis of variance (MANOVA) followed by a post-hoc Tukey's HSD test. The daily calcium data, in addition to the data of fluoride released on days 1, 2, 3, and 8, were used as the dependent variables and the seven experimental groups as the fixed factor. The level of significance was set at α = 0.05.

RESULTS

Statistically significant differences were found between the treatment groups in the net cumulative calcium change as well as the cumulative calcium loss and uptake after the 15-day pH-cycling (ANOVA, $p < 0.0001$; Figure 1, Table 1). The net cumulative calcium change revealed a protective effect that showed a dose-response relationship with the fluoride concentrations, irrespective of the type of fluoride. SDF 4.1% F$^-$ was as effective as KF 4.1% F$^-$, SDF 1.025% F$^-$ was as effective as KF 1.025% F$^-$, and SDF 0.26% F$^-$ was as effective as KF 0.26% F$^-$; all were significantly more effective than no treatment (control) (Table 1). Significant net remineralization was observed in the dentin lesions treated with 4.1% F$^-$ (SDF and KF), followed by 1.025% F$^-$ (SDF and KF). Although demineralization was found in the dentin lesions treated with 0.26% F$^-$ (SDF and KF), both treatments
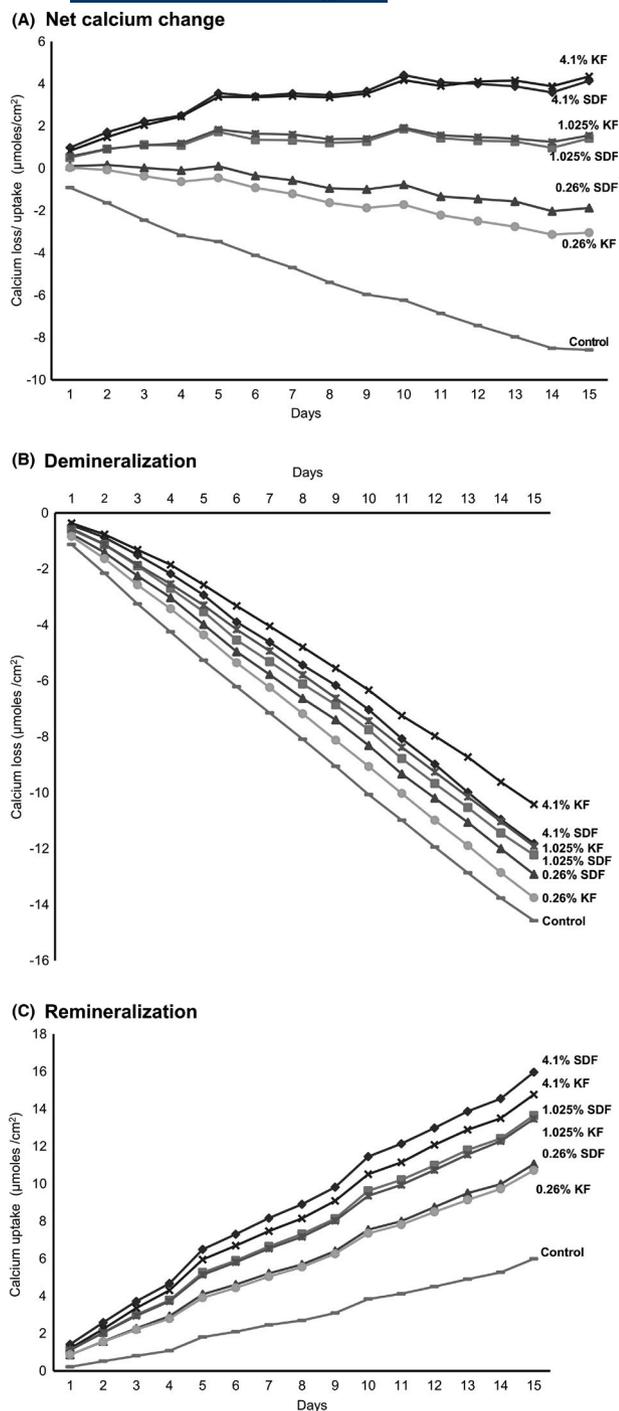
FIGURE 1 Cumulative net calcium changes (combined data), calcium loss, and calcium uptake during the 15 days of pH-cycling. Before pH-cycling, each group received a single fluoride treatment or no treatment (control). The figures show data from (A) net calcium change, (B) demineralization cycles, and (C) remineralization cycles

TABLE 1 Mean cumulative calcium data (μmoles/cm2) of (A) net calcium change, (B) demineralization cycles, and (C) remineralization cycles during the 15-day pH-cycling

Experimental group	Mean[*] ± SD	Significance of the difference[**]
(A) Net calcium change		
4.1% KF	+4.3 ± 0.8	a
4.1% SDF	+4.1 ± 0.9	a
1.025% KF	+1.5 ± 0.9	b
1.025% SDF	+1.4 ± 0.5	b
0.26% SDF	−1.9 ± 0.9	c
0.26% KF	−3.1 ± 0.8	c
Control	−8.6 ± 0.7	d
(B) Demineralization cycles		
4.1% KF	−10.4 ± 0.4	a[***]
4.1% SDF	−11.8 ± 0.6	b[***]
1.025% KF	−11.9 ± 1.1	b
1.025% SDF	−12.2 ± 0.8	b
0.26% SDF	−12.9 ± 0.9	b, c
0.26% KF	−13.8 ± 0.3	c, d
Control	−14.6 ± 0.8	d
(C) Remineralization cycles		
4.1% SDF	+15.9 ± 0.5	a
4.1% KF	+14.8 ± 1.1	a, b
1.025% SDF	+13.6 ± 0.8	b
1.025% KF	+13.5 ± 0.9	b
0.26% SDF	+11.1 ± 1.8	c
0.26% KF	+10.7 ± 0.7	c
Control	+5.9 ± 0.7	d

*A positive figure means calcium uptake by the mineral; a negative figure means calcium release by the mineral.

**a,b,c,d represent statistically significant differences at $p < 0.05$ (ANOVA, Tukey post hoc test).

***Demineralization between 4.1% F⁻ as either KF or SDF is statistically different.

significantly inhibited demineralization compared to the control group (Table 1). In the demineralization cycles, KF 4.1% F⁻ inhibited demineralization significantly better than did the other groups, with KF 0.26% F⁻ inhibition not reaching statistical significance (Table 1). At the concentration of 1.025%, SDF and KF were equally effective (Table 1). In the

remineralization cycles, remineralization was significantly enhanced in all fluoride groups compared to the control group in a dose-response relationship, with no differences between the equal fluoride concentrations of SDF and KF (Table 1).

Analysis of the daily net calcium change (Figure 2A) confirmed that there was no statistically significant difference between the same fluoride concentrations of SDF and KF, except for days 3, 9, and 12, where 0.26% F⁻ as SDF performed better than 0.26% F⁻ as KF (data indicated with closed dots in Figure 2A); on day 12, however, 4.1% F⁻ as KF performed better than the same fluoride concentration as SDF (indicated with open dots in Figure 2A). Furthermore, the effectiveness of all treatments, despite declining over time, continued over the full experimental period. The results of the daily demineralization cycles showed that there were no statistically significant differences between the control group
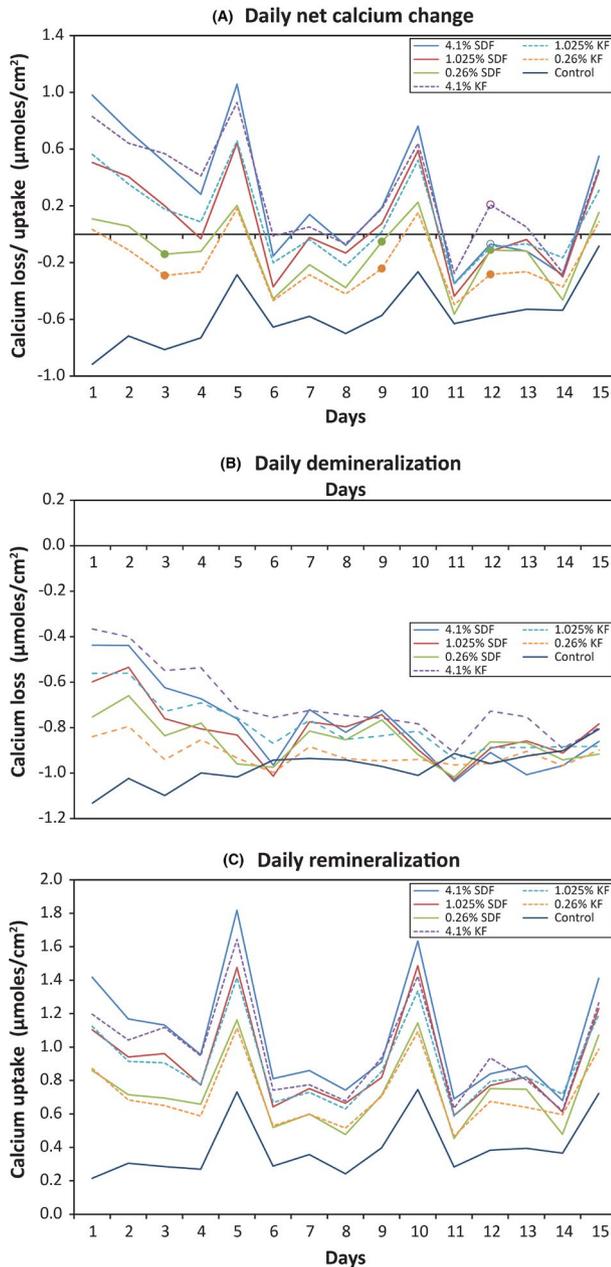
FIGURE 2 Daily net calcium change, calcium loss, and calcium uptake during the experiment. Before pH-cycling, each group received either a single fluoride treatment or no treatment (control). The figures show data regarding (A) daily net calcium change (closed and open dots show the days where there were differences between the fluoride compounds), (B) daily demineralization, and (C) daily remineralization. The high values observed on days 5, 10, and 15 regarding daily net calcium change (panel A) and daily remineralization (panel C) are related to the inclusion of the weekend's remineralization period in these data
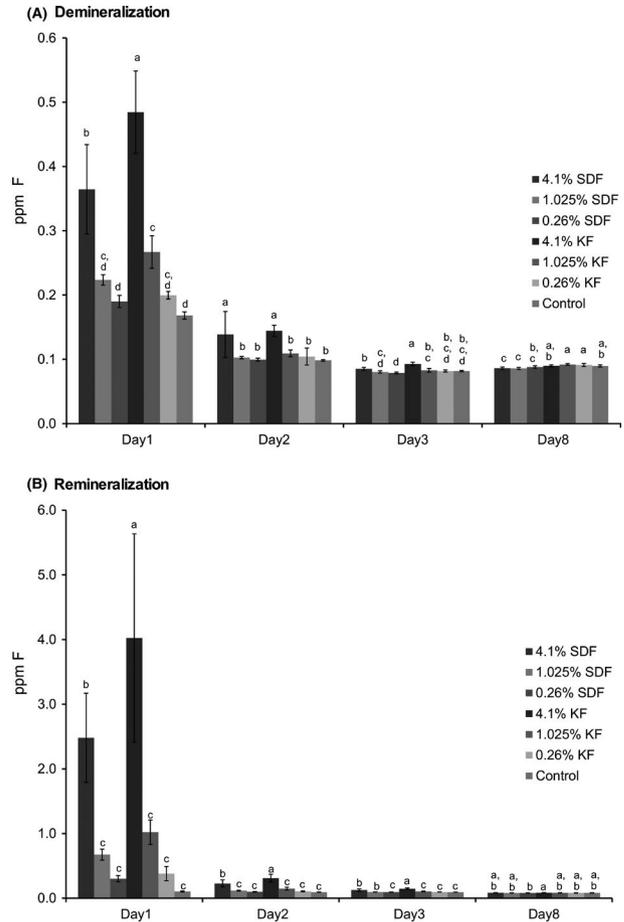

FIGURE 3 Fluoride analysis on days 1, 2, 3, and 8 in the (A) demineralization cycles and (B) remineralization cycles. Y-axis represents the fluoride content in ppm, and X-axis represents cycling days. Different characters represent statistically significant differences at $p < 0.05$ (post hoc Tukey test)

and the 0.26% F⁻ groups from day 5 onward (except for day 9 for SDF 0.26% F⁻) and for the KF 1.025% F⁻ group from day 8 onward (Figure 2B). On day 11, the statistical significance of the differences between the control and all other fluoride groups disappeared as well, except for KF 4.1% F⁻, which

remained significantly effective until day 14. The differences between the fluoride compounds were not statistically significant at the same fluoride concentrations, except for day 6, where SDF performed worse than KF, and day 9, where SDF performed better than KF. The results for the remineralization cycles confirmed that there was no statistically significant difference between treatments with SDF and KF, and the effectiveness of all treatments, although declining, continued over the full experimental period (Figure 2C).

The fluoride analysis showed significant differences between the groups in the amount of fluoride released in the de- and remineralization cycles on days 1, 2, 3, and 8 of pH-cycling (Figure 3). In the demineralization cycles on day 1, statistically significantly more fluoride was released from the dentin lesions treated with 4.1% F⁻ as KF than from the other groups, followed by 4.1% F⁻ as SDF, and 1.026% F⁻ as KF. On day 2, fluoride releases exceeding those of the control group were only observed in the 4.1% F⁻ of SDF and KF groups. On day 3, only a minimally larger fluoride release was observed for the 4.1% F⁻ as KF group than for the control

group. In the remineralization cycles, dentin lesions treated with 4.1% F⁻ as KF showed significant fluoride release on day 1 and 2, followed by SDF 4.1% F⁻, in comparison with the control group. On day 3, only a slightly larger fluoride release was observed for the 4.1% F⁻ as KF group than for the control group. No association was observed between the amount of fluoride released in the buffers and the inhibition of demineralization or enhancement of remineralization.

DISCUSSION

All fluoride treatments enhanced remineralization and inhibited demineralization more effectively than no treatment. However, the degree of enhancement of dentin remineralization or inhibition of dentin demineralization could be attributed to the concentration of the fluoride and not to the type of treatment. This means that the higher the fluoride concentration, the greater the treatment benefit.

The current study compared SDF to KF instead of NaF because of insufficient solubility of the NaF salt in water (at room temperature, 40.4 g/L). It was not possible to reach the same fluoride concentration as that of 38% SDF to make comparisons between SDF and NaF at this high concentration of fluoride. The solubility of KF in water is much higher than that of NaF, which made comparisons between SDF and KF possible. The method of pH-cycling used in the present study has been used for several decades in in-vitro studies to compare anti-caries products applied to enamel and dentin. [16,18] The current pH-cycling model showed that a single application of fluoride treatment was sufficient to protect the dentin lesions against chemical demineralization and to enhance dentin remineralization. By analyzing the daily calcium loss and calcium uptake, it was also possible to measure the duration of the effect of the fluoride treatment.

Irrespective of the type of fluoride treatment, the higher fluoride concentrations of 4.1% F⁻ and 1.025% F⁻ resulted in net remineralization throughout the whole experimental period. In contrast, the lower fluoride concentration of 0.26% F⁻ protected against net demineralization compared to the control group, with no enhancement of net remineralization. These results indicate that the type of fluoride treatment is not as important as the fluoride concentration. The cumulative effects of the demineralization cycles at the end of the experiment showed that 4.1% F⁻ as KF was more effective than 4.1% F⁻ as SDF (Table 1). Analysis of the daily cycles suggested that this was probably related to the fact that the effect of 4.1% KF continued over 13 days of the experiment, while the effect of SDF continued only over 10 days (Figure 2B). In the remineralization cycles, this difference between 4.1% F⁻ either as SDF or KF was not observed. Analysis of the daily cycles revealed that both treatments were effective over the whole period of the experiment and to the same

extent. From the current pH-cycling results, it is clear that SDF had no superior effect over KF when the fluoride concentration of both treatments was the same.

The control group also showed remineralization in the remineralization cycles. This observation is inconsistent with a previous finding by the current authors (unpublished data). In that experiment, with a comparable de- and remineralization protocol for the control group, the control group showed demineralization in the remineralization cycles. For this reason, the current pH of the de- and remineralization buffers was changed from 4.8 and 7.1 to 5.0 and 7.2, respectively.

In the current experiment, six specimens were used. This number was based on our previous pH-cycling experiments mostly with enamel, but also with dentin lesions.[15,16,19] Furthermore, we wished to have all specimens in one pH-cycling experiment to avoid inter-experiment variation. Power calculations (G*power 3.1.9.7; [20]) for a two-sided t-test for independent samples (as a surrogate for the Tukey HSD-test) revealed that assuming a power of 80%, α of 5%, and six samples for each group, the effect size would be 1.79. Effect size is the difference between the two groups divided by the standard deviation. In our experiments, the standard deviations for the calcium data in the fluoride groups were, on average, 5.65% of the means in the demineralization cycles and 7.65% in the remineralization cycles. This implied that the significant differences were 1.79*5.65 = 10.1% in the demineralization cycles and 1.79*7.65 = 13.7% in the remineralization cycles. We thought that smaller differences would not be important for showing that one method is superior to the other. In fact, the differences for which we could not reject the null hypothesis were 4.5% and 4% in the demineralization and remineralization cycles, respectively. Considering these results, we are confident in our outcomes where we did not reject the null hypothesis of no difference.

This experiment showed that, in the demineralization cycles, the effect of the lowest concentration continued over the first 4 days of the experiment and, for the higher concentrations, the effect continued even longer (Figure 2B); meanwhile, in the remineralization cycles, the fluoride effects continued over the whole experiment, even though the treatments were applied only once (Figure 2C). In comparison to the control group, the released fluoride from dentine specimens extended no further than the first two or three days (Figure 3). Therefore, it is speculated that, in the present experiment, the effect of the fluoride treatment beyond the first two or three days was not due to the fluoride released in the buffers but to the presence of fluoride deposits on the dentin surface (CaF₂-like material) and the incorporated fluoride in different layers of the dentin lesions (fluoridated hydroxyapatite crystals) [21].

On day 1, the fluoride concentration in the demineralization solution in the control group was unexpectedly high (Figure 3). We believe, however, that this is mainly due to

a difficulty in measuring the fluoride concentration. Due to a technical problem, we had to repeat the measurements for day 1, but the volume of sample that was left was far less than the required 1 ml. Therefore, we introduced a multiplication factor. The measured values in the control group were comparable to the values recorded during the other days.

Our results suggest that silver ions in SDF had no added benefits under non-microbial de- and remineralization conditions, implying that the high fluoride concentration in SDF is the main reason for its superior clinical effect when compared to another fluoride product that contains a lower fluoride concentration. The current study focused mainly on the anti-demineralizing effects of SDF to completely understand the role of high fluoride concentration and silver ions in a non-microbial model. However, we did not study the alleged anti-microbial effect of the silver ions in SDF. It would be interesting to study both the anti-demineralizing and anti-microbial effects of SDF in a single model. For this reason, further experiments are needed to prove the role of silver ions in SDF as an anti-microbial agent before translating any alleged effects into clinical practice.

The present study only analyzed the chemical data of the dentin lesions treated with either SDF or KF; however, examining the mineral content and silver penetration in the dentin lesions may provide more information on the mineral distribution and deposition. In addition, examination of mineral content and silver penetration may offer an overview of the possible role of silver in influencing the mineral deposition within the lesion. Therefore, further studies using transversal microradiography analysis are warranted to examine the possible differences between SDF and KF in the dentin lesions.

In conclusion, under the chosen conditions, equal fluoride concentrations of SDF and KF continually and equally inhibited demineralization and/or enhanced the remineralization of artificial dentin lesions during the experimental period. These findings indicate that high fluoride concentration in the SDF product is an important factor for its superior effect (compared to products with lower fluoride concentrations) when SDF is applied to dentin lesions clinically. Under the current non-microbial conditions, silver did not add any extra protective properties to the effects of fluoride.

CONFLICT OF INTERESTS
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
Marwa Alhothali: Conceptualization, Methodology, Investigation, Formal analysis, Writing -original draft; **Mark Buijs:** Investigation; **Rob Exterkate:** Conceptualization, Methodology, Investigation, Formal analysis; **Maxim Lagerweij:** Conceptualization, Methodology; **Cor van Loveren:** Conceptualization, Methodology, Formal analysis, Writing - review & editing; **Guus van Strijp:** Conceptualization, Methodology, Formal analysis, Writing - review & editing.

ORCID
Marwa Alhothali https://orcid.org/0000-0001-9265-5474
Rob Exterkate https://orcid.org/0000-0002-6472-3051
Maxim Lagerweij https://orcid.org/0000-0003-4212-9606
Mark Buijs https://orcid.org/0000-0003-4746-4104
Cor van Loveren https://orcid.org/0000-0002-3588-2977
Guus van Strijp https://orcid.org/0000-0002-5842-2121

REFERENCES
1. Gao SS, Zhang S, Mei ML, Lo EC, Chu CH. Caries remineralisation and arresting effect in children by professionally applied fluoride treatment – a systematic review. BMC Oral Health. 2016;16:201–10.
2. Innes NP, Frencken JE, Bjørndal L, Maltz M, Manton DJ, Ricketts D, et al. Managing carious lesions: consensus recommendations on terminology. Adv Dent Res. 2016;28:49–57.
3. van Strijp G, van Loveren C. No removal and inactivation of carious tissue: non-restorative cavity control. Monogr Oral Sci. 2018;27:124–36.
4. Li Y, Liu Y, Psoter WJ, Nguyen OM, Bromage TG, Walters MA, et al. Assessment of the silver penetration and distribution in carious lesions of deciduous teeth treated with silver diamine fluoride. Caries Res. 2019;53:431–40.
5. Yamaga R, Nishino M, Yoshida S, Yokomizo I. Diammine silver fluoride and its clinical application. J Osaka Univ Dent Sch. 1972;12:1–20.
6. Lou YL, Botelho MG, Darvell BW. Reaction of silver diamine fluoride with hydroxyapatite and protein. J Dent. 2011;39:612–8.
7. Lansdown AB. Silver in health care: anti-microbial effects and safety in use. Curr Probl Dermatol. 2006;33:17–34.
8. Hu S, Meyer B, Duggal M. A silver renaissance in dentistry. Eur Arch Paediatr Dent. 2018;19:221–7.
9. Milgrom P, Horst JA, Ludwig S, Rothen M, Chaffee BW, Lyalina S, et al. Topical silver diamine fluoride for dental caries arrest in preschool children: a randomized controlled trial and microbiological analysis of caries associated microbes and resistance gene expression. J Dent. 2018;68:72–8.
10. Mitwalli H, Mourao MDA, Dennison J, Yaman P, Paster BJ, Fontana M. Effect of silver diamine fluoride treatment on microbial profiles of plaque biofilms from root/cervical caries lesions. Caries Res. 2019;53:555–66.
11. Mei ML, Chu CH, Lo EC, Samaranayake LP. Fluoride and silver concentrations of silver diamine fluoride solutions for dental use. Int J Paediatr Dent. 2013;23:279–85.
12. Crystal YO, Niederman R. Silver diamine fluoride treatment considerations in children's caries management. Pediatr Dent. 2016;38:466–71.
13. Wierichs RJ, Stausberg S, Lausch J, Meyer-Lueckel H, Esteves-Oliveira M. Caries-preventive effect of NaF, NaF plus TCP, NaF plus CPP-ACP, and SDF varnishes on sound dentin and artificial dentin caries in vitro. Caries Res. 2018;52:199–211.
14. Thanatvarakorn O, Islam M, Nakashima S, Sadr A, Nikaido T, Tagami J. Effects of zinc fluoride on inhibiting dentin demineralization and collagen degradation in vitro: A comparison of various topical fluoride agents. Dent Mater J. 2016;35:769–75.

15. ten Cate JM, Buijs MJ, Damen JJ. pH-cycling of enamel and dentin lesions in the presence of low concentrations of fluoride. Eur J Oral Sci. 1995;103:362–7.

16. ten Cate JM, Exterkate RAM, Buijs MJ. The relative efficacy of fluoride toothpastes assessed with pH cycling. Caries Res. 2006;40:136–41.

17. van Loveren C, Moorer WR, Buijs MJ, van Palenstein Helderman WH. Total and free fluoride in toothpastes from some non-established market economy countries. Caries Res. 2005;39:224–330.

18. Mukai Y, Lagerweij MD, ten Cate JM. Effect of a solution with high fluoride concentration on remineralization of shallow and deep root surface caries in vitro. Caries Res. 2001;35:317–24.

19. ten Cate JM, Buijs MJ, Miller CC, Exterkate RAM. Elevated fluoride products enhance remineralization of advanced enamel lesions. J Dent Res. 2008;87:943–7.

20. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39:175–91.

21. Rølla G. On the role of calcium fluoride in the cariostatic mechanism of fluoride. Acta Odontol Scand. 1988;46:341–5.