Remineralisation of enamel with silver diamine fluoride and sodium fluoride

Ollie Yiru YU[A] Email: yuyiru@hku.hk

May Lei MEI[A] Email: mei1123@hku.hk

Irene Shuping ZHAO[A] Email: zhao110@hku.hk

Quan-Li LI[B] Email: ql-li@126.com

Edward Chi-Man LO[A] Email: hrdplcm@hkucc.hku.hk

Chun-Hung CHU[A] E-mail: chchu@hku.hk

[A.] Faculty of Dentistry, The University of Hong Kong, Hong Kong

[B.] Key Laboratory of Oral Diseases Research of Anhui Province, Stomatological Hospital & College, Anhui Medical University

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Correspondence to : Prof. C. H. Chu
Faculty of Dentistry,
The University of Hong Kong.
34 Hospital Road, Hong Kong SAR, China.
Tel: +852 2859 0287
Fax: +852 2858 7874
E-mail: chchu@hku.hk

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Objectives: *To evaluate the remineralising effect of the adjunctive application of 38% silver diamine fluoride (SDF) solution and 5% sodium fluoride (NaF) varnish on artificial enamel caries lesions.*

Methods: *Forty-eight demineralised enamel specimens were allocated into four groups. Group 1 received 38% SDF and 5% NaF; Group 2 received 38% SDF; Group 3 received 5% NaF; and Group 4 received deionized water. After pH cycling, the surface morphology and fluoride content of the specimens were studied via scanning electron microscopy (SEM)/energy dispersive spectroscopy (EDS). The lesion depth and crystal characteristics were assessed using micro-computed tomography and X-ray diffraction (XRD) respectively. The crystallization reaction was performed by incubating hydroxyapatite powder with NaF or SDF for 48 hours. The precipitates were studied via transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS).*

Results: *SEM demonstrated the destruction of the enamel surface in Group 4. EDS revealed that the mean fluoride weight percentage of Groups 1 to 4 were 1.28±0.15, 1.33±0.19, 1.03±0.09 and 0.87±0.04 respectively. The mean lesion depths of Groups 1 to 4 were 129±14μm, 131±16μm, 153±10μm and 181±21μm respectively. The addition of NaF to SDF did not reduce the lesion depths (p=0.779). XRD revealed that silver chloride formed as a main product in Groups 1 and 2. Meanwhile, TEM analysis indicated that silver nanoparticles were incorporated into hydroxyapatite crystal in SDF-treated hydroxyapatite. XPS spectra suggested that the chemical state of the silver was metallic.*

Significance: *The adjunctive application of SDF and NaF varnish had a similar remineralising effect to that of SDF on enamel caries.*

1. Introduction

The topical application of fluoride varnishes provides a non-invasive method for caries control and prevention. It is cost effective and easily operated. Therefore, fluoride varnishes have been widely used to treat dentine hypersensitivity, prevent caries and remineralise early caries. The Food and Drug Administration (FDA) of the United States recognized fluoride varnish in the late 1990s [1]. Fluoride compounds such as stannous fluoride (SnF_2), titanium tetrafluoride (TiF_4) and sodium fluoride (NaF) are used as topical fluoride products in dentistry. SnF_2 is chemically unstable. It could only be reserved for a short time. The discoloration of teeth after application of SnF_2 and its metallic taste limited its utilization. In addition, the precipitation on teeth surface after applying SnF_2 might interfere with the remineralisation [2]. TiF_4 was proved to be effective in remineralising caries lesions in several *in vitro* studies. However, but the number of clinical (in situ) trials is limited [3]. Fluoride varnishes contain 5% sodium fluoride is one of the most common fluoride product in the market.

The fluoride concentration of 5% NaF varnish is 22,600 parts per million (ppm), which was considered to be a cariostatic amount of fluoride [4]. The potential of the fluoride varnish for remineralising caries has been discussed in a Cochrane review [5]. It reported that 5% NaF varnishes could stop approximately 43% of caries in permanent teeth and 37% of caries in primary teeth [6]. Therefore, the caries-arresting effect of the 5% NaF varnish is not satisfactory [7].

To enhance the caries-arresting effects, anti-caries products with higher fluoride concentrations were introduced for caries control. Silver diamine fluoride (SDF) is a translucent solution used for professional topical fluoride application. The concentration of the most commonly used SDF was 38%, which contains 44,800 ppm fluoride. *Ex vivo* studies demonstrated that SDF increased the microhardness and mineral density of the caries lesions [8, 9]. SDF is also shown to inhibit the growth of cariogenic bacteria and the formation of cariogenic biofilm [10, 11].

The FDA approved the use of SDF as a desensitizing agent in 2014 [12]. It is relatively new, and a consensus on using SDF has not yet reached. Although a protocol of applying SDF has been published recently [12], it is more experience based rather than evidence based. Several concerns exist with regards to this protocol. One is that SDF is a solution with high

fluidity. The contact time of SDF with the surface of the caries lesion can be limited in the fluctuant oral environment. Another concern is that saliva might dilute the fluoride concentration of SDF. Although the inactive ingredients of NaF varnish, such as colophony, provide favorable stickiness of the agents to the caries lesions, it may protect the SDF from being washed away by the saliva. The contact time of SDF and caries lesions can be prolonged with the cover of NaF varnish [13]. In addition, the remineralising effect of an SDF solution can be enhanced with an additional source of fluoride from NaF varnish [14]. Even though studies revealed that SDF was more effective in remineralising dental caries compared with NaF varnish [15, 16], it is unknown whether a synergetic effect of the adjunctive application of an SDF solution and sodium fluoride varnish on caries management exists. Thus, the objective of this study was to evaluate the remineralising effect of the topical application of 5% NaF varnish, 38% SDF solution and the adjunctive application of NaF and SDF on enamel caries lesions.

2. Materials and methods

2.1 pH cycling of enamel caries lesion with SDF and NaF

2.1.1 Sample preparation

The Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (IRB UW 17-089) approved this study. Forty-eight human enamel specimens (4×2×2 mm^3) were prepared from 12 molars. All of the enamel specimens were polished with micro-fine 4000 grid sanding paper. The set of four specimens from the same molar was excluded if cracks or other defects were found via a stereomicroscope. The half surface of each specimen was covered with an acid-resistant nail varnish (Clarins, Paris, France). The enamel specimens were stored in 0.5% thymol solutions prior to demineralisation. Then, the specimens were demineralised using an acetate buffer solution containing 50 mM acetate, 2.2 mM calcium chloride, 2.2 mM potassium dihydrogen phosphate, 0.02% sodium azide and 0.02 ppm fluoride at 25°C. The pH of the buffer was adjusted to 4.5. Each specimen was immersed in 1 mL of an acetate buffer solution in a 24-well plate. The acetate buffer solution was refreshed daily for six days to create subsurface caries lesions. The specimens were then examined via micro-computed tomography (micro-CT) (SkyScan 1172; SkyScan, Antwerp, Belgium) to check the depth of the demineralised lesions.

2.1.2 Experimental treatment

Four specimens prepared from one molar were randomly allocated to four groups. The sample size in each group was 12. The specimens in each group received the following experimental treatment. The specimens in Group 1 received a topical application of 38% SDF solution (Saforide, Toyo Seiyaku Kasei, Osaka, Japan) followed by 5% NaF varnish (Duraphat; Colgate-Palmolive Co., New York City, NY, USA). The specimens in Group 2 received a topical application of 38% SDF solution. The specimens in Group 3 received a topical application of 5% NaF varnish. The specimens in Group 4 received deionized water (negative control). All of the agents were left on the surface of the specimen for 60 min. The redundant SDF solution and/or NaF varnish were removed before being subjected to a pH-cycling process for 21 days.

2.1.3 pH-cycling

The pH-cycling used in this study was roughly based on the one used in Lippert et al. [17]. The process was repeated for 21 days. The cyclic treatment regimen for each day consisted of one four-hour demineralisation period and one 20-hour remineralisation period. The pH-cycling phase was conducted at room temperature without stirring. The composition of the demineralisation solution was 50 mM acetic acid, 2.25 mM calcium chloride dihydrate ($CaCl_2 \times 2\ H_2O$), 1.35 mM potassium dihydrogen phosphate (KH_2PO_4) and 130 mM potassium chloride (KCl). The pH of the demineralisation solution was adjusted to 4.5 using potassium hydroxide (KOH). The remineralisation solution was composed of 1.5 mM $CaCl_2 \times 2\ H_2O$, 0.9 mM KH_2PO_4, 150 mM KCl, 20 mM HEPES. The pH of the remineralisation was adjusted to 7.0 using KOH. Following pH cycling, the specimens were collected for the assessment of demineralisation. The flowchart of this is present in Figure 1.

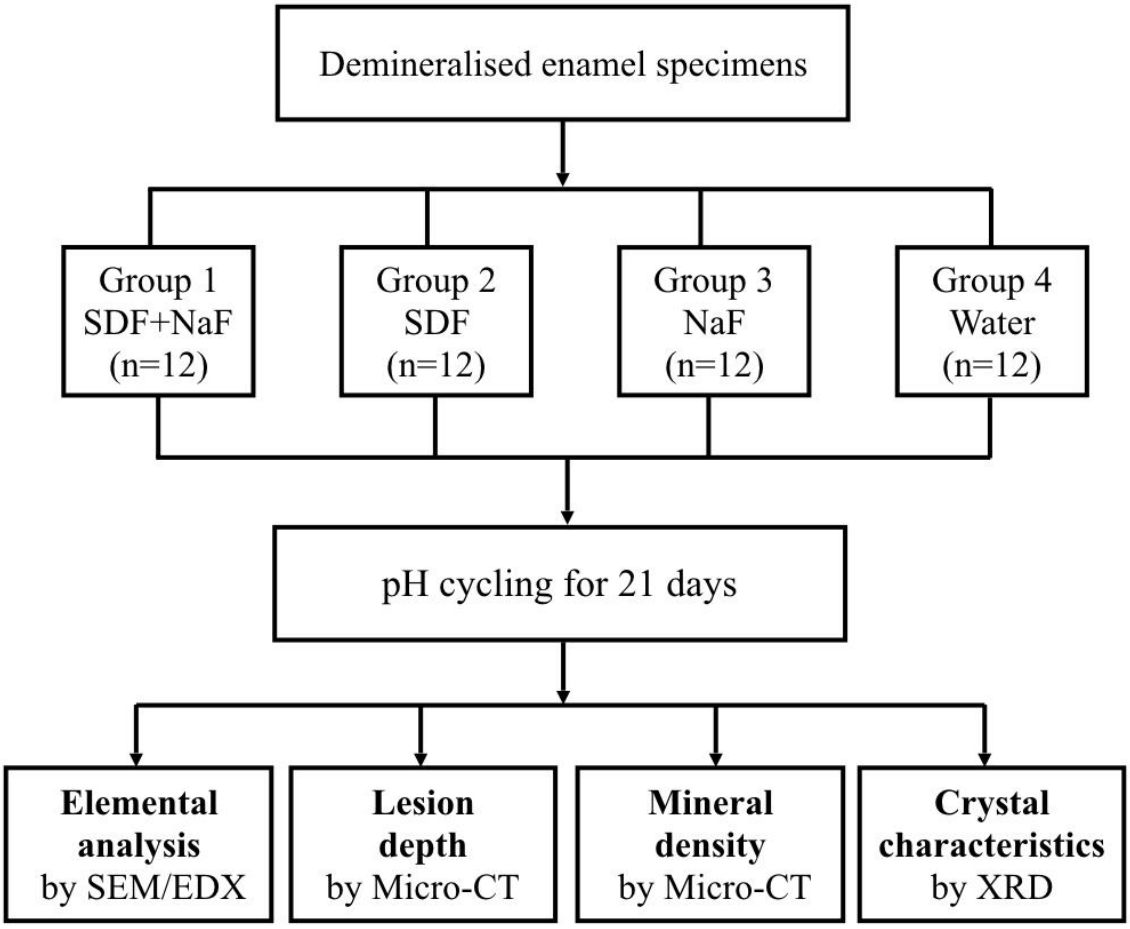

Fig. 1 - Flowchart of the de-/remineralisation on enamel.

SEM- scanning electron microscopy; EDS- energy-dispersive X-ray spectroscopy; Micro-CT- micro-computed tomography; XRD- X-ray diffraction

2.1.4 Assessment of enamel demineralisation

2.1.4.1 Surface morphology and fluoride content

Two enamel specimens in each group were dehydrated in a series of ethanol solutions, critical point dried in a desiccator and finally sputter coated with gold. The surface morphologies of the specimens were then observed under scanning electron microscopy (SEM) (Hitachi S-4800 FEG Scanning Electron Microscope, Hitachi Ltd., Tokyo, Japan) at 5 kV in high-vacuum mode.

An elemental assessment was carried out on the surfaces of six specimens in each group. The levels of fluoride (F) ions were evaluated by energy-dispersive X-ray spectroscopy (EDS) under SEM. The elemental assessment was performed by measuring five 5×5 μm^2 square areas on the enamel surface in each sample, and the mean weight percentage of F was calculated.

2.1.4.2 Lesion depth and mineral density

Ten specimens in each group were used for the assessment of lesion depth and mineral loss by micro-CT. The X-ray source was operated at a source voltage of 80 kV and a current of 100 μA. The pixel size of the image was set at 8 μm. The scanning results were reconstructed using the NRecon reconstruction software (SkyScan, Antwerp, Belgium). The reconstructed three-dimensional images were viewed and analyzed using software CTAn (SkyScan, Antwerp, Belgium). Cross-sectional images of each enamel specimen were obtained from the reconstructed images. From these cross-sectional images, 15 were randomly selected to evaluate the lesion depth. Image analysis was conducted using software Image J (National Institutes of Health, MD, USA). An image area with a grayscale value of more than 95% was defined as sound enamel [18]. An area of demineralised enamel was determined, and the depths of the lesions were measured. In addition, two standard mineral cylindrical phantoms (Bruker, Kontich, Belgium) with MDVs of 0.25 $g_{HAp}cm^{-3}$and 0.75 $g_{HAp}cm^{-3}$were scanned for calibration of the greyscale of the specimens. The greyscale value of the images was calibrated using commercially available image analysis software (CTAn, Skyscan NV, Kontich, Belgium), the demineralised area was then calculated into the mineral density value (MDV, $g_{HAp}cm^{-3}$) using the same software [19]. The mean MDV of the demineralised area of each specimen was evaluated.

2.1.4.3 Precipitate characteristics

Two specimens from each group were used for X-ray diffraction (XRD) analysis. The XRD data were collected with an X-ray diffractometer (Bruker D8 Advance; Bruker AXS, Karlsruhe, Germany) with CuKa (l = 1.5418 A˚) radiation. The data were collected in a range of 20-60° 2θ, at a step size of 0.02° and at a scan speed of 40 seconds/step. The International Centre for Diffraction Data (ICDD, PDF-2 Release 2004) was used to check the phase purity and to index the chemical phase. The Bruker DIFFRAC plus EVA program (Bruker AXS, Karlsruhe, Germany) was used to analyze the diffraction patterns.

2.2 Chemical reaction of hydroxyapatite with SDF and NaF

2.2.1 Specimen preparation

SDF, NaF and SDF+NaF were added into hydroxyapatite powder (Sigma-Aldrich, Missouri, USA) and mixed at room temperature for 48 hours as follows: Group SDF+NaF – 20 mg hydroxyapatite powder + 80μL 38% SDF solution +8 mg NaF in 4 mL deionized water; Group SDF – 20 mg hydroxyapatite powder + 80μL 38% SDF solution in 4 mL deionized water; Group NaF – 20 mg hydroxyapatite powder + 8 mg NaF in 4 mL deionized water; Group

Control: 20 mg hydroxyapatite powder in 4 mL deionized water. Following mixing for 48 hours, the hydroxyapatite powder was then washed ultrasonically three times followed by re-suspending in absolute ethanol. The flowchart of the mineral reaction is presented in Figure 2.

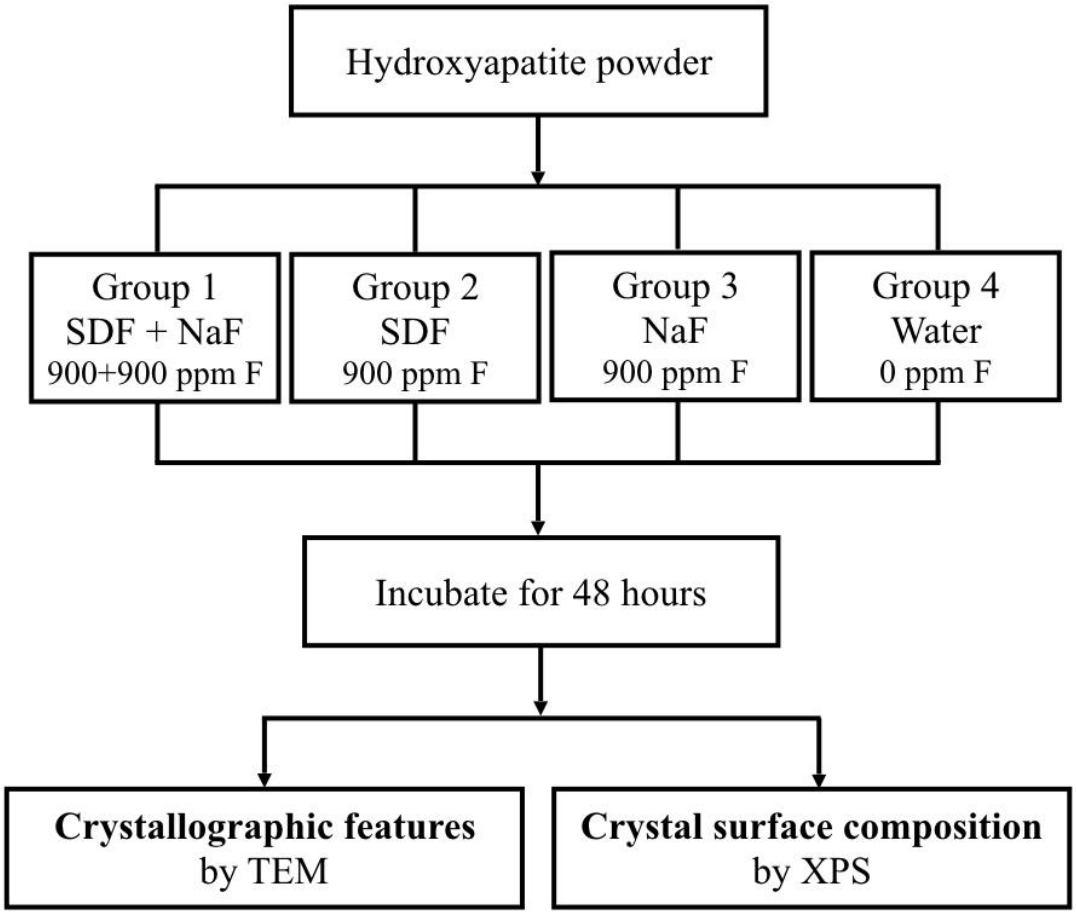

Fig. 2 - Flowchart of the mineral reaction.

TEM- transmission electron microscopy; XPS- X-ray photoelectron spectroscopy

2.2.2 Crystallographic features

The suspension was dropped onto the formvar/carbon-coated 200-mesh transmission electron microscopy (TEM) grids (Agar Scientific, Essex, UK). The grids were then rinsed with Milli-Q water, blotted against filter paper and air dried overnight. TEM analysis was performed with a Technai F20 (FEI) equipped with a field emission gun and a Tietz CCD camera (8 × 8 k; Beniash et al. 2005). Selected-area electron diffraction (SAED) was performed to evaluate the diffraction pattern of the crystal, which is used to characterize the crystal structure. Three areas in each group were imaged and analyzed. EDS was used for the elemental analysis of the crystal.

2.2.3 Chemical state

The suspension was dropped onto a silicon disk, and X-ray photoelectron spectroscopy (XPS) analysis was performed with a Thermo ESCALAB 250 with an Al Kα X-ray source (1486.6 eV photons). A wide-scan survey spectrum over a binding energy (BE) range of 0-1400 eV was recorded at a pass energy of 100 eV to estimate the chemical elemental composition and 30 eV for high-resolution detailed scans. The system was calibrated using the C1s peak at 284.8 eV. All spectra were recorded at a takeoff angle of 45 degrees. The maximum information depth of the XPS method was not more than 10 nm.

2.3 Statistical analysis

Shapiro–Wilk test was adopted to evaluate the normality of data. A two-way analysis of a variance test with a Bonferroni post hoc test was applied to compare the lesion depths, mineral densities and fluoride concentrations among the four experimental groups. All of the analyses were performed with the software SPSS 21.0 (IBM Corporation, Armonk, USA). The significance level was set at 5 %.

3. Results

3.1 pH cycling of enamel caries lesion with SDF and NaF

SEM images of the enamel lesion surface are displayed in Figure 3. The surfaces of enamel treated with SDF and NaF remained relatively dense and intact compared with other groups. The enamel surfaces in the group treated with NaF revealed a larger inter-crystalline space compared with SDF+NaF and the SDF group. On the contrary, the enamel rods fractured and displayed sparsely in groups treated with water. The mineral content analysis via EDS revealed that the fluoride weight percentage ± standard deviation (SD) from Groups 1 to 4 were 1.28 ± 0.15, 1.33 ± 0.19, 1.03 ± 0.09 and 0.87 ± 0.04 respectively. No statistically significant interaction effect on the fluoride percentage was detected between the SDF treatment and NaF treatment ($p=0.073$). Groups with SDF treatment (Group 1 and Group 2) presented higher fluoride percentages than groups without SDF did (Group 3 and Group 4) ($p<0.001$). No significant difference in fluoride percentage was found between Group 1 and Group 2 ($p=0.339$).

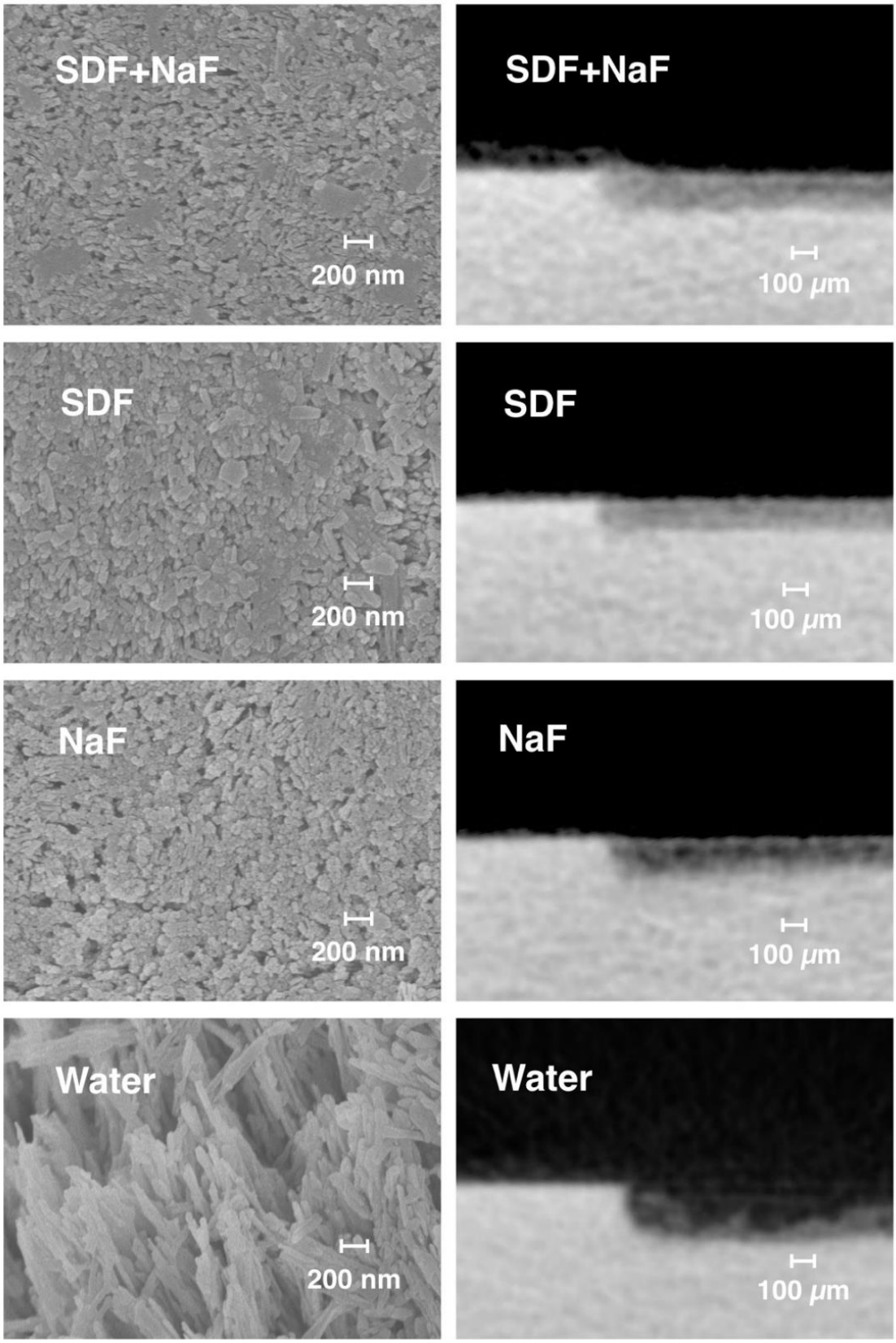

Fig. 3 - Representative scanning electron micrographs of the enamel surface morphology (images in the left column), and typical images of micro-computed tomography images (images in the right column) of the enamel caries lesions of the four treatment groups. SDF- silver diamine fluoride; NaF- sodium fluoride

Representative micro-CT images are presented in Figure 3. The lesion depths ± SD of Groups 1 to 4 were 129 ± 14 μm, 131 ± 16 μm, 153 ± 10μm and 181 ± 21 μm respectively. An interaction effect was found on the lesion depths between SDF treatment and NaF treatment (p=0.013). A Bonferroni post hoc test revealed no significant difference in the lesion depths between the SDF+NaF group (Group 1) and SDF group (Group 2) (p=0.779). Group 1 and Group 2 showed less lesion depths than Group 3 (p=0.002) did. Group 3 had a significantly smaller lesion depth than Group 4 did (p<0.001).

The MDVs of the lesions in the four experimental groups were 1.67±0.27$g_{HAp}cm^{-3}$, 1.66±0.15$g_{HAp}cm^{-3}$, 1.38±0.19$g_{HAp}cm^{-3}$ and 1.07±0.22$g_{HAp}cm^{-3}$ respectively. An interaction effect on MDVs was found between SDF treatment and NaF treatment (p=0.031). No significant difference was detected between the NaF+SDF group (Group 1) and SDF group (Group 2) (p=0.917). The MDVs of the NaF+SDF group (Group 1) was higher than that of the NaF group (Group 3) (p=0.003).

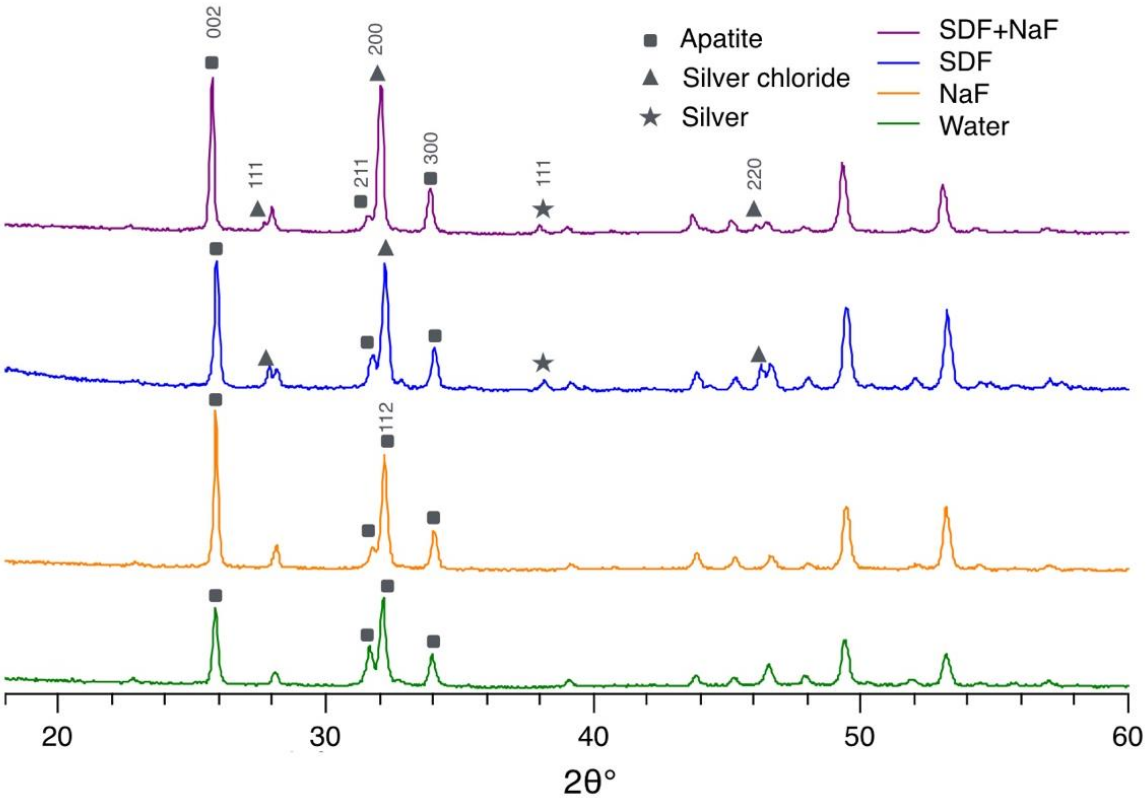

Fig. 4 - Typical X-ray diffraction patterns of the enamel in the four treatment groups.

The typical XRD spectra of the four experimental groups are displayed in Figure 4. Diffraction peaks were detected at 2θ = 25.8°, 31.8°, 32.2°, and 32.8° corresponded to the reflections of (002), (211), (112) and (300) for hydroxyapatite, suggesting that the crystals were hydroxyapatite. The reflections of (002) and (112) in the SDF+NaF group, SDF group and NaF were sharper than those in the water group were; this indicated that the hydroxyapatite in these groups were better crystallized compared with the water group. The crystals were oriented along their c axis. In addition, the strong diffraction reflections were detected at 27.9°, 32.2° and 46.3° in the SDF+NaF group and SDF group, which were coincident with silver chloride (AgCl) (111), (200) and (220). This indicated that AgCl was formed in these two groups. Apart from AgCl, the peak of Ag (111) in group SDF+NaF and group SDF suggests the formation of metallic silver.

3.2 Chemical reaction of hydroxyapatite with SDF and NaF

The TEM images revealed the morphology of the formed crystals and correspondent SEAD and EDS results (Figure 5). Shortplate-shaped to rod-shaped apatite crystals were found in all treatment groups; All the three selected area show the same diffraction pattern, SEAD revealed the typical reflections corresponding to the (110), (001), and (100) planes, confirming that these crystals were made of hydroxyapatite. Nanoparticles (diameter around 5 nm) were detected on the hydroxyapatite crystals in the groups treated with SDF (arrows in Figures 5A and 5D), and they were confirmed to be silver via EDS (Figure 5C and 5F). Round-shaped crystal was observed in the NaF-treated groups (Figure 5G). EDS indicated that the major component of this structure was calcium and fluoride, with the diffraction pattern of the crystal showing characteristics of (220) and (111) plates (Figure 5H). This confirmed that the crystal was made of calcium fluoride.

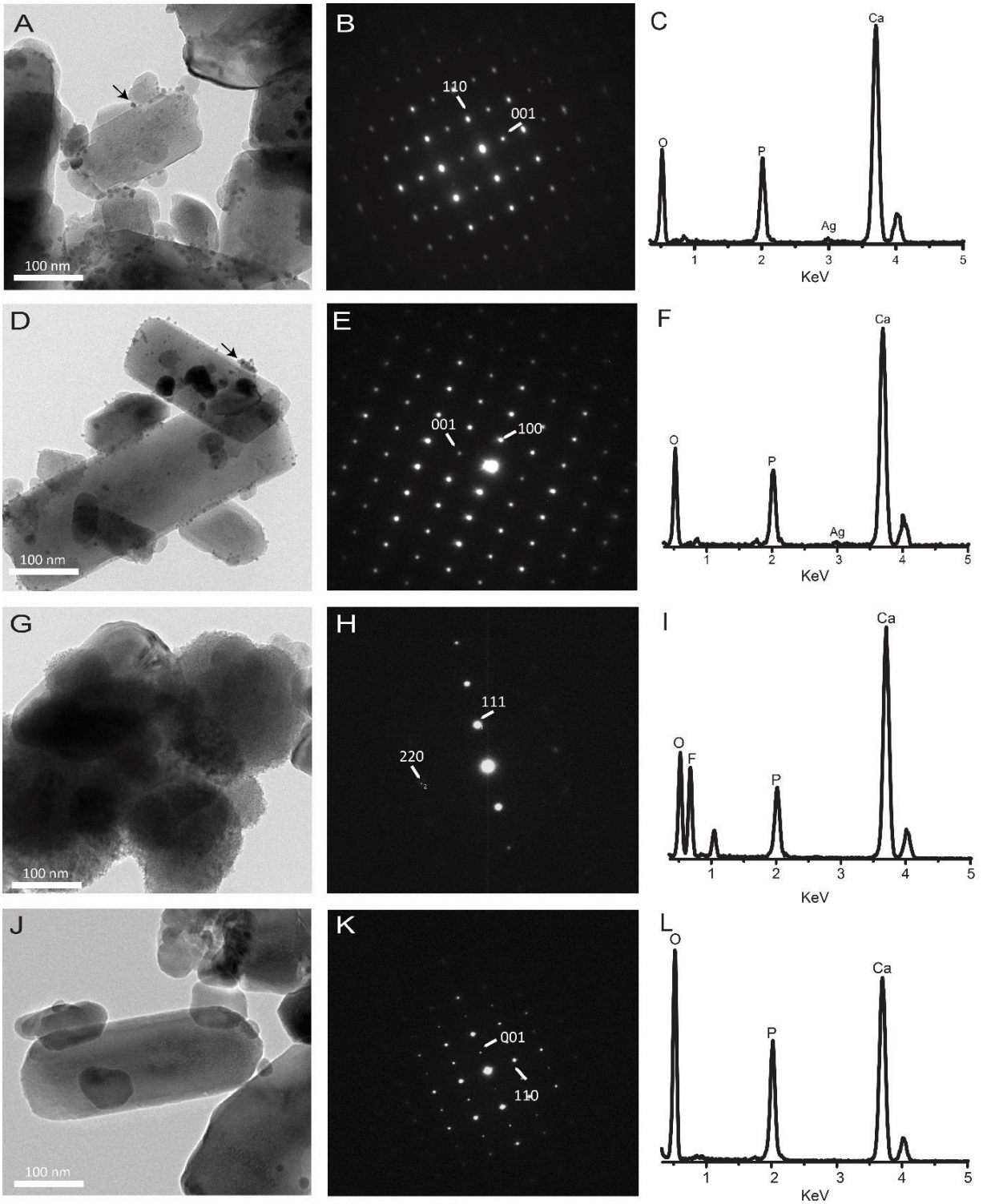

Fig. 5 - Transmission electron microscopy data of experimental groups. SDF+NaF group: (A) morphology, (B) SAED pattern, (C) EDS spectra. SDF group (D) morphology, (E) SAED pattern, (F) EDS spectra. NaF group (G) morphology, (H) SAED pattern, (I) EDS spectra. Water group (J) morphology, (K) SAED pattern, (L) EDS spectra.
EDS- energy-dispersive X-ray spectroscopy; F- fluoride; O- oxygen; P- phosphorus; Ag- silver. SAED- selected-area electron diffraction; SDF- silver diamine fluoride; NaF- sodium fluoride

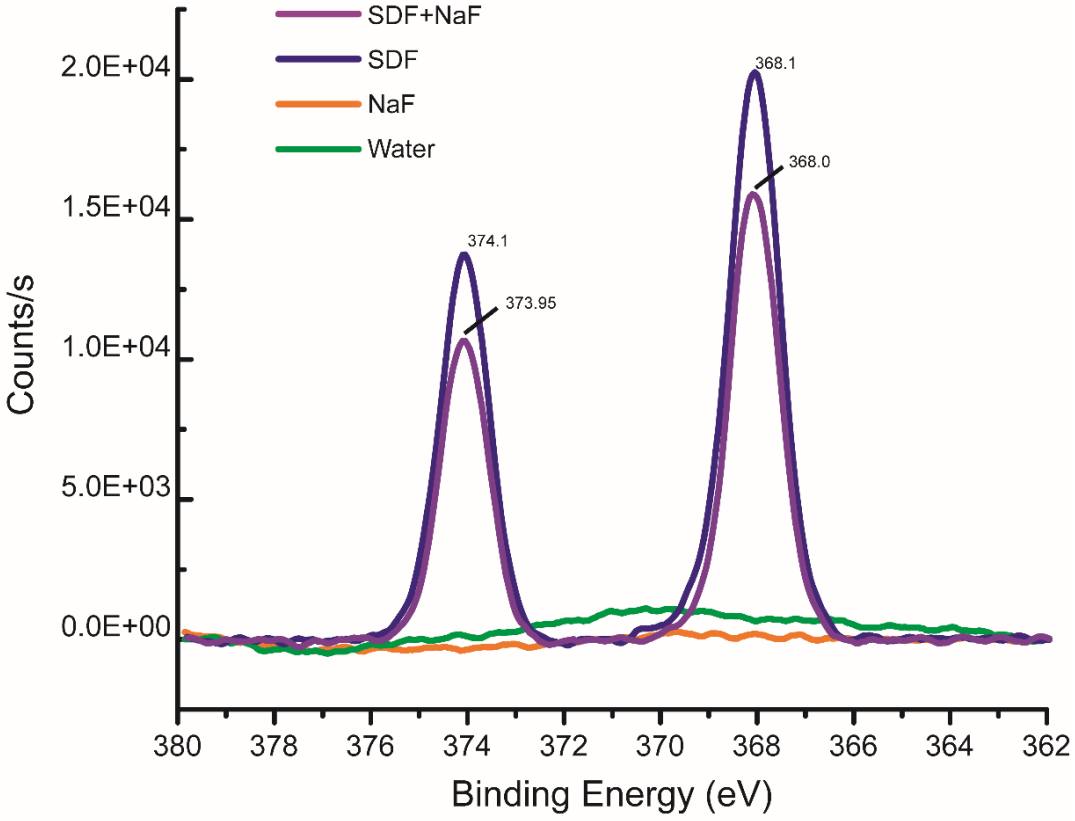

Fig. 6 - The X-ray photoelectron spectroscopy high-resolution spectra of Ag3d of experimental groups.

The chemical state of silver was determined via XPS. The XPS Ag3d peaks were clearly present in the groups treated with SDF (SDF+NaF and SDF), whereas two specific peaks with the binding energies of 374 eV and 368 eV were attributed to the $Ag3d_{5/2}$ and $Ag3d_{3/2}$ electrons of Ag^0 respectively. The spin energy separation was identified as 6.0 eV, indicating that the silver on the hydroxyapatite was metallic Ag^0.

4. Discussion

This study was the first one to investigate the remineralising effect of the adjunctive application of 38% SDF solution and 5% NaF varnish on artificial enamel caries lesions. It revealed that the adjunctive application of SDF and NaF varnish had a similar remineralising effect to that of SDF on enamel caries. In this study, we adopted a pH cycling model to investigate the remineralising effect on an artificial enamel caries lesion as well as on a hydroxyapatite powder and fluoride solution chemical model to investigate the changes of the hydroxyapatite crystal after SDF or NaF treatment. This is different from a real situation;

caution should be exerted in data interpretation. Another limitation of the chemical system is the lack of a biological component, in which the role of silver could be underestimated.

In the current study, the enamel specimens were demineralised using a modified chemical model before fluoride agent application to mimic the early caries formation. A concentration of 0.02 ppm fluoride was added into the acetate buffer solution in this model. The addition of the low concentration of fluoride could preserve the surface integrity of the enamel specimens. In addition, the surface layer with a higher mineral density was formed using the modified chemical model in our previous study [20]. Although the demineralised enamel specimens would be used to test the effect of fluoride products, such a low concentration of fluoride in the modified chemical models would not affect the results of the experiment. Previous research demonstrated that the surface layer of a natural caries lesion contains a low concentration of fluoride [21]. This low level of fluoride in the oral cavity is from different sources, such as drinking water, food or saliva. The fluoride of low concentration in oral cavity can react with the dissolved hydroxyapatite when pH of oral cavity drops below critical pH. The formation of less soluble fluorapatite deposit on the surface of the teeth, lowering the critical pH value and the dissolution of enamel [22]. Therefore, fluoride works as the natural inhibitor of demineralization. The similarity of the artificial caries lesions to natural caries lesions increased due to the addition of fluoride. The clinical situations of the early caries were better simulated in this chemical caries model.

It is worth noting that no significant difference in fluoride intake was found between the SDF+NaF group and SDF group via the SEM/EDS assessment. Namely, the fluoride content on the specimen surface did not increase with the addition of NaF. The reason could be related to the mechanism of fluoride on demineralised enamel. The topical application of fluoride induces the unstable fluoride present at the tooth-oral fluid interface. The unstable fluoride exists in the form of calcium fluoride and calcium fluoride-like materials. The dissolution of these calcium fluoride deposits is pH dependent. When the pH value drops to some extent (normally below 5), the labile fluoride is released and is stable bound. As a result, the fluoride hydroxyapatite crystals are formed [23]. As mentioned above, the first step in fluoride working as a demineralisation inhibitor is for it to combine with the ionized calcium. Nevertheless, in the present study, the number of calcium ions on the surfaces of the teeth might be limited. When the amount of fluoride exceeded the capacity of the calcium, the redundant fluoride would not function. In summary, the bioavailable calcium might be

deficient and thus limit the intake ability of fluoride in the SDF+NaF group. Therefore, even though the NaF varnish provided an additional source of fluoride in the SDF+NaF group, it did not uptake more fluoride than the SDF group did.

The SEM/EDS result revealed that the NaF group had a lower fluoride weight percentage than the SDF group did. The amounts of fluoride applied to the surfaces of the specimens in the SDF group and NaF group were inequivalent. In fact, the SDF group received evidently less fluoride than the NaF group did. The average amount of SDF applied on the surface of the specimen with a micro-brush was about 0.22 mg; it contained about 8.8 μg fluoride [24]. The average amount of sodium fluoride varnish applied on one specimen was about 7 mg; it contained 0.35 mg NaF. The fluoride content from NaF applied to each specimen was about 158 μg. Thus, specimens in the SDF group received 26 μg fluoride, whereas specimens in the NaF group received 158 μg fluoride. However, the fluoride intake in the SDF group was significantly higher than that in the NaF group. This result might be attributed to the flowability of the SDF solution. The liquidity of SDF allowed for the full contact of SDF and the enamel surface in a relatively short time. The vehicle of NaF was varnish. Although it contained more fluoride, the viscidity of varnish might have slowed down the diffusion of fluoride ions into the surface of the enamel. Furthermore, to simulate the removal of the fluoride in a clinic, we removed the fluoride agent after one hour because the fluoride agents were normally removed by the drinking, eating or rinsing behavior of the patients after half an hour to one hour in a clinical situation. The limited contact time might have further reduced the fluoride intake of enamel specimens in the NaF group.

The lesion depths and the MDVs of the enamel caries lesion did not reveal a statistical difference in the SDF+NaF group and SDF group. Lesion depths and MDVs revealed degrees of mineralisation. The results demonstrated that the degrees of remineralisation in the SDF+NaF group and SDF group were similar. The remineralisation of enamel was not linearly related to the concentration of fluoride when fluoride products of high concentration were used [25]. SDF contains 44,800 ppm fluoride. The fluoride concentration is the highest among all of the commercially available fluoride agents in dentistry. Thus, the supplement of NaF revealed no additional impact on enamel remineralisation in the present study. The result was consistent with the data from EDS tests, which proved that the fluoride uptake in the SDF+NaF group and SDF group was similar.

SDF showed stronger remineralising effects than NaF did in the results of the micro-CT assessment. It concurred with previous studies [6, 10]. However, the mechanism of SDF on dental caries was not fully understood. The arresting action of SDF on enamel caries could be explained by its high concentration of fluoride, its alkaline property and the presence of silver [26]. Fluoride promotes the remineralisation of hydroxyapatite. The alkaline property is favorable to the remineralisation process and inactive dentine collagenases [27]. The effect of silver in SDF on enamel caries was explained by the insolubility of the formed silver composite and the antibacterial effect of silver in previous studies [26, 27]. In the present study, the formation of insoluble AgCl and metallic silver was confirmed via the XRD. AgCl has a low solubility (8.9×10^{-5} g/100 mL), which may also contribute to the increased hardness of a caries lesion.

Ogard et al. proposed that fluoride may react with apatite in several ways: the exchange of fluoride ions for hydroxyl ions, the crystal growth of fluorapatite from supersaturated solutions, or apatite dissolution with calcium fluoride formation [13]. A recent study adopted a chemical system to simulate the salivary environment using calcium and phosphate ions provided in Tris-buffered saline solution. The study found SDF reacted with the system and formed fluoroapatite after a 24-hour incubation period [28]. The current study revealed calcium fluoride formation in the NaF-treated group, whereas no calcium fluoride was found in the SDF-treated groups. A recent published review hypothesized that silver in SDF may diffuse into the hydroxyapatite crystal and produce silver-containing hydroxyapatite [26]. This silver-containing hydroxyapatite has been shown to reduce bacterial adhesion and to minimize tissue cytotoxicity [29]. The formula of silver-containing hydroxyapatite is $Ca_{10-x}Ag_x(PO_4)_6(OH)_2$ with $0.0 \leq x \leq 0.5$, with a very small number of calcium ions substituted with silver ions. This minute amount of silver incorporation makes the characterisation challenging, especially in biological specimens such as enamel. Therefore, we developed a basic chemical model to simulate the reaction between hydroxyapatite and SDF. The reaction solution went through an ultrasonic to remove all of the loosely bound matters prior to subsequent testing. Nanoparticles were detected on the hydroxyapatite crystals in the groups treated with SDF (Figures 5A and 5D), and they were confirmed to be silver via EDS. The XPS results revealed that the silver on the hydroxyapatite was metallic Ag0. This study approved the hypothesis that silver can be doped in a hydroxyapatite crystal. This silver-containing hydroxyapatite can suppress bacterial adhesion and thus contribute to the caries arrest progress [29].

5. Conclusion

This study revealed that the adjunctive application of SDF and NaF varnish had a similar remineralising effect to SDF on enamel caries. Silver from SDF can be doped into hydroxyapatite structure.

Acknowledgments

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