Caries-arresting effects of silver diamine fluoride and sodium fluoride on dentine caries lesions

Short title: Caries-arresting effect on dentine caries

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Objectives: To investigate the remineralising effect and bacterial growth inhibition of 38% silver diamine fluoride (SDF) solution and 5% sodium fluoride (NaF) varnish on artificial dentine caries lesions.

Methods: Demineralised dentine blocks were treated with SDF+NaF (Group 1), SDF (Group 2), NaF (Group 3) and water (Group 4) and subjected to a *Streptococcus mutans* biofilm challenge. Lesion depth, precipitates' characteristics and matrix (collagen)-to-mineral ratio were evaluated by micro-computer tomography (micro-CT), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR), respectively. The biofilm kinetics, viability and topography were assessed by counts of colony forming units (CFUs), confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM), respectively. Data were analysed by two-way ANOVA test.

Results: The lesion depths of Groups 1 to 4 were 170±28μm, 160±32μm, 353±38μm and 449±24μm, respectively. The addition of NaF to SDF did not show better remineralisation than SDF ($p=0.491$). Metallic silver and silver chloride were found in Groups 1 and 2. The amide I-to-hydrogen phosphate ratios of the four groups were 0.14±0.02, 0.14±0.01, 0.29±0.05 and 0.49±0.16, respectively, and the addition of NaF to SDF did not offer better protection against collagen exposure than SDF ($p=0.986$). The Log_{10} CFUs of Groups 1 to 4 were 5.75±0.56, 4.49±0.57, 6.55±0.39 and 6.40±0.38, respectively. The presence of NaF reduced the antibacterial effect of SDF ($p<0.001$). The SEM and CLSM images supported the findings.

Conclusion: Application of SDF with or without NaF reduced the demineralisation of dentine caries, but SDF exerted stronger inhibition of biofilm growth than SDF with NaF.

Clinical Significance： NaF varnish affects the antibacterial effects of SDF, the adjunctive application of SDF solution and NaF varnish is not recommended to arrest dentine caries in clinic.

1. Introduction

Sodium fluoride (NaF) varnish is one of the most concentrated fluoride products available commercially. It is relatively new in the United States, although it has been widely used in Europe for more than 50 years. Although NaF varnishes have different compositions and delivery systems, most of them contain 5% NaF (22,600 ppm fluoride) in a natural colophony base, which allows the varnish to adhere to tooth surfaces in the presence of saliva [1]. Because of its adherent nature, NaF varnish can stay in contact with the tooth surface for several hours. NaF varnish is one of the most common topical fluorides for prevention of dental caries. Apart from caries prevention, dentists also use NaF varnish to arrest dental caries [2]. A recent systematic review concluded that 5% NaF varnish (22,600 ppm fluoride) can arrest enamel caries [3]. However, whether NaF varnish can effectively arrest dentine caries was inconclusive [4].

Unlike caries on enamel, which is basically highly mineralised tissue, dentine caries is more complex. The complexity of dentine caries can be attributed to its structural composition; it contains approximately 50% organic materials and water by volume [5]. Compared to enamel, dentine in general has smaller hydroxyapatite crystallites, higher carbonate and magnesium content and a more porous structure [6]. Thus, dentine is more vulnerable than enamel to dental caries. Caries in dentine is a biochemical process starting with the dissolution of dentine's mineral content by acid [7]. When the minerals are lost, organic matrix—which is basically collagen—is exposed. It is subsequently degraded by bacteria- and host-derived enzymes [7].

Some dentists have used silver diamine fluoride (SDF) to arrest cavitated dentine caries [8, 9]. The most commonly used concentration of SDF is 38% (44,800 ppm F) [10]. Mechanistic studies have found that SDF can remineralise carious dentine [11, 12]. A highly remineralised layer with high calcium and phosphate content was found on arrested dentine caries lesions

after SDF treatment [13]. SDF also inhibited the degradation of dentine organic matrix, which mainly consisted of Type I collagen [14, 15]. Although SDF has been used in some countries in Asia and South America for many years, the US Food and Drug Administration only approved its use for the management of dental hypersensitivity in 2014. More evidence is required for the use of SDF in caries management, and a use protocol for SDF based on scientific evidence is essential. Although the School of Dentistry of the University of San Francisco has proposed a protocol for SDF use [16], the protocol was developed basically through specialists' experience. Because SDF is a clear liquid solution and its contact time with the caries lesion is limited due to its high fluidity, some clinicians have applied 38% SDF solution followed by 5% NaF varnish. The NaF varnish is believed to not only protect the SDF from being washed away by the saliva but also provide additional fluoride for an extended period of time. However, whether the adjunctive application of SDF and NaF (SDF+NaF) has a superior caries-arresting effect on dentine caries lesions has not been studied. Thus, this study investigates the antibacterial and remineralising effects of 38% SDF solution followed by 5% NaF varnish on dentine caries.

2. Materials and methods:

2.1 Sample preparation

Twenty-six dentine slices were prepared from human third molars with the patients' consent. Ethics approval was obtained from the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (IRB number UW 12-221). The dentine slices were polished, and each slice was cut into four blocks ($3\times3\times2$ mm^3). A total of 104 dentine blocks were prepared. Half of the surfaces of 48 blocks were covered by acid-resistant nail varnish (Clarins, Paris, France) as internal controls to study mineral content (dentine demineralisation). The remaining 56 blocks were used for biofilm assessment. The dentine blocks were sterilised by autoclave. *Streptococcus mutans* (*S. mutans*) American Type Culture

Collection (ATCC) 35668 was anaerobically cultivated on blood agar plates at 37°C for 2 days. A single colony was picked and cultivated in brain heart infusion (BHI) at 37°C in anaerobic conditions. Subsequently, bacterial cell pellets were collected and re-suspended in BHI with 5% sucrose. The concentration of the bacteria suspension was adjusted to McFarland 2 (6×10^8 cells/mL). Then, 1 mL bacteria culture was incubated with each dentine block in a well of a 24-well plate. The plates were incubated anaerobically at 37°C for three days to create carious lesions that were approximately 80 μm in depth on the exposed surfaces of the dentine blocks [15]. The biofilm on the dentine surface was then removed by ultrasonication.

2.2 Experimental treatment

Four dentine blocks prepared from the same slice were randomly allocated into four experimental groups. The blocks in Group 1 received a topical application of 38% silver diamine fluoride (SDF) (Saforide; Toyo Seiyaku Kasei Co., Ltd., Osaka, Japan) followed by a 5% sodium fluoride (NaF) varnish (Duraphat; Colgate-Palmolive Co., New York City, NY, USA). Group 2 received a topical application of SDF. Group 3 received a topical application of NaF. The dentine blocks in Group 4 received sterile deionised water. A microbrush (Micro Applicator—Regular; Premium Plus International Ltd., Hong Kong, China) was used to apply SDF solution and NaF varnish to the dentine surface. The SDF solution and/or fluoride varnish were left on the dentine surface for 60 min before they were subjected to the *S. mutans* biofilm challenge described above for 7 days. After the cariogenic challenge, the blocks were used to study dentine demineralisation (n = 12 per group) and biofilm (n = 14 per group). The flowchart of the experiment is shown in Figure 1.

2.3 Assessment of dentine demineralisation

2.3.1 Lesion depth

Ten dentine blocks from each group were scanned by X-ray micro-computed tomography (Micro-CT) (SkyScan 1172; SkyScan, Antwerp, Belgium). The X-ray source voltage and current were set at 80 kV and 100 µA, respectively. The image pixel size was 8 µm. The images of the dentine blocks were reconstructed using the NRecon reconstruction software (SkyScan, Antwerp, Belgium). The software CTAn (SkyScan, Antwerp, Belgium) was used to view and analyse the reconstructed three-dimensional images. Fifteen reconstructed cross-sectional images from each block were selected for assessment. The lesion depth was measured by Image J (National Institutes of Health, MD, USA) using the internal control as a reference line.

2.3.2 Precipitates' characteristics

Two blocks from each group was used to study the characteristics of the precipitates by X-ray diffraction (XRD) analysis. The data was collected with an X-ray powder diffractometer (D8 Advance; Bruker AXS, Karlsruhe, Germany) equipped with a CuKa (l = 1.5418 Å) radiation detector. The accelerating voltage and the current of the X-ray generator were 40 kV and 40 mA, respectively. The data was collected with 2θ ranges from 20° to 60°, step size = 0.02°, scan speed = 30 second/step. The phase purity and indexing of the chemical phase were checked and matched with the International Center for Diffraction Data database (ICDD, PDF-2 Release 2004). The X-ray diffraction patterns were processed with the program BRUKER DIFFRAC plus EVA (Bruker Corporation, Massachusetts, US).

2.3.3 Collagen degradation

Ten dentine blocks from each group were sectioned transversely to analyse potential changes in the chemical structure, including the organic content of the dentine caries lesions, using Fourier transform infrared (FTIR) spectroscopy (UMA 500, Bio-Rad Laboratories, Hercules, CA, USA) equipped with an attenuated total reflection element. The wavelength number of the infrared radiation ranged from 650 cm^{-1} to 4000 cm^{-1} [17]. Spectra of the dentine caries lesions

were acquired with an infrared spot of 32 μm. The extent of demineralisation of the dentine caries lesions was studied by evaluating the ratio of the integrated area of collagen amide I absorbance (between 1585 and 1720 cm^{-1}) to that of HPO_4^{2-} absorbance (between 900 and 1200 cm^{-1}) [17].

2.4 Assessment of biofilm

2.4.1 Growth kinetics of biofilm

Colony-forming units (CFUs) of the *S. mutans* on ten blocks from each group were evaluated to determine the growth kinetics of the biofilm. The specimens were gently rinsed with phosphate-buffered solutions. Serial 10-fold dilutions of homogenised biofilm samples in sterile BHI solution were plated in duplicate with a spiral plater (Autoplate 4000; Spiral Biotech Inc., Norwood, MA, USA). Horse blood agar plates were used to determine the CFU counts (Defib Horse Blood; Hemostat Laboratories, Dixon, CA, USA). These plates were cultivated anaerobically for 72 hours before counting the CFUs.

2.4.2 Biofilm surface topography

Dentine blocks with the biofilm were fixed in 2.5% glutaraldehyde solution after being rinsed with 1% PBS. They were dehydrated with ethanol solutions, dried in a desiccator and sputter coated with gold. The topographical features of the biofilm surface were observed under scanning electron microscopy (SEM) (Hitachi S-4800 FEG Scanning Electron Microscope; Hitachi Ltd., Tokyo, Japan).

2.4.3 Viability of the bacteria

The viability of the bacteria in the biofilms was studied via confocal laser scanning microscopy (CLSM). The biofilms were labelled in situ using two fluorescent probes: PI and SYTO-9 (LIVE⁄DEAD BacLight Bacterial Viability Kit; Molecular Probes, Eugene, OR, USA). The

dead cells were labelled red with the PI probe, whereas the live cells were labelled green with the SYTO-9 probe. The dentine blocks were kept in the dark for 30 min [18]. Four cellular images of each biofilm specimen were obtained after labelling by CLSM and viewed with a Fluoview FV 1000 (Olympus, Tokyo, Japan). The red-to-green ratio is the ratio of dead-to-live bacteria, which indicates the antimicrobial effects of the experimental treatment. The ratio was studied using the Image J image-analysis software (National Institutes of Health, Bethesda, MD, USA).

2.5 Statistical analysis

The characteristics of the precipitates by XRD, the surface morphology by SEM and the viability of the bacteria by CLSM were observational and not subjected to statistical analyses. The lesion depth, ratio of amide I:HPO_4^{2-}, $\log_{10}$ CFU and dead-live ratio were analysed using two-way ANOVA test and Bonferroni post hoc test. All of the analyses were conducted using IBM SPSS Version 20.0 software (IBM Corporation, Armonk, New York, USA), and the level of significance for the analyses was set at 5%.

3. Results

The typical micro-CT images and the reconstructed three-dimensional images of the dentine blocks of the four experimental groups are shown in Figure 2. The mean lesion depths (± standard deviation) of Groups 1 to 4 were 170 ± 28 µm, 160 ± 32 µm, 353 ± 38 µm and 449 ± 24 µm, respectively. Both SDF and NaF treatment had statistically significant effects on lesion depth when compared to water treatment ($p < 0.001$). An interaction effect on lesion depth was found between NaF and SDF treatment ($p < 0.001$). In groups treated with NaF, the addition of SDF (Group 1) significantly decreased the lesion depth over NaF treatment alone (Group 3) ($p < 0.001$). Lesion depth in the SDF groups with (Group 1) or without (Group 2) NaF treatment did not show significant differences ($p = 0.491$).

Typical XRD spectra of the four groups are presented in Figure 3. The diffraction peaks were detected at 2θ = 25.8°, 2θ = 31.8°, 2θ = 32.2° and 2θ = 32.8°, which corresponded to the peaks for hydroxyapatite (002, 211, 112 and 300). The lower intensity of peaks 211, 112 and 300 in the spectra of Group 4 indicated the loss of crystallinity of dentine due to the dissolution of the hydroxyapatite crystal structure. In addition, prominent diffraction peaks were detected at 32.2° and 46.3° in the spectra of Groups 1 and 2, which corresponded to silver chloride (200 and 220), indicating the presence of silver chloride. The peak at 2θ = 38.18° represented the detection of silver (111) in Groups 1 and 2, implying the presence of metallic silver.

The mean values ± standard deviation of the ratio of amide I:HPO_4^{2-} absorbance in Groups 1 to 4 were 0.14 ± 0.02, 0.14 ± 0.01, 0.29 ± 0.05 and 0.49 ± 0.16, respectively. Figure 4 shows the representative FTIR spectra of the four experimental groups. An interaction effect of the SDF and NaF treatments was detected ($p < 0.001$). In the presence of SDF, the addition of NaF (Group 1) or not (Group 2) had no significant effects on amide I:HPO_4^{2-} ratios ($p = 0.986$).

The Log_{10} CFU values of *S. mutans* for the four treatment groups after seven days are shown in Table 1. Two-way ANOVA analysis revealed an interaction effect on Log_{10} CFU between SDF and NaF treatment ($p = 0.001$) (Figure 5). The pairwise comparison results showed Log_{10} CFUs in the NaF group (Group 3) and water group (Group 4) were not significantly different ($p = 0.493$). The *S. mutans* counts of SDF with NaF treatment (Group 1) were significantly higher than those of SDF treatment (Group 2) ($p < 0.001$). The SDF+NaF group (Group 1) exhibited a superior antibacterial effect to the NaF group (Group 3) ($p = 0.001$). The SEM images corroborated the findings (Figure 6). The CLSM images revealed that the dead (red) bacterial cells dominated in Group 2 (Figure 6). The red-to-green ratios representing the dead-live ratios of the bacteria cells are presented in Table 1. The pairwise comparisons of the dead-live ratios from the Bonferroni test supported the Log_{10} CFU results.

Discussion

Children with systemic overexposure to fluoride before six years of age can develop dental fluorosis. Therefore, some people – particularly those who are against the use of fluoride – have raised concerns about the use of products with high fluoride concentrations on young children. Mild dental fluorosis affects mainly the aesthetics of the teeth, but more severe forms can adversely affect enamel development. The clinical manifestation of dental fluorosis includes white horizontal lines, yellow or light brown stained area or substantial loss of enamel, to different extents [19]. Thus, children should avoid receiving frequent and redundant doses of fluoride in dental clinics. The fluoride products 38% SDF (44,800 ppm F) and 5% NaF (22,600 ppm) have very high fluoride concentrations. Clinicians must use them with caution in caries management. Some clinicians have proposed applying 38% SDF solution followed by 5% NaF varnish to protect the SDF from being washed away by the saliva and to provide additional fluoride over extended periods of time. However, this study does not demonstrate the additional benefit of the adjunctive application of SDF and NaF in remineralising dentine caries lesions.

This study used a single species (*S. mutans*) of biofilm because of its consistency in both composition and structure [20]. The main advantage of using this biofilm model in this study was that the bacteria cell growth and accumulation rates as well as the physiological characteristics of the biofilm could be accurately compared among the four experimental groups. This study used *S. mutans* as a cariogenic challenge because of its acidogenic and aciduric properties. It is a major etiological agent in the development of dentine caries [21, 22]. Nevertheless, the microbiota in dentine caries *in vivo* is very complex. Apart from *Mutans Streptococci*, there were many other species of bacteria such as *Actinomyces* and *Lactobacillus* being detected in dentine caries [22, 23]. *Candida albicans* might also exist in dentine caries lesions. It was suggested that there was potential synergistic alliance of *S. mutans* and *C.*

albicans to cause virulent tooth decay in children [24]. *C. albicans* is also commonly found in root caries among middle-aged adults and the elderly [25]. Interactions of these species could not be considered because a mono-species biofilm was adopted in the present study.

In this study, dentine caries treated with SDF presented superior reductions in the lesion depth of the dentine blocks than the NaF group and water group. The results suggest that SDF promoted the remineralisation of hydroxyapatite in dentine and concurred with a previous study [14]. SDF at 38% has high fluoride content and thus has the ability to promote remineralization. SDF promotes the formation of insoluble calcium fluoride, which dissolves in a salivary environment to release calcium and fluoride ions [14]. This process facilitates the subsequent replacement of the hydroxyl ion of hydroxyapatite by fluoride to form acid-resistant fluroapatite. SDF is alkaline, and its high alkalinity creates ideal conditions for ion exchange, which promotes the formation of fluroapatite [26, 27]. One of the crucial factors affecting the formation of fluroapatite is bioavailable apatite, or apatite that can react with fluoride bio-actively. This could explain why SDF + NaF was not better than SDF alone in remineralising the demineralised dentine, although the former had more fluoride content than the latter.

Silver phosphate is supposed to be the insoluble product of the chemical reaction between SDF and hydroxyapatite in dentine [8]. However, no significant silver phosphate could be detected in the present study. The XRD patterns of the four experimental groups confirmed the formation of silver chloride in the SDF+NaF and SDF groups. Silver chloride can be synthesised through the reaction of silver phosphate and chloride ions in alkaline environments because the solubility of silver phosphate is higher than that of silver chloride. The formation of silver chloride in this study might have contributed to the caries-arresting effect of SDF because it precipitated on the surface of the demineralised dentine. As a result, the insoluble

silver chloride formed would work as a protective layer and decrease the loss of calcium and phosphate from the dentine [26].

The ratio of amide I:HPO_4^{2-} absorbance of the four experimental groups represented more mineral precipitation and less collagen exposure in the SDF+NaF group and SDF group than the NaF and water groups. Collagen exposure fostered collagen degradation and further mineral loss from the demineralised dentine. The organic matrix of dentine contained inactive host-derived collagenases such as matrix metalloproteinases (MMPs) and cysteine cathepsins, which are activated in acidic environments [28]. Once the dentine collagens were exposed after bacterial acid attack, the activated host-derived collagenase would break down the telopeptide region of the collagen molecules [28]. The exposed collagen in demineralised dentine functioned as a scaffold for the mineral crystals' deposition [29]. More minerals were dissolved with the degradation of this scaffold; as a result, more collagens were exposed. SDF inhibited the activities of proteases as well as collagen degradation and protected the collagen from subsequent exposure and degradation [26]. A previous study suggested that the inhibitory effect of SDF might be attributed to silver rather than to fluoride [30]. The antibacterial action of silver was related to its combined intracellular and extracellular properties [31]. This study supports the study by Gao et al [31] because the additional fluoride from NaF had no added value in the inhibition of collagen exposure by SDF alone.

This study found that NaF varnish did not suppress the growth of *S. mutans* biofilm because the bacterial count of the NaF group was similar to that of the water group (negative control). However, SDF had significant anti-microbial effect because the SDF group had a lower Log_{10} CFU value than the water group. Both fluoride and silver in SDF could inhibit biofilm formation [17]. Fluoride in high concentrations could adversely influence the bacterial enzymes involved in carbohydrate metabolism and sugar uptake [17]. Silver also had

antibacterial effects on the biofilm's growth. The XRD patterns in this study also confirmed the formation of metallic silver after SDF application. A study found nanoscopic metallic silver particles in SDF-treated dentine [14]. Silver nanoparticles can effectively kill cariogenic bacteria [13]. Silver in SDF also existed in the form of ionised silver. The silver ions could inhibit the formation of *S. mutans* biofilm at a concentration of 20 ppm [32].

While the addition of NaF varnish had no additional benefit to SDF in promoting remineralisation of the demineralised dentine, the addition of NaF varnish could adversely affect the antibacterial properties of SDF. This might be due to the reduction of bioactive silver ions. The ingredients of NaF varnish might bind to and inactivate the silver ions, so they can no longer bind with bacterial cells. Since NaF varnish affects the antibacterial effects of SDF, the adjunctive application of SDF solution and NaF varnish is not recommended to arrest dentine caries.

4. Conclusion

From the results of the limited study, adjunctive application of SDF and NaF is no better than SDF in promoting the remineralisation of demineralised dentine. However, the addition of NaF varnish reduced the antibacterial effects of SDF. Thus, adjunctive application of SDF solution and NaF varnish is not recommended to arrest dentine caries.

References

[1] C.H. Chu, M.L. Mei, E. Lo, Use of fluorides in dental caries management, Gen. Dent. 58(1) (2010) 37-43; quiz 44-5, 79-80.

[2] P. Milgrom, M. Rothen, A. Spadafora, E. Skaret, A case report: arresting dental caries, J. Dent. Hyg. 75(3) (2001) 241-3.

[3] S.S. Gao, S.N. Zhang, M.L. Mei, E.C.M. Lo, C.H. Chu, Caries remineralisation and arresting effect in children by professionally applied fluoride treatment - a systematic review, BMC Oral Health 16 (2016).

[4] C.H. Chu, E.C.M. Lo, H.C. Lin, Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children, J. Dent. Res. 81(11) (2002) 767-770.

[5] Y.C. Chien, A.K. Burwell, K. Saeki, A. Fernandez-Martinez, M.K. Pugach, G. Nonomura, S. Habelitz, S.P. Ho, M. Rapozo-Hilo, J.D. Featherstone, S.J. Marshall, G.W. Marshall, Distinct decalcification process of dentin by different cariogenic organic acids: Kinetics, ultrastructure and mechanical properties, Arch. Oral Biol. 63 (2016) 93-105.

[6] N. Takahashi, B. Nyvad, Ecological Hypothesis of Dentin and Root Caries, Caries Res. 50(4) (2016) 422-31.

[7] M.L. Mei, E. Lo, C. Chu, Clinical use of silver diamine fluoride in dental treatment, Compend Continu Educa Dent 37(2) (2016) 93-98.

[8] C.H. Chu, E.C.M. Lo, Promoting Caries Arrest in Children With Silver Diamine Fluoride: A Review, Oral Health & Preventive Dentistry 6(4) (2008) 315-321.

[9] A. Rosenblatt, T.C.M. Stamford, R. Niederman, Silver diamine Fluoride: A Caries "Silver-Fluoride Bullet", J. Dent. Res. 88(2) (2009) 116-125.

[10] M.L. Mei, C.H. Chu, E.C.M. Lo, L.P. Samaranayake, Fluoride and silver concentrations of silver diammine fluoride solutions for dental use, Int. J. Paediatr. Dent. 23(4) (2013) 279-285.

[11] C.H. Chu, L. Mei, C.J. Seneviratne, E.C. Lo, Effects of silver diamine fluoride on dentine carious lesions induced by Streptococcus mutans and Actinomyces naeslundii biofilms, Int. J. Paediatr. Dent. 22(1) (2012) 2-10.

[12] M.L. Mei, Q.L. Li, C.H. Chu, E.C. Lo, L.P. Samaranayake, Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries, Ann. Clin. Microbiol. Antimicrob. 12 (2013) 4.

[13] M.L. Mei, L. Ito, Y. Cao, E.C.M. Lo, Q.L. Li, C.H. Chu, An ex vivo study of arrested primary teeth caries with silver diamine fluoride therapy, J. Dent. 42(4) (2014) 395-402.

[14] M.L. Mei, L. Ito, Y. Cao, Q.L. Li, E.C.M. Lo, C.H. Chu, Inhibitory effect of silver diamine fluoride on dentine demineralisation and collagen degradation, J. Dent. 41(9) (2013) 809-817.

[15] M.L. Mei, C.H. Chu, K.H. Low, C.M. Che, E.C.M. Lo, Caries arresting effect of silver diamine fluoride on dentine carious lesion with S-mutans and L-acidophilus dual-species cariogenic biofilm, Medicina Oral Patologia Oral Y Cirugia Bucal 18(6) (2013) E824-E831.

[16] J.A. Horst, H. Ellenikiotis, P.M. Milgrom, U.S.C.A. Committee, UCSF protocol for caries arrest using silver diamine fluoride: rationale, indications, and consent, Journal of the California Dental Association 44(1) (2016) 16.

[17] M.L. Mei, Q.L. Li, C.H. Chu, E.C.M. Lo, L.P. Samaranayake, Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries, Ann. Clin. Microbiol. Antimicrob. 12 (2013).

[18] C.J. Seneviratne, R.W.K. Wong, L.P. Samaranayake, Potent anti-microbial activity of traditional Chinese medicine herbs against Candida species, Mycoses 51(1) (2008) 30-34.

[19] P. Denbesten, W. Li, Chronic fluoride toxicity: dental fluorosis, Monogr. Oral Sci. 22 (2011) 81-96.

[20] O.Y. Yu, I.S. Zhao, M.L. Mei, E.C.-M. Lo, C.-H. Chu, A Review of the Common Models Used in Mechanistic Studies on Demineralization-Remineralization for Cariology Research, Dentistry Journal 5(2) (2017) 20.

[21] S.R. Brailsford, B. Shah, D. Simons, S. Gilbert, D. Clark, I. Ines, S.E. Adams, C. Allison, D. Beighton, The predominant aciduric microflora of root-caries lesions, J. Dent. Res. 80(9) (2001) 1828-33.

[22] J.A. Aas, A.L. Griffen, S.R. Dardis, A.M. Lee, I. Olsen, F.E. Dewhirst, E.J. Leys, B.J. Paster, Bacteria of dental caries in primary and permanent teeth in children and young adults, J. Clin. Microbiol. 46(4) (2008) 1407-1417.

[23] D. Preza, I. Olsen, J.A. Aas, T. Willumsen, B. Grinde, B.J. Paster, Bacterial profiles of root caries in elderly patients, J. Clin. Microbiol. 46(6) (2008) 2015-2021.

[24] H. Koo, W.H. Bowen, Candida albicans and Streptococcus mutans: a potential synergistic alliance to cause virulent tooth decay in children, Future Microbiol. 9(12) (2014) 1295-1297.

[25] M.L. Zaremba, W. Stokowska, A. Klimiuk, T. Daniluk, D. Rozkiewicz, D. Cylwik-Rokicka, D. Waszkiel, G. Tokajuk, A. Kierklo, S. Abdelrazek, Microorganisms in root carious lesions in adults, Adv. Med. Sci. 51 Suppl 1 (2006) 237-40.

[26] I.S. Zhao, S.S. Gao, N. Hiraishi, M.F. Burrow, D. Duangthip, M.L. Mei, E.C.M. Lo, C.H. Chu, Mechanisms of silver diamine fluoride on arresting caries: a literature review, Int. Dent. J. 369 (2017) 10.

[27] M.L. Mei, F. Nudelman, B. Marzec, J.M. Walker, E.C.M. Lo, A.W. Walls, C.H. Chu, Formation of Fluorohydroxyapatite with Silver Diamine Fluoride, J. Dent. Res. 96(10) (2017) 1122-1128.

[28] L. Tjaderhane, M.A.R. Buzalaf, M. Carrilho, C. Chaussain, Matrix Metalloproteinases and Other Matrix Proteinases in Relation to Cariology: The Era of 'Dentin Degradomics', Caries Res. 49(3) (2015) 193-208.

[29] F.R. Tay, D.H. Pashley, Guided tissue remineralisation of partially demineralised human dentine, Biomaterials 29(8) (2008) 1127-1137.

[30] M.L. Mei, L. Ito, Y. Cao, Q.L. Li, C.H. Chu, E.C.M. Lo, The inhibitory effects of silver diamine fluorides on cysteine cathepsins, J. Dent. 42(3) (2014) 329-335.

[31] S.S. Gao, I.S. Zhao, S. Duffin, D. Duangthip, E.C.M. Lo, C.H. Chu, Revitalising Silver Nitrate for Caries Management, Int. J. Environ. Res. Public Health 15(1) (2018).

[32] G.M. Knight, J.M. McIntyre, G.G. Craig, Mulyani, P.S. Zilm, N.J. Gully, Inability to form a biofilm of Streptococcus mutans on silver fluoride- and potassium iodide-treated demineralized dentin, Quintessence Int. 40(2) (2009) 155-161.

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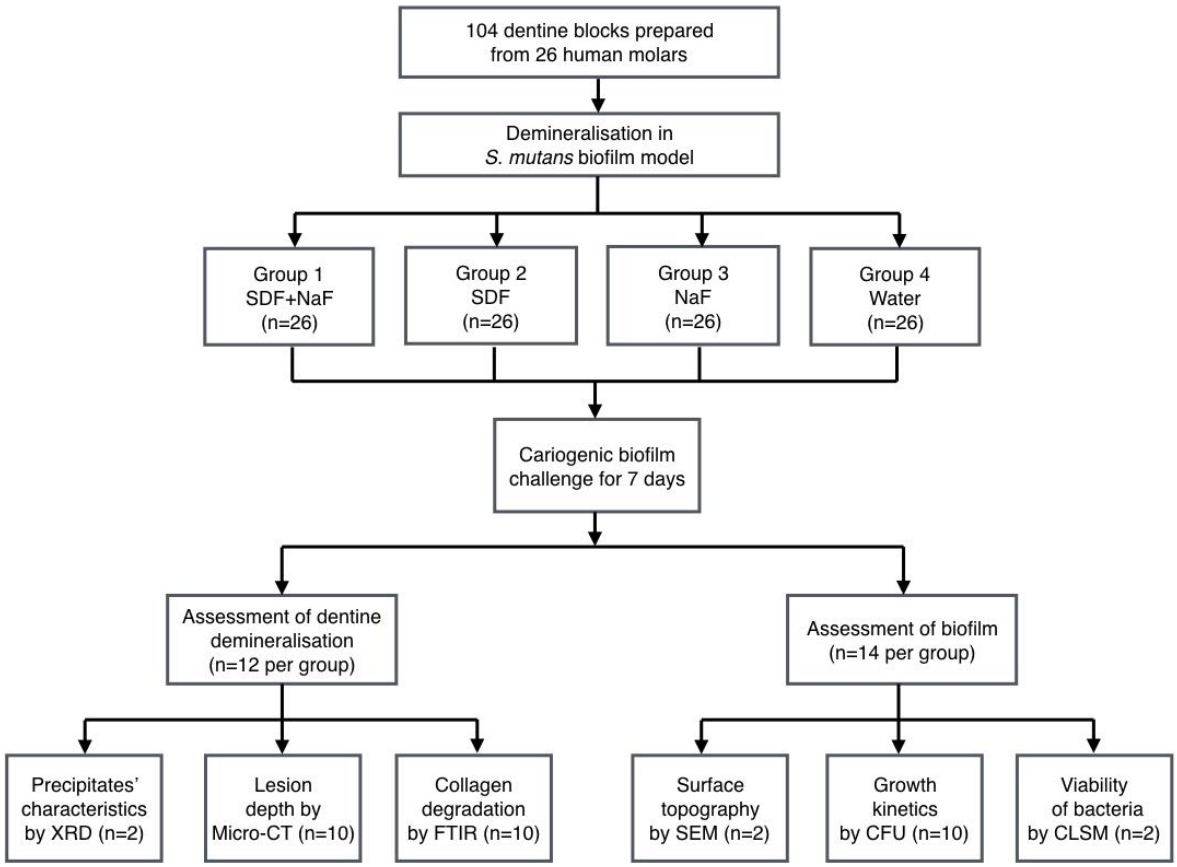

Figure 1 Flow chart of the study

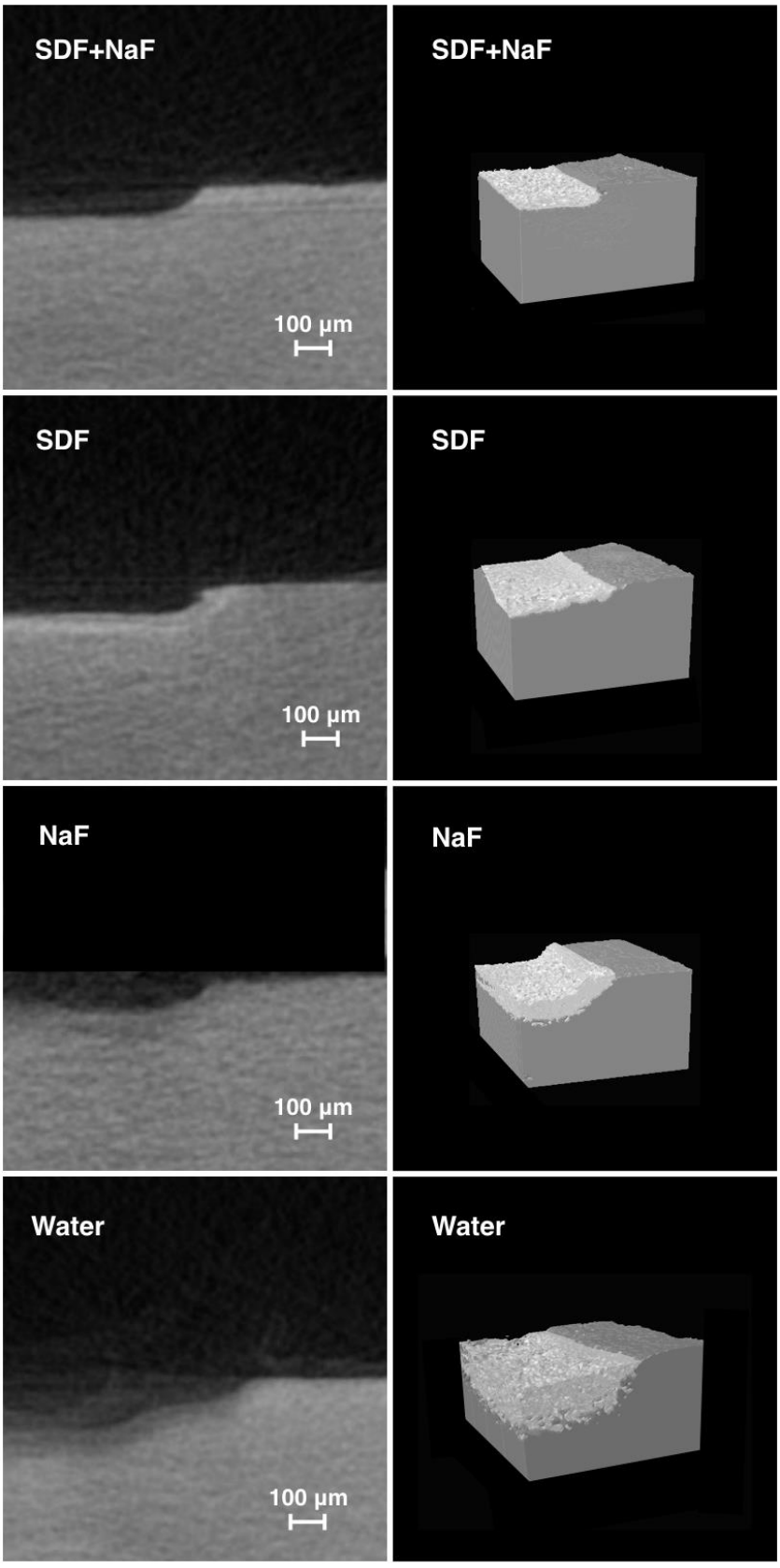

Figure 2 Representative micro-computed tomographs (images in the left column) and reconstructed three-dimensional images (images in the right column) of artificial dentine carious lesions of the treatment groups (SDF – 38% silver diamine fluoride solution, NaF – 5% sodium fluoride varnish

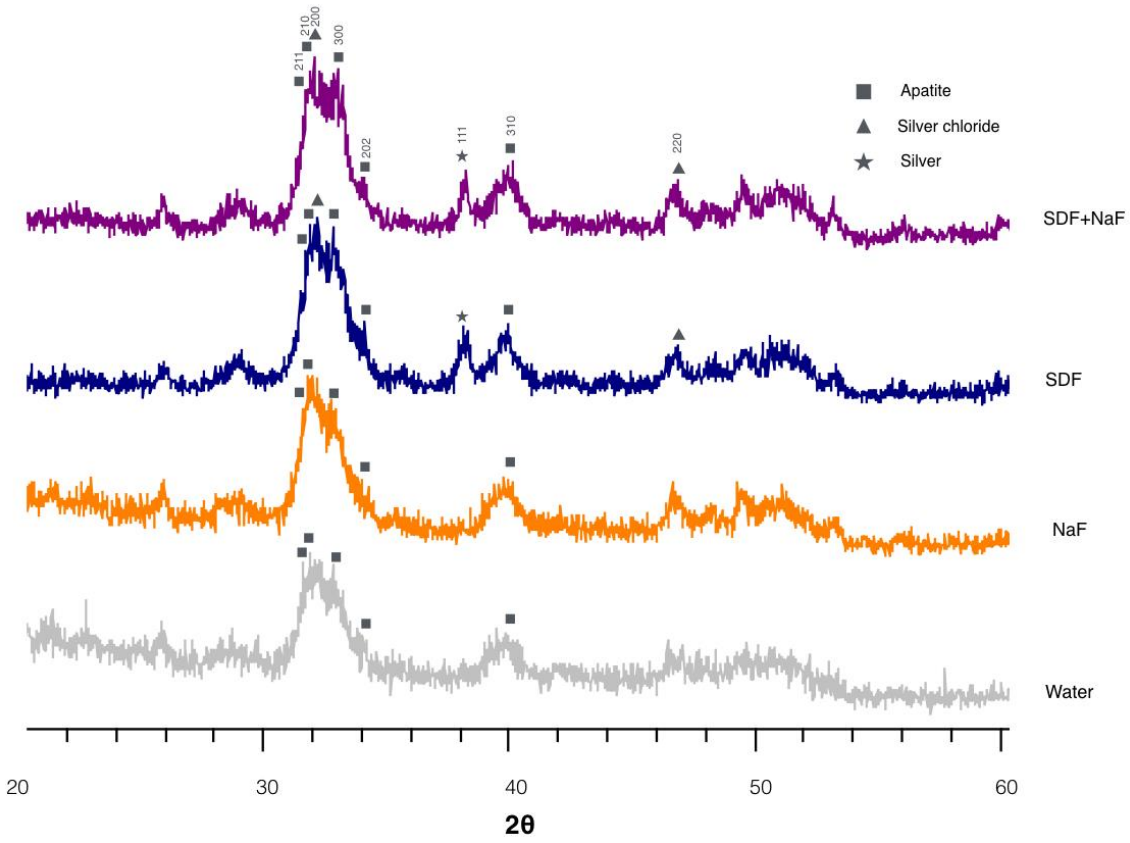

Figure 3 Typical X-ray diffraction patterns of the dentine in the four treatment groups

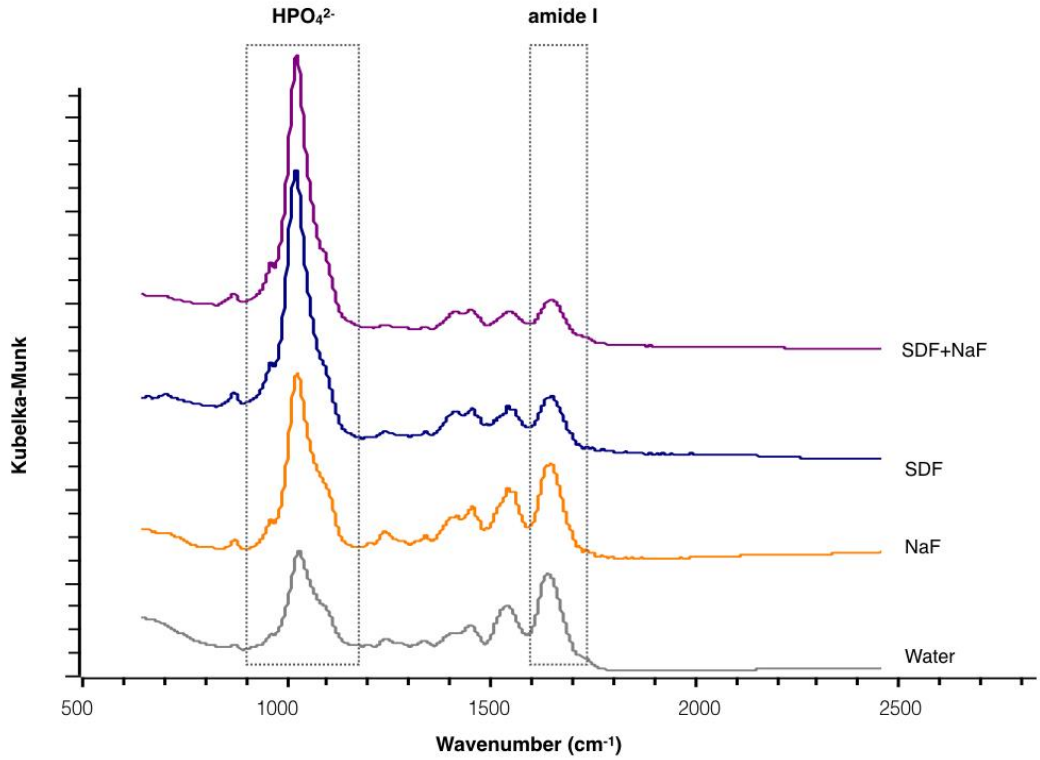

Figure 4 Typical Fourier transform infrared spectra of the artificial dentine caries lesions by treatment groups

The peaks corresponding to wavenumbers 1585–1720 cm^{-1} represent amide I absorbance, and the peaks between 900 and 1200 cm^{-1} represent HPO_4^{2-} absorbance.

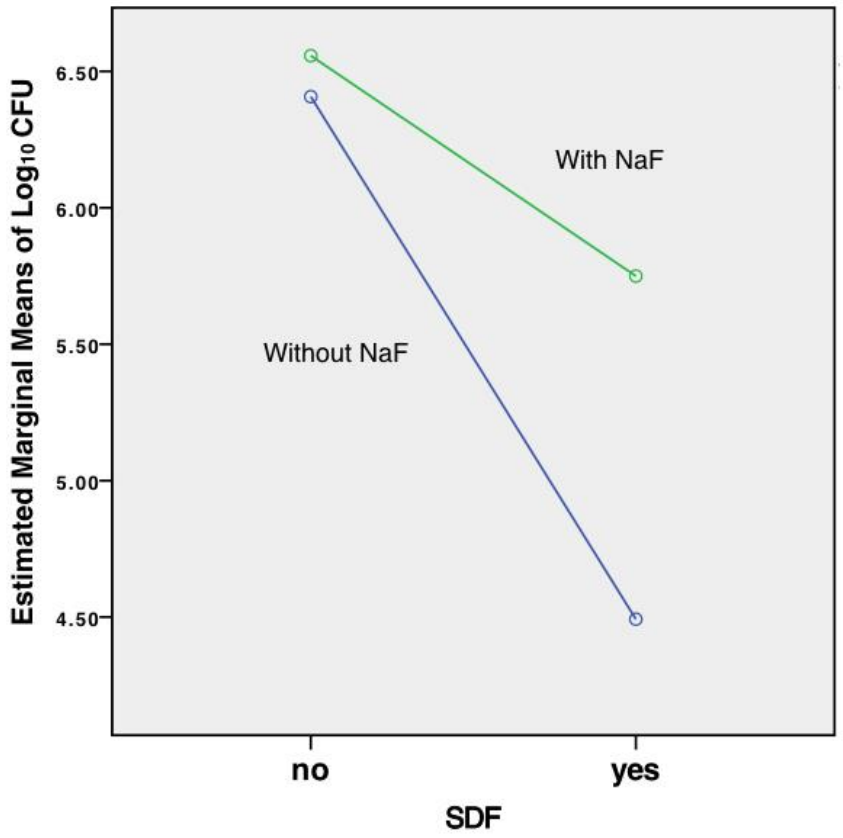

Figure 5 Effects of the SDF treatment and NaF treatment on Log_{10} CFU

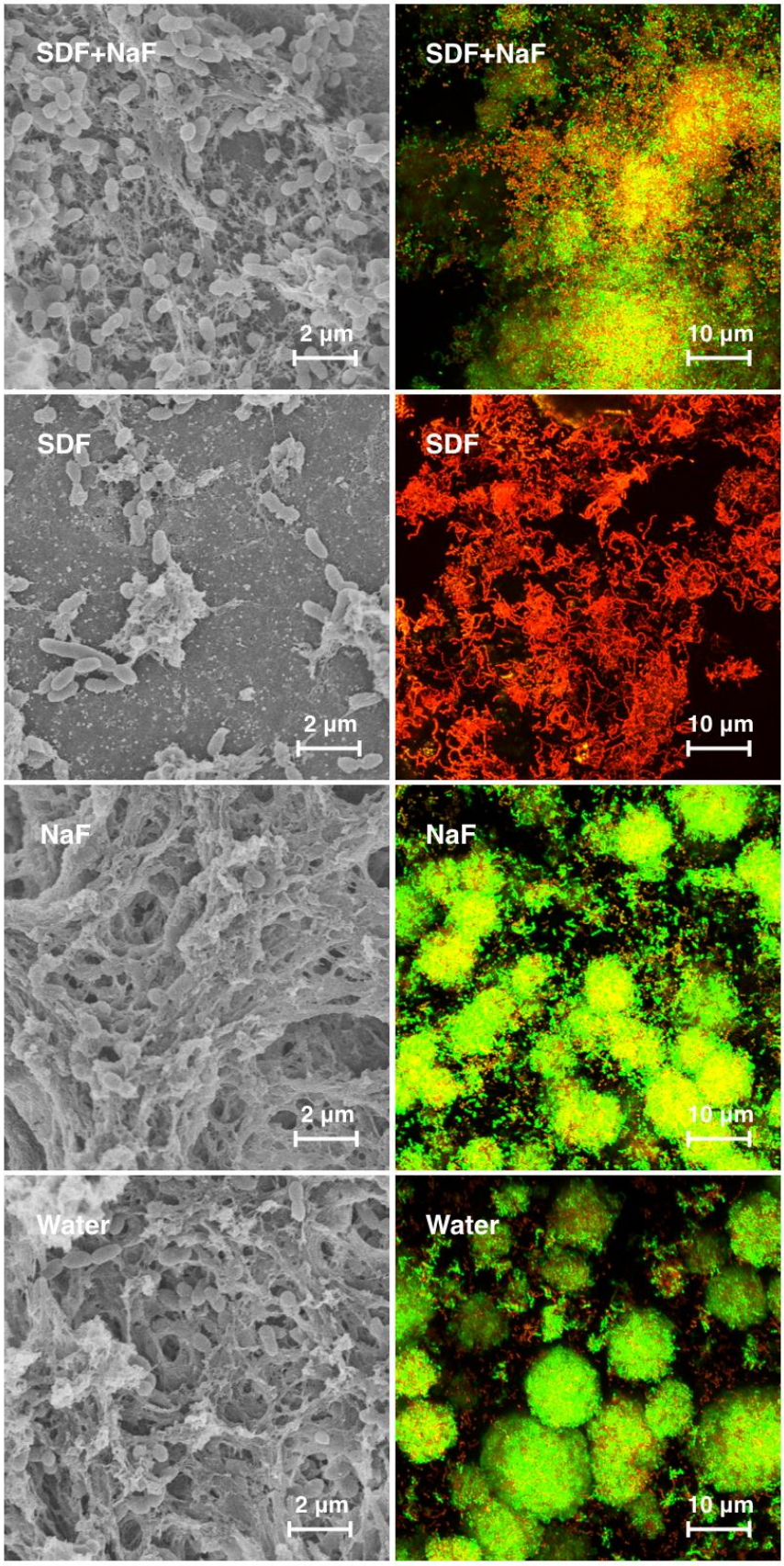

Figure 6 Typical scanning electron micrographs (images in the left column) of the biofilm topography, and typical confocal laser scanning microscopy images* (images in the right column) of the *Streptococcus mutans* biofilm of the four treatment groups

* Dead bacterial cells are marked red, and live cells are marked green (magnification x1000).