Micro-CT assessment of the effect of silver diammine fluoride on inhibition of root dentin demineralization

Miyuki SHIMIZU1, Naoko MATSUI1, Mahmoud SAYED1, Hidenori HAMBA1,2, Sho OBAYASHI1, Motoi TAKAHASHI1, Yuka TSUDA1, Tomohiro TAKAGAKI1,3, Toru NIKAIDO1,3 and Junji TAGAMI1

1 Department of Cariology and Operative Dentistry, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
2 Department of Operative Dentistry, Cariology and Pulp Biology, Tokyo Dental College, 2-9-18, Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan
3 Department of Operative Dentistry, Division of Oral Functional Science and Rehabilitation, School of Dentistry, Asahi University, 1851-1 Hozumi, Mizuhoku, Gifu 501-0296, Japan

Corresponding author, Naoko MATSUI; E-mail: Matsui.ope@gmail.com

This study evaluated the ability of different types of silver diammine fluoride (SDF) to inhibit dentin demineralization using micro-focused X-ray computed-tomography (µCT). Dentin specimens were divided into five groups (n=10): no-treatment (control), 3.8% SDF (RC), 38% SDF, 38% SDF with potassium iodide (SDF/KI), and potassium fluoride (KF). The treated-dentin surfaces were subjected to demineralization for 7-days and assessed using µCT to determine mineral loss (ML) values. Specimens were also analyzed with scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). The ML values of the SDF and KF groups were significantly lower than those of the RC and SDF/KI groups. EDS detected fluoride ions in the SDF and KF groups but not in the RC and SDF/KI groups. It was concluded that 38% SDF demonstrated a high ability to inhibit dentin demineralization while additional application of KI may diminish the inhibitory effect of SDF. The amount of dentin demineralization with SDF treatments was material dependent.

Keywords: SDF, Fluoride, Micro-CT, Dentin, Anti-demineralization

INTRODUCTION

In aging societies nowadays, a greater proportion of older people are retaining their natural teeth1. Gingival recession leads to exposure of root dentin which can increase the risk of root caries because dentin is more susceptible to demineralization at higher pH values. Therefore, in current dental practice, the prevention and treatment of root caries are becoming increasingly important. Preventive strategies are needed to manage the damage associated with caries, especially on root surfaces. Amongst the various strategies for caries prevention, fluoride therapy is widely accepted2. Topical fluoride agents have been extensively studied with regard to their anti-caries effects in dentin root surfaces. Fluoride treatments use a variety of agents such as NaF, acidulated phosphate fluoride (APF), and silver diammine fluoride (SDF); SDF was reported to be the most effective3,4. Therefore, SDF application is expected to play an important role for dentin remineralization and demineralization.

Since 2014, the FDA has approved SDF as it was proven to be a viable method to reduce caries progression5. However, SDF exhibits a black discoloration on exposure to light due to the formation of a silver oxide layer; this is a major esthetic concern despite its benefits6-8. Some methods to overcome the SDF discoloration have been reported, such as the use of a saturated solution of potassium iodide (KI) immediately after SDF application or the additon of a reducing agent such as Glutathione9,10.

Micro-focused X-ray computed tomography (µCT) imaging can capture three-dimensional (3D) architectural information from samples non-destructively11,12. µCT has been frequently used in experiments exploring mineral density and the structure of mineralized tissues, such as bone and teeth13-16. Mineral density (MD) assessment of teeth is important in demineralization and remineralization studies, providing an insight into the changes associated with spatial distribution of mineral within lesions. Thus, the use of µCT is a promising method for assessing demineralization or remineralization in enamel and dentin17-20.

Despite several studies investigating the efficacy of SDF, the understanding of how SDF exerts its preventive effect on root caries has not yet been established21. As mentioned above, studies have shown that SDF is effective in arresting and preventing dentin caries, however, there is little information about the effect of...
different types of SDF materials with regards to the inhibition of dentin demineralization. Thus, the purpose of this study was to evaluate the effect of different types of SDF-containing materials on the inhibition of dentin demineralization using µCT for the quantitative analysis of mineral density (MD). In addition, scanning electron microscopy (SEM) was used to observe material depositions on the surface and within the dentinal tubules.

MATERIALS AND METHODS

Materials
The materials used in this study are listed in Table 1. Three different types of SDF materials: Saforide 38% [SDF] and Saforide 3.8%•RC [RC] (Bee Brand Medico Dental, Osaka, Japan), and Riva Star [SDF/KI] (SDI, Bayswater, Australia). Riva Star is composed of 38% SDF (1st liquid) and a saturated solution of potassium iodide (KI) (2nd liquid). Additionally, potassium fluoride solution (FUJIFILM Wako Pure Chemical Industries, Osaka, Japan) was used as a positive control (KF).

Specimen preparation
Nine teeth (bovine incisors) were used in this study. Teeth were cut at the cemento–enamel junction and dentin specimens were obtained from the cervical portion of the root using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under running water. A total of 50 dentin blocks (3×5×3 mm) were embedded in epoxy resin (EpoxiCure 2, Buehler, Lake Bluff, IL, USA) then the dentin surfaces were ground flat till 1500-grit silicon carbide paper (Fuji Star, Sankyo Rikagaku, Saitama, Japan) under running water. Dentin specimens were randomly assigned into the five groups (n=10) according to material application, namely: control, 38% SDF (SDF), 3.8% SDF (RC), SDF/KI and KF.

Specimen surfaces were covered with nail varnish (CO11, Revlon, New York, NY, USA), leaving a window (2×4 mm) exposed, then the materials were applied to all specimens. The specimen preparations were performed at room temperature with the avoidance of strong light exposure.

Treatment protocol
38% SDF (SDF), 3.8% SDF (RC) and KF were applied on the dentin surface with a micro-brush for 10 s. SDF/KI was applied as 38% SDF (1st liquid) for 10 s, then immediately after KI (2nd liquid) was applied until a change of solution color from milky white to clear occurred. All specimens were left for 3 min after applying each material and rinsed for 10 s with copious amounts of distilled water, then air dried for 10 s using strong pressure from a dental air syringe. In the control specimens, the surface was left untreated.

Specimens were then immersed in 125 mL of a buffered demineralizing solution containing 2.2 mmol/L CaCl₂, 2.2 mmol/L NaH₂PO₄, and 50 mmol/L acetic acid adjusted to pH 4.5, then removed from the solution after 7 days demineralization, rinsed, after which µCT scanning was performed (Fig. 1).

µCT scanning
Each specimen was scanned twice with µCT, once before material application on dentin surface “as baseline measurement” and a second time after the demineralization challenge. The mineral density (MD) values of the specimens were evaluated using µCT (InspeXio SMX-100CT, Shimadzu, Kyoto, Japan). For each specimen, the same scanning parameters were applied as follows: 100 kV, 140 µA, 400 s, and 7.5 µm isotropic voxel dimensions. The specimens were rotated through 360° with 0.6° steps. The distances from the X-ray source to the detector and from the X-ray source to the specimen were 300 and 73.5 mm, respectively. A brass 0.2 mm-thick filter was positioned in the beam path to cut off low-energy X-rays, to decrease any beam-hardening effects. A MD calibration curve was obtained by scanning three reference phantoms: two hydroxyapatite disks (0.60 and 0.80 gHAp cm⁻³) (Phantoms, S/N-1001-39, Ratoc System Engineering, Tokyo, Japan) and a pure aluminum wire (0.9 mm diameter, 1.55 gHAp cm⁻³) which were scanned using the same setup and parameters as the tooth specimens. In order to avoid

Table 1  Materials used in the study

| Code | Brand Name | Composition                      | F (ppm)  | pH     | Manufacturer                     |
|------|------------|----------------------------------|----------|--------|----------------------------------|
| SDF  | Saforide38%| 38% Silver diamine fluoride      | 44,800   | 9–10   | Bee Brand Medico Dental, Osaka, Japan |
|      |            | F=44,880 ppm, Ag=253,870 ppm     |          |        |                                  |
| RC   | Saforide3.8%•RC | 3.8% Silver Diamine Fluoride | 4,480    | 9–10   | Bee Brand Medico Dental          |
|      |            | F=4,488 ppm, Ag=25,387 ppm       |          |        |                                  |
| SDF/KI| Riva Star | 1st liquid : 38% Silver Diamine Fluoride | 44,800   | 9–10   | SDI, Bayswater, Australia        |
|      |            | 2nd liquid : Potassium Iodide (KI) | —        | 7      |                                  |
| KF   | Potassium Fluoride | —                             | 48,000   | 7      | FUJIFILM Wako Pure Chemical Industries, Osaka, Japan |
dehydration of the specimens, scanning was performed under 100% humidity where the specimens were put in a vial with wet cotton.

To analyze µCT data, a 3D-digital object was reconstructed from 140 2D-images (16-bit), with 1,024×1,024 pixels resolution, using a program software (TRI/FCS-BON ver.10.01, DIF, TMD, Ratoc System Engineering). The computed tomography (CT) values were converted to MD (gHAp cm$^{-3}$) using a calibration curve derived from a linear regression ($R^2>0.999$) based on the phantom scans. The assessment was performed with a volume of interest (VOI) of 600×600×600 voxels at the center of the test window.$^{23}$

The MD values were calculated from the VOI every 7.5 µm isotropic voxel dimensions, and then the MD profile was plotted in relation to the lesion depth. The mineral loss (ML) (vol%µm) values in each group were calculated from the MD profile by subtracting the area under the curve for the treated-dentin from that of sound-dentin. The reference point of the depth axis (0 µm) in the mineral profile was set at the axial position of the sound dentin surface at the baseline. These calculations were performed by importing the MD data into a spreadsheet software package (Microsoft Excel 2016 for Windows, Microsoft, Redmond, WA, USA)$^{23}$.

Statistical analysis
Distribution of data was analyzed by the Shapiro-Wilk test. The mineral loss (ML) values calculated from the µCT analysis were analyzed by Kruskal Wallis test as a primary statistical test followed by Dunn’s test with Bonferroni correction for pairwise comparison. All the statistical tests were performed with a computerized statistical program (SPSS ver. 22.0, IBM, Chicago, IL, USA). The significance level of all the tests was set at $\alpha=0.05$.

RESULTS
µCT analysis
Typical two-dimensional (2D) images taken for the specimens before and after demineralization are shown in Fig. 2. The µCT images showed the effect of the demineralizing solution on the dentin surface in the different groups after 7-days demineralization (Figs. 2b–d). The SDF and KF groups had greater ability to protect the dentin surface by demonstrating shallower demineralization depths than the RC and SDF/KI groups. (Figs. 2c and d).

Mineral density profiles
Figure 3 shows the MD profile of demineralized dentin in each experimental group. The baseline profile was measured before exposure to the demineralization solution. The MD profiles of all groups shifted to the right side after demineralization, demonstrating mineral loss
on the dentin surface. The further right shift of profile exhibited more demineralization. The no-treatment group revealed the highest mineral loss pattern within all experimental groups. In the SDF/KI and RC groups, the MD profiles showed similar patterns of mineral loss, however, the RC group revealed a greater mineral loss than the SDF/KI group. Both SDF and KF groups showed the least percentage of mineral loss compared with the other groups. The profile pattern of the SDF group exhibited remarkably less demineralization by demonstrating a peak on the mineral density axis near the surface, whereas the RC and SDF/KI groups showed high mineral loss near the surface through demonstration of a relatively straight MD profile with no peaks. In the KF group, the MD profiles showed an increase in the mineral volume especially at a depth of 50 µm.

**Mineral loss**
The ML values for each group were calculated from the MD profiles and are shown in Table 2. The no treatment group (control) demonstrated the greatest mineral loss, followed by RC, SDF/KI, SDF and KF. However, there were no significant differences in ML between RC and SDF/KI groups. Also, there were no significant differences between SDF and KF groups (p<0.05). The SDF and KF groups showed significantly less ML than the no treatment and RC groups (p<0.05).

**SEM/EDS observation**
The SEM images of the dentin surfaces of each group before and after demineralization are shown in Fig. 4. In the no treatment group, a smear layer was observed on the surface before demineralization (Fig. 4a), however, it was removed after demineralization, and the dentinal tubules were opened (Fig. 4b). In the RC group, the dentin surface showed less deposition of silver crystals than SDF before demineralization (Fig. 4c), and the surface depositions on the dentin decreased further after demineralization (Fig. 4d). Similarly, the SDF/KI group showed the same surface morphology as the
RC group before demineralization (Fig. 4e) and after demineralization (Fig. 4f). In the SDF group, multiple crystal deposits were observed on the dentin surface before demineralization (Fig. 4g) and after demineralization (Fig. 4h), and inhibition of demineralization was evident (Fig. 4h). In the KF group, crystal deposits were not observed before demineralization (Fig. 4i) or after demineralization (Fig. 4j). However, some surface deposition was observed resulting in partial occlusion of the dentinal tubules before and after demineralization compared with the no-treatment group. Figure 5 shows the cross-sectional SEM images of dentin specimens after demineralization. The no-treatment group showed funnel-shaped openings of the dentinal tubules (Fig. 5a). The SDF/KI group showed small crystal deposits within the tubules compared to the SDF group (Fig. 5b). The SDF group showed multiple crystal deposits (0.3–1.5 µm) that occluded the dentin tubules and increased inhibition of demineralization (Fig. 5c). The KF group showed narrowing of dentinal tubules which may be related to the precipitates inside the dentinal tubules, enhancing the inhibition of demineralization (Fig. 5d).

Fig. 4 SEM micrographs of the dentin surface of each treatment group before and after demineralization; No treatment (a and b), RC (c and d), SDF/KI (e and f), SDF (g and h), KF (i and j) (×5,000). Arrow refers to silver crystals.

Fig. 5 Cross-sectional SEM images of the dentin specimen for each group before and after application; No treatment (a and b), RC (c and d), SDF/KI (e and f), SDF (g and h), KF (i and j) (×5,000). Arrow refers to silver crystals.
Typical EDS analysis results of the dentin surface after application of the solutions are shown in Fig. 6. The no treatment group showed obvious peaks of carbon (C) and oxygen (O) but no F peak (Fig. 6a). F peak at around 0.7 KeV was detected in the SDF group along with C and O peaks (Fig. 6b), however, the SDF/KI group showed only peaks of C and O. The RC group showed the similar pattern with the SDF/KI group (not shown in Fig. 6). Also, the KF group showed high peaks of O, C and F, the same as the SDF group (not shown in Fig. 6). In this study, Ag peak was difficult to detect due to the low voltage operating condition (5 kV), while the power condition increased the ability to detect F ions which is more relevant to this study.

DISCUSSION

Several concentrations of SDF have been investigated in relation to dental tissue reactions. SDF is a metal ammine complex of silver fluoride, which contains a high amount of fluoride and silver ions. When hydroxyapatite reacts with SDF, calcium fluoride (CaF₂), silver phosphate and ammonium hydroxide are produced. SDF can increase the microhardness of existing caries lesions and promote remineralization. In addition, the silver diammine component is reported to inhibit caries formation via inhibition of cysteine cathepsins, matrix metalloproteinases (MMP) and binding to collagen.

Saforide® contains 38% SDF, which has been widely accepted for prevention of primary caries in pediatric dentistry. Saforide RC® contains 3.8% SDF, which was developed as a root canal irrigant or inter-appointment dressing during endodontic treatment because of its capability to penetrate into root canal dentin as well as its antimicrobial activity. However, SDF application is often clinically limited due to the formation of dark stains within tooth structure caused by silver deposition, that can lead to esthetic concerns. Riva Star® is composed of two liquids: 38% SDF and potassium iodide (KI). Potassium iodide was introduced in an attempt to solve the color change problem associated with SDF application, silver iodide (AgI) crystals are formed and precipitate on the surface as a creamy white precipitate, which can reduce the amount of free silver ions that form AgI and discolor. Also, a high concentration of potassium fluoride at 48,800 ppm F adjusted at pH 7.0 (KF) was prepared and used as a comparative material to 38% SDF according to work from a previous study.

The effect of the different types of SDF materials on inhibition of root dentin demineralization was evaluated using µCT. From the MD profile and ML values (Fig. 3 and Table 2), the demineralization depth in the no treatment group was around 200 µm, while the MD profiles of the treatment groups (38% SDF, 3.8% SDF, SDF/KI and KF) indicated inhibition of root dentin demineralization, however, the effect was material dependent. The SDF and KF groups demonstrated the greatest effect to prevent demineralization compared with RC and SDF/KI, because of a higher concentration of fluoride (44,880 ppm) of each material. Previous studies evaluated the prevention of demineralization efficacy by topical application of fluoride agents on root dentin. It was reported that 38% SDF and KF (48,000 ppm F) had equivalent efficacy in inhibition of dentin demineralization. Interestingly, the current results showed that the KF group presented a MD profile pattern different from that of SDF, showing an increase of the mineral volume up to a depth of 50 µm after the demineralization cycle and featured a subsurface layer. On the other hand, a highly opaque superficial layer was observed in the SDF group (Fig. 2c), which may be due to deposition of mineral and more likely silver compounds which may enhance the inhibition against dentin demineralization. On the other hand, the RC and SDF/KI groups had poor effects to prevent demineralization. The SDF concentration of RC is one-tenth that of 38% SDF. The SDF/KI group had an equivalent fluoride concentration as the SDF group, however, the prevention of demineralization by SDF/KI was almost same as that of RC.

The representative SEM micrographs indicated morphological differences of the dentin surfaces after the
mended. The agitation process may reduce the subsequent application of KI, resulting in formation of silver crystals. Sayed et al. observed silver crystals on the dentin surface via SEM analysis and also showed crystal deposition occurred dentinal tubule orifices after 38% SDF application. On the other hand, the RC group showed smaller amounts of accumulated crystals than the SDF group. It was previously reported that deposition of Ag on the dentin surface treated with 38% SDF was higher than that with 3.8% SDF, suggesting an increase in acid resistance with more silver precipitates present. The SDF/KI group demonstrated a completely different morphology from the SDF group. The SEM images of the SDF+KI group exhibited silver crystals on the dentin surface and also in the dentinal tubules but were smaller and less in number than the SDF group. In the instructions of the SDF/KI group, the initial application of SDF may be agitated with the subsequent application of KI, resulting in formation of AgI. However, the agitation process may reduce the Ag and F ions on the dentin surface. Therefore, the additional application of the KI solution might adversely affect the demineralization efficacy of SDF. On the other hand, crystal formation on the dentin surface was not observed in the KP group both before and after the demineralization procedure. However, partially occluded dentinal tubule orifices and the narrowing of the dentinal tubules were observed, which can explain the high anti-demineralization efficacy of the KP group.

The current EDS analysis of the dentin surfaces showed that fluorine atoms were detected in the SDF group, but not in the SDF/KI group. This fact indicated that few fluoride ions remained on the dentin surface treated with the SDF/KI group. For the SDF group, CaF$_2$ may be formed on the dentin surface. For, the SDF/KI group, CaF$_2$ formed at the first application of SDF may be removed mechanically, when rubbing the dentin surface with the micro-brush during the application of the KI solution. Finally, the demineralization preventive effect of the 38% SDF/KI group was greatly reduced.

Although it was mentioned in a previous study that a considerable amount of silver ions might be removed from the surface after rinsing, still the effect of silver ion deposition into dentin and its relation to the mineral density should be investigated in future studies.

Based on the current results, inhibition of dentin demineralization was attributed to fluoride ions, and Ag ions may reinforce the prevention of demineralization efficacy physically; however, such efficacy was dependent on the concentration of SDF. It was strongly suggested that 38% SDF is the most effective in prevention of root caries among the SDF-containing materials tested, while the demineralization prevention efficacy was weakened by the additional application of KI despite it solving the problem of tooth discoloration.

**CONCLUSION**

Within the limitations of the current research, the following conclusion was achieved:

The degree of root dentin demineralization inhibition with SDF was material dependent. 38% SDF demonstrated the strongest capability to inhibit root dentin demineralization. However, the additional application of KI may diminish the inhibitory effect of SDF.

**ACKNOWLEDGMENTS**

This research was supported by the Japan Society for the Promotion of sciences (16H05515).

**REFERENCES**

1) Fure S, Zickert I. Prevalence of root surface caries in 55, 65, and 75-year-old Swedish individuals. Community Dent Oral Epidemiol 1990; 18: 100-105.
2) Walls AW, Meurman JH. Approaches to caries prevention and therapy in the elderly. Adv Dent Res 2012; 24: 36-40.
3) Tan HP, Lo EC, Dyson JE, Luo Y, Corbet EF. A randomized trial on root caries prevention in elders. J Dent Res 2010; 89: 1086-1090.
4) Yee R, Holmgren C, Mulder J, Lampa D, Walker D, van Palenstein Helderman W. Efficacy of silver diamine fluoride for arresting caries treatment. J Dent Res 2009; 88: 644-647.
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) Mazzoni A, Tjaderhane L, Checchi V, Do Lenarda R, Salo T, Tay FR, et al. Role of dentin MMP's in caries progression and bond stability. J Dent Res 2015; 94: 241-251.
7) Mei ML, Ito L, Cao Y, Li QL, Lo EC, Chu CH. Inhibitory effect of silver diamine fluoride on dentin demineralization and collagen degradation. J Dent 2013; 41: 809-817.
8) Thanatvarakorn O, Islam MS, Nakakshima 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: 769-775.
9) Rosenblatt A, Stamford TC, Niederman R. Silver diamine fluoride: a caries "silver-fluoride bullet". J Dent Res 2009; 88: 116-125.
10) Chu CH, Lo EC, Lin HC. Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children. J Dent Res 2002; 81: 767-770.
11) Sayed M, Matsui N, Hiraishi N, Nikaido T, Burrow MF, Tagami J. Effect of glutathione bio-molecule on tooth discoloration associated with silver diamine fluoride. Int J Mol Sci 2018; 19: 1392.
12) Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. Inability to form a biofilm of Streptococcus mutans on silver fluoride- and potassium iodide-treated demineralized dentin. Quintessence Int 2009; 40: 155-161.
13) Elliott JC, Dover SD. X-ray microtomography. J Microsc 1982; 126: 211-213.
14) Stock SR, Barss J, Dahl T, Veis A, Almer JD. X-ray absorption
microtomography (microCT) and small beam diffraction mapping of sea urchin teeth. J Struct Biol 2002; 139: 1-12.

15) Stock SR, Vieira AE, Delbem AC, Cannon ML, Xiao X, Carlo FD. Synchrotron micro-computed tomography of the mature bovine dentin-enameel junction. J Struct Biol 2008; 161: 129-132.

16) Zhou XZ, Zhang G, Dong QR, Chan CW, Liu CF, Qin L. Low-dose X-irradiation promotes mineralization of fracture callus in a rat model. Arch Orthop Trauma Surg 2009; 129: 1179-1184.

17) Kinney JH, Balooch M, Haupt DL Jr, Marshall SJ, Marshall GW Jr. Mineral distribution and dimensional changes in human dentin during demineralization. J Dent Res 1995; 74: 1179-1184.

18) Anderson P, Elliott JC, Bose U, Jones SJ. A comparison of the mineral content of enamel and dentine in human premolars and enamel pearls measured by X-ray microtomography. Arch Oral Biol 1996; 41: 281-290.

19) Songsiripradubboon S, Hamba H, Trairatvorakul C, Tagami J. Sodium fluoride mouthrinse used twice daily increased incipient caries lesion remineralization in an in situ model. J Dent 2014; 42: 271-278.

20) Hamba H, Nikaido T, Inoue G, Sadr A, Tagami J. Effects of CPP-ACP with sodium fluoride on inhibition of bovine enamel demineralization: a quantitative assessment using microcomputed tomography. J Dent 2011; 39: 405-413.

21) Peng JJ, Botelho MG, Matinlinna JP. Silver compounds used in dentistry for caries management: a review. J Dent 2012; 40: 531-541.

22) Nakamura K, Hamba H, Nakashima S, Sadr A, Nikaido T, Oikawa M, et al. Effects of experimental pastes containing surface pre-reacted glass ionomer fillers on inhibition of enamel demineralization. Dent Mater J 2017; 36: 482-490.

23) Zan KW, Nakamura K, Hamba H, Sadr A, Nikaido T, Tagami J. Micro-computed tomography assessment of root dentin around fluoride-releasing restorations after demineralization/ remineralization. Eur J Oral Sci 2018; 126: 390-399.

24) Hamba H, Nikaido T, Sadr A, Nakashima S, Tagami J. Enamel lesion parameter correlations between polychromatic micro-CT and TMR. J Dent Res 2012; 91: 586-591.

25) Chu CH, Lo EC. Microhardness of dentine in primary teeth after topical fluoride applications. J Dent 2008; 36: 387-391.

26) Mei ML, Ito L, Cao Y, Li QL, Chu CH, Lo EC. The inhibitory effects of silver diamine fluorides on cysteine cathepsins. J Dent 2014; 42: 329-335.

27) Mei ML, Li QL, Chu CH, Yiu CK, Lo EC. The inhibitory effects of silver diamine fluoride at different concentrations on matrix metalloproteinases. Dent Mater 2012; 28: 903-908.

28) Llodra JC, Rodriguez A, Ferrer B, Menardia V, Ramos T, Morato M. Efficacy of silver diamine fluoride for caries reduction in primary teeth and first permanent molars of schoolchildren: 36-month clinical trial. J Dent Res 2005; 84: 721-724.

29) Hiraishi N, Yiu CK, King NM, Tagami J, Tay FR. Antimicrobial efficacy of 3.8% silver diamine fluoride and its effect on root dentin. J Endod 2010; 36: 1026-1029.

30) Savas S, Kucukylmaz E, Celik EU, Ates M. Effects of different antibacterial agents on enamel in a biofilm caries model. J Oral Sci 2015; 57: 367-372.

31) Nguyen V, Neill C, Felsenfeld J, Primus C. Potassium iodide. The solution to silver diamine fluoride discoloration? Adv Dent Oral Health 2017; 5: 555655.

32) Craig GG, Knight GM, McIntyre JM. Clinical evaluation of diamine silver fluoride/potassium iodide as a dentine desensitizing agent. A pilot study. Aust Dent J 2012; 57: 308-311.

33) Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. Differences between normal and demineralized dentine pretreated with silver fluoride and potassium iodide after an in vitro challenge by Streptococcus mutans. Aust Dent J 2007; 52: 16-21.

34) Mei ML, Ito L, Cao Y, Lo EC, Li QL, Chu CH. An ex vivo study of arrested primary teeth caries with silver diamine fluoride therapy. J Dent 2014; 42: 395-402.

35) Zander V, Chan D, Sadr A. Microcomputed tomography evaluation of root dentin caries prevention by topical fluorides and potassium iodide. Sensors (Basel) 2019; 19: 874.

36) Sayed M, Matsui N, Hiraishi N, Inoue G, Nikaido T, Burrow MF, et al. Evaluation of discoloration of sound/demineralized root dentin with silver diamine fluoride: In-vitro study. Dent Mater J 2019; 38: 143-149.

37) Sayed M, Matsui N, Okumura S, Nikaido T, Burrow MF, et al. Morphological and elemental analysis of silver penetration into sound/demineralized dentin after SDF application. Dent Mater J 2019; 35: 1718-1727.

38) Ko AK, Matsui N, Nakamoto A, Ikeda M, Nikaido T, Burrow MF, et al. Effect of silver diammine fluoride application on dentin bonding performance. Dent Mater J 2020; 39: 407-414.