Effect of Silver Fluoride in Preventing the Formation of Artificial Dentinal Caries Lesions in vitro

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Objective: *To investigate the effect of silver fluoride in preventing the development of artificial caries lesions in root dentine using an artificial mouth system (AMS).*

Methods: *A total of 34 extracted intact human premolars were embedded individually in blocks with one root dentine surface exposed. Among these, 32 were randomly divided into four groups (eight each) and the remaining two tooth blocks were used in the baseline evaluation of the cariogenic biofilm after bacterial inoculation. The interventions (topical application of 2.36 M solutions) were applied after inoculation as follows: group-1, silver fluoride (AgF); group-2, potassium fluoride (KF); group-3, silver nitrate ($AgNO_3$); and group-4, deionised water (control). Subsequently, the tooth blocks were transferred into the AMS to start an artificial caries challenge (5% sucrose was delivered three times per day). After 10 days, the formed biofilm was assessed via colony forming unit (CFU) counts, confocal laser scanning microscopy, and scanning electron microscopy. The artificial dentinal caries lesion was evaluated using micro-computed tomography.*

Results: *After the interventions and the 10-day challenge in the AMS, the median CFUs ($AgF < AgNO_3 < KF = control$, $P < 0.001$) and the median live-to-dead bacteria ratios ($AgF < AgNO_3 < KF = control$, $P < 0.005$) of the biofilm differentiated significantly among the groups. Scattered bacterial cells were found in the tooth blocks of the AgF and $AgNO_3$ groups, while a confluent biofilm layer was observed in the tooth blocks of the KF and control groups. The median lesion depth in the AgF group was significantly lower than in the $AgNO_3$ ($P = 0.016$), KF ($P = 0.016$) and control ($P = 0.009$) groups.*

Conclusion: *The combined use of silver and fluoride ions in the AgF solution significantly protected dentine against the development of caries lesions.*

Key words: *silver fluoride, dental biofilm, artificial mouth system, dentine caries lesion, prevention*

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The silver diamine fluoride (SDF) solution, considered the 'silver-fluoride bullet' in the management of dental caries[1], is an important tool for achieving the World Health Organisation (WHO) Millennium Goal for oral health. However, it has some disadvantages such as containing high concentrations of fluoride, having unpleasant metallic taste and causing black staining of the teeth, which are major drawbacks to its use. A clear understanding of the role that each ion (F^- and Ag^+) plays in the SDF solution is crucial in the management of dental caries. Previous studies reported that fluoride ions can increase the acid resistance of dentine, and that enamel CaF_2 concentrations are highly correlated with reduced demineralisation[2-8].

Although still controversial, it has been suggested that fluoride has a direct effect on oral bacteria, and this plays a role in the prevention of caries disease[9-10]. However, fluoride is required at a rather high concentration. A recent study[11] used an artificial mouth model

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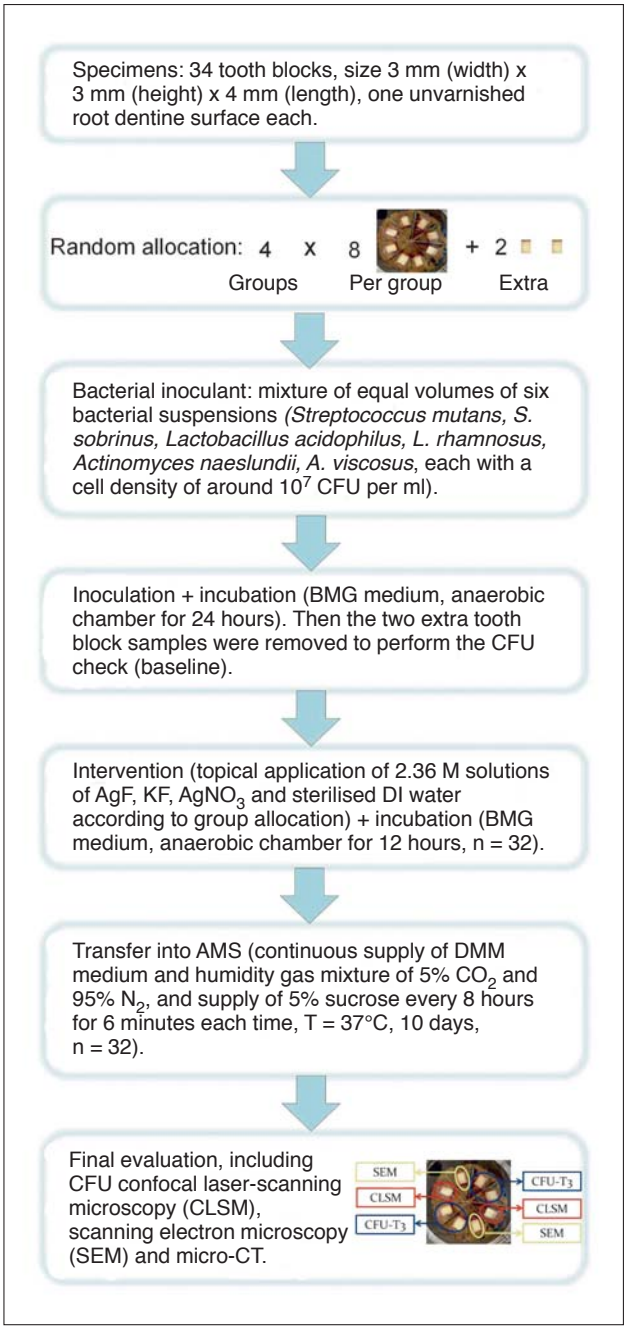

Fig 1 Flow chart of the experimental procedures used in the present study. 34 sterilised tooth blocks with an unvarnished root dentine surface were prepared from extracted sound human premolars. They were randomly allocated into four groups (eight each) to receive different intervention (topical application of 2.36 M solutions): AgF, KF, $AgNO_3$ and deionised water (control). All tooth blocks were then transferred into the artificial mouth system (AMS) to induce artificial caries. The biofilm formed was evaluated via CFU counts, CLSM, and SEM and the dentine artificial caries lesions were evaluated using micro-CT to compare the role of the different ions in the intervention solutions.

and found that fluoride-containing mouthrinse demonstrated no inhibitory effect against a cariogenic biofilm containing four putative root-caries pathogens.

Regarding the effect of silver ions on the dental hard tissue, a previous study observed that silver ions did not play any significant role in the prevention of enamel demineralisation[12], and there was no evidence that they played an independent or synergistic effect in promoting the remineralisation of caries lesions[13].

The antimicrobial activity of SDF against single-, dual- or multi-species cariogenic biofilms has been postulated to be mainly due to the silver ions[14-16]. However, it is still not clear whether the fluoride ions in the SDF solution provide significant, independent or synergistic, effect against biofilms, which improves caries prevention.

Furthermore, the damaging potential of another solution containing silver ($AgNO_3$) to the tooth hard tissue should be taken into account when formulating solutions combining both silver with fluoride ions; however, studies addressing this issue are still scarce in the literature. The potential chemical reactions of $AgNO_3$ with the tooth mineral hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) suggests that it may be used as an agent to enhance dentine demineralisation[7]. The following chemical reaction has been suggested:

$$Ca_{10}(PO_4)_6(OH)_2 + 20AgNO_3 \rightarrow 6Ag_3PO_4 + 10Ca(NO_3)_2 + Ag_2O + H_2O$$

Ag_3PO_4 and Ag_2O may react with alkali chlorides to precipitate silver chloride (AgCl), which may promote the reaction to occur from the left to the right direction. $Ca(NO_3)_2$ has been described to be soluble in saliva once formed, leading to the loss of calcium from the dentine (same effect as demineralisation). This initial demineralisation allows the solution to penetrate deeper into the dentine and cause further demineralisation, as described by a pilot research study[17].

Clearly, the specific independent or combined effect of the two ions (F^- and Ag^+) in the SDF solution in the prevention of dental caries has not yet been fully understood. As previously suggested, the benefit of using silver with fluoride together should be further examined, but meanwhile, silver and fluoride should be used separately[7].

The specific objective of this study was to describe the relative contribution of silver and fluoride ions in the prevention of artificial caries lesions.

Materials and methods

This is an in vitro, randomised, controlled study conducted in an artificial mouth model designed to compare three intervention groups – using testing solutions applied topically containing different combinations of F^- and Ag^+ ions (AgF, $AgNO_3$ and KF) and a negative control group (deionised water) – on the ability of F^- and Ag^+ ions, alone or in combination, to prevent caries lesions in prepared root dentine blocks. The ethical approval for the extracted tooth collection was obtained from the Institutional Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB).

Study procedures

The design and flow chart of the experimental procedures are summarised in Figure 1 and described below.

Tooth collection

Intact premolars extracted due to orthodontic reasons were selected at the Faculty of Dentistry, University of Hong Kong. The patient's informed consent was obtained before the collection of the teeth. The teeth were placed in a 6% sodium hypochlorite solution for 24 hours after extraction, and all soft tissues, stains and plaque were removed. They were then stored in a 0.5% thymol solution at 4°C.

Tooth block preparation

Tooth sectioning was performed by using a copper cutting disc mounted on a home-made microtome cutting machine. Firstly, the premolars were sectioned transversely at the level of the crown-root junction and parallel to this at 3 mm towards the apical direction. The root trunks in between these two cuts were used to prepare the specimen. Secondly, thin layers of sections were cut off from the mesial or distal surfaces of the root trunks to expose fresh root dentine surfaces. Thirdly, the root trunks were then cut into tooth blocks of 4 mm × 3 mm × 3 mm. Surfaces of size 4 mm × 3 mm from each tooth block were stored to be used at a later stage. These surfaces were examined using a stereomicroscope (10X magnification). The targeted surface of the tooth blocks containing cracks, hypoplasia or other development defect were excluded. The targeted fresh dentine surfaces were left exposed while all other surfaces of the prepared tooth blocks were coated with a red-coloured acid-resistant nail varnish (Nicole 044, O.P.I. Products, Hollywood, USA). In total, 34 tooth blocks were prepared.

The prepared tooth blocks were randomly divided into four groups, with eight blocks being allocated per group; the two remaining blocks were used for the baseline evaluation of the cariogenic biofilm. The 32 tooth blocks, from the four groups, were embedded into four plastic discs made from epoxy resin according to their group allocation. The exposed dentine surfaces of the tooth blocks were left exposed during and after the embedding. An even distribution of the eight tooth blocks in each plastic disc was achieved. The size of the resin disc fitted that of the glass holder in the artificial mouth system (AMS). To assist evaluations during the later experimental steps, the embedded tooth blocks in each plastic disc were separated by equal sections of the plastic disc. The sectors (plastic section with tooth block embedded) were then spliced back by sticking them onto one plastic coverslip of the same size as the plastic disc. This was to recover their shape and size to allow the plastic disc to be put into the glass holder of the AMS. The two extra tooth blocks were also embedded and separated into sectors without being spliced back. The prepared tooth blocks were then sterilised by autoclave before the interventions were performed.

Bacterial inoculation

A bacterial mixture composed of six cariogenic species (*Streptococcus mutans*, *S. sobrinus*, *Lactobacillus acidophilus*, *L. rhamnosus*, *Actinomyces naeslundii*, *A. viscosus*) was prepared[18]. A single colony of each bacterial species was picked from a 48-hour blood agar plate culture and transferred separately into a centrifuge tube containing 10 ml of basal mineral glucose (BMG) medium (ThermoFisher Scientific, Waltham, USA) supplemented with 5% sucrose. They were then incubated under anaerobic conditions for 24 hours at 37°C. Bacterial pellets were obtained by centrifuging the tubes and washing cells twice with phosphate buffered saline (PBS) (ThermoFisher Scientific). A bacterial suspension of a cell density of around 10^7/ml was prepared in BMG broth for each bacterial species. Equal volumes from each of the six bacterial suspensions were then mixed together to obtain the bacterial inoculant.

The four sterilised plastic discs with tooth blocks embedded were carefully put into a 6-well microplate separately by using sterilised tweezers. The discs were positioned with the exposed dentine surfaces faced up. The extra two tooth blocks embedded in resin sections were put into another well. Using a syringe, 3 ml of inoculant was added onto the exposed dentine surface

of each tooth block. The resin discs were then transferred to a new microplate and 6 ml of BMG broth were gently added to each well. The microplates were then placed under anaerobic conditions for 24 hours at 37°C.

Intervention and artificial mouth simulation

After the 24-hour incubation, the inoculated microplates were removed from the anaerobic chamber. The extra two tooth blocks were also removed from the anaerobic chamber for the baseline evaluation of the cariogenic biofilm. The four sterilised plastic discs with tooth blocks embedded were transferred carefully into a new microplate. Sterilised paper discs were used to absorb the extra broth solution on the resin discs without disturbing the biofilm. The intervention solutions were then topically applied on each tooth block according to their group allocation.

The four solutions (prepared freshly by using sterilised deionised water) were as follows: group-1, 2.36 M (mole/l) silver nitrate ($AgNO_3$) (Sigma-Aldrich, St. Louis, MO, USA); group-2, 2.36 M silver fluoride (AgF) (Fluka, Pittsburgh, USA); group-3, 2.36 M potassium fluoride (KF) (Sigma-Aldrich); and group-4, control (deionised water). The concentrations of silver ions in the AgF and $AgNO_3$ solutions were the same, and the concentrations of fluoride ions in the AgF and KF solutions were also the same. A concentration of 2.36 M of a commercial product with SDF (Saforide, Toyo Seiyaku Kasei, Osaka, Japan) was used according to previous clinical studies. The same molar concentration was used for all the solutions to ensure the study groups were comparable.

Using a micropipette, 5 µl of the respective intervention solution was pipetted on each exposed dentine surface. One minute after the intervention solution was applied on the dentine surfaces, the plastic discs with the tooth blocks embedded were transferred to another new 6-well microplate and 6 ml of BMG broth was added to each well; the microplate was then incubated in an anaerobic chamber for 12 hours at 37°C. Subsequently, the plastic discs with the tooth blocks embedded were transferred carefully into four glass holders located in four separate stations in the AMS to start the artificial mouth simulation. To simulate a real-life dietary situation, 5% sucrose solution was supplied every 8 hours for 6 minutes at a flow rate of 15 ml/hour by the computer program LabVIEW (Version 2.2). Simulated oral fluid (defined medium mucin, DMM) was continuously supplied at 0.06 ml/minute to simulate salivary flow[19]. A humidified gas mixture of 5% carbon dioxide and 95% nitrogen was continuously supplied at 60 ml/minute. The temperature inside the incubator was maintained at 37ºC. The simulation started once the plaque holders were mounted in position in the AMS. The bacterial inoculum on the tooth blocks was allowed to grow on the exposed dentine surface, after the interventions, for 10 days.

Evaluations

1. Biofilm on the tooth blocks

Immediately after the 24-hour anaerobic incubation of the originally inoculated bacteria, the two extra tooth blocks embedded in free resin sections were used for the baseline evaluation of the cariogenic biofilm. The biofilm covering the exposed dentine surface of the two tooth blocks was collected and suspended separately in PBS solution and homogenised. Serials of 10-fold dilutions were prepared for each of the two bacterial samples. Using a spiral plater (Autoplate 4000, Spiral Biotech, Norwood, MA), 100 µl of the bacterial suspension from each dilution were plated, in duplicate, on horse blood agar (Defib Horse Blood, Hemostat Laboratories, Dixon, USA). After a 72-hour anaerobic incubation, colony forming unit (CFU) were counted to evaluate the growth of the biofilm. The average CFU value of the two samples was considered as the result of the baseline inoculation. At the end of the experiments, immediately after the tooth blocks were removed from the AMS, four tooth blocks in each group were randomly selected. The biofilms covering each of the four tooth blocks of each group were evaluated in the same way as the baseline. The median CFU values obtained among the four groups were compared. The Sabouraud Dextrose Agar (SDA) (ThermoFisher) was used to monitor fungi contamination at the baseline as well as final biofilm evaluations.

A confocal laser scanning microscope (CLSM) (FluoView FV1000, Olympus, Tokyo, Japan) was used to assess the bacterial viability in the biofilm. The biofilm on exposed dentine surfaces of two randomly selected tooth blocks from each group were labelled in situ using two fluorescent probes, propidium iodide (PI) and SYTO 9 (LIVE/DEAD BacLight bacterial viability kit, Molecular Probes, Eugene, OR, USA). The PI probe specifically labelled the dead cells in red, whereas the SYTO-9 probe labelled the live cells in green. After labelling, the blocks were incubated in the dark for 30 minutes. Subsequently, cell images of the biofilm were obtained using CLSM. Independent series of CLSM images (100X magnification) were obtained at two representative sites of the biofilm covering each tooth block. The image displaying the richest fluorescent signal and the two adjacent images (the image

before and the image after) were extracted from each series of the CLSM images. The ratio of green fluorescence (live cells) to red fluorescence (dead cells) on each selected image was calculated using the image analysis software Image J (National Institutes of Health, USA). The ratio represented the proportion of live/dead bacteria; ie, live-to-dead ratio of bacteria in the biofilm. This value was used as an indirect representation of the antimicrobial effect[20]. The median live-to-dead ratio of the bacteria from four sites of the biofilm, from each of the four groups, were compared.

The last two specimens of each group were analysed via scanning electron microscopy (SEM) (SU1510, Hitachi, Tokyo, Japan) to assess the surface topographies of the biofilm. Immediately after removing the samples from the artificial mouth station, they were rinsed with 4% (vol/vol) formaldehyde and 1% (vol/vol) phosphate buffer, and placed in a 1% osmium tetroxide solution for 1 hour; they were then washed with distilled water, followed by dehydration in a series of ethanol solutions at various concentrations (70% for 10 minutes, 95% for 10 minutes and 100% for 20 minutes). After dehydration they were air dried in a desiccator prior to the sputter coating with gold for SEM scanning. A high-vacuum mode at 20 kV was used during the SEM scan.

2. Artificial caries lesions

At the end of the experiments, following biofilm evaluation, all tooth blocks except the two send for SEM evaluation were analysed via micro-computed tomography (micro-CT) (SkyScan 1172, Antwerp, Belgium). The tooth blocks were positioned vertically in a 1.7-ml centrifuge tube with the exposed dentine surface located laterally. They were immersed in 1 ml of deionised water during the scan to retain moisture. The micro-CT was operated at an X-ray source voltage of 120 kV and electrical current of 80 μA. A spatial resolution of 8 μm was used, which yielded around 500 cross-sectional images along the long axis of each tooth block. A signal-to-noise ratio of five was used, and a 1 mm aluminium filter was used to cut off the softest X-rays. Among all the images showing the dentine caries lesion of each tooth block, a total of 20 were selected by using systematic random sampling. The lesion depth in each selected image was measured using the line measurement tool from the data analysis software CTAn (SkyScan Company). Three measurements from the deepest portion of the lesion were collected for each image. Overall, 60 measurements were collected for the 20 selected images from each tooth block. The average value of these 60 measurements was calculated to represent the depth of the artificial caries lesion in that specific tooth block. The medians of lesion depths in tooth blocks among the four groups were compared.

Data analysis

The effect of F^- and Ag^+ ions, alone or in combination, in preventing the artificial caries lesion in root dentine blocks, as well as their possible working mechanism, were evaluated and analysed by comparing the study parameters related to the biofilms and artificial caries lesions among the four parallel groups. Both quantitative data analysis and qualitative description were employed in this study. The quantitative data analysis mainly focused on the biofilm evaluation, and thus the sample size estimation was conducted based on this. With an estimation of a standardised effect size of 2 (ie, two times of the standard deviation values of CFU distribution in the biofilm) at α error probability equal to 0.05, to achieve a testing power of 80%, the sample size was calculated to be 8; ie, two samples needed for each of the four groups. The small sample size compromised the data distribution and thus, a nonparametric test was used for statistical analysis. The Kruskal-Wallis one-way ANOVA was used for the comparison of the data obtained from the CFU, CLSM, and micro-CT evaluations. The statistical significance level was set at 0.05.

Results

Approximately 1.8×10^7 CFU were inoculated in each unvarnished dentine surface at baseline. After the interventions and a 10-day challenge in the AMS, the median CFU in the four groups was 1×10^2 (AgF), 1.3×10^3 ($AgNO_3$), 4.3×10^6 (KF), and 5.5×10^6 (control), respectively. The distribution ($P < 0.001$) and the medians ($P < 0.001$) of CFU values among the four groups differed significantly. Pairwise comparisons showed that the relationships of these medians were AgF < $AgNO_3$ < KF = control (Table 1).

From the CLSM images (Fig 2), the median live-to-dead ratio of bacteria covering the tooth blocks were 0.27 (AgF), 0.69 ($AgNO_3$), 2.90 (KF) and 3.83 (control). The distribution ($P = 0.005$) and the median ($P = 0.001$) of the live-to-dead ratio among the four groups differed significantly. Pairwise comparisons showed that the relationships of these medians were AgF < $AgNO_3$ < KF = control (Table 1).

The SEM scanning showed scattered bacterial plaque covering the tooth blocks of the AgF and $AgNO_3$ groups, while a confluent biofilm layer was observed on the tooth blocks of the KF and control groups (Fig 2).

Table 1 Comparisons of medians of colony forming unit (CFU) counts, live-to-dead ratio and lesion depth among the four groups – AgF and $AgNO_3$ were effective against the cariogenic biofilm, while only AgF was effective in the prevention of lesion-depth development.

	AgF	$AgNO_3$	KF	Control	Comparison* (Relationship)
Median CFU counts at baseline (n = 2)	1.8×10^7				–
Median CFU counts after experiment (n = 4 for each group)	1.0×10^2	1.3×10^3	4.3×10^6	5.5×10^6	Overall distribution: $P < 0.001$ (AgF < $AgNO_3$ < KF = Control)
Live-to-dead ratio† (n = 2 for each group)	0.27	0.69	2.90	3.83	Overall distribution: $P = 0.005$ (AgF < $AgNO_3$ < KF = Control)
Median lesion depth‡ (μm) (n = 2 for each group)	134.00	200.08	264.42	232.53	Overall distribution: $P = 0.007$ (AgF < $AgNO_3$, KF, Control)

*Comparisons were performed using Kruskal-Wallis1-way ANOVA using the software SPSS 20.0. †Calculated using CLSM images and using the software ImageJ. ‡Measured using micro-CT images and using the software CTAn; The statistical significance level was set at 0.05.

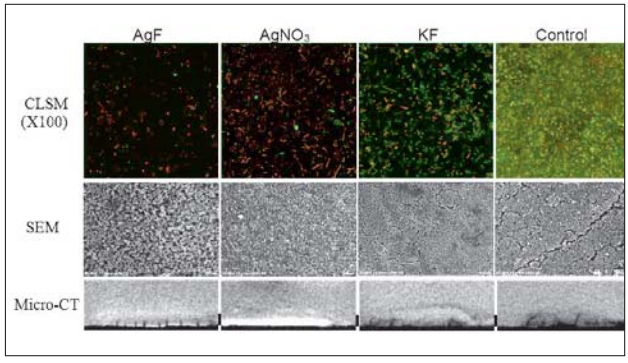

Fig 2 Representative images of each group. The upper row shows the CLSM images (100X magnification) – most bacteria died in the AgF and $AgNO_3$ groups while the bacterial viability in the KF group was not significantly affected. The middle row shows the SEM images – scattered bacterial plaque covered the tooth blocks in the AgF and $AgNO_3$ groups, while a confluent biofilm layer was detected in the KF and control groups. The lower row shows the micro-CT images (the black bar in between the images represents a depth of 200 μm in the image) – the lesion in the AgF group was the shallowest and the lesion depth in the KF group was similar to the control group, showing a thick mineralised surface layer.

From the micro-CT images (Fig 2), the median lesion depth of the artificial dentinal caries lesions in the tooth blocks were 134.00 μm (AgF), 200.11 μm ($AgNO_3$), 264.42 μm (KF), and 232.54 μm (control). The distribution ($P = 0.007$) and the medians ($P = 0.008$) of the lesion depth among the four groups differed significantly. Pairwise comparisons showed that the median lesion depth in the AgF group was significantly lower than in the $AgNO_3$ ($P = 0.016$), KF ($P = 0.016$) or control ($P = 0.009$) groups. No other statistically significant differences were found among the groups (Table 1). The lesion in the KF group had a thick mineralised surface layer compared with the lesion from the control group. A more obvious and thicker opaque outer layer was observed in the images of the $AgNO_3$ group compared with the AgF group. In both AgF and $AgNO_3$ groups, an opacity greater than normal was observed in the dentine even beyond the main lesion, of around 150 μm deep.

Discussion

This in vitro study was designed to provide insights into the independent and combined role of the main ions in a silver fluoride solution used for the prevention of dentine caries. The independent effect of Ag^+ or F^- ions against the cariogenic biofilm did not significantly reduce the caries lesion-depth progression. On the other hand, the co-existence of both ions enhanced the effects significantly.

A rough estimation of the standardised effect size of 2 in differences of the CFU counts was used here for sample size calculation; this was verified to be satisfactory based on data obtained in the present study. Although small, the sample size was regarded as sufficient to perform the main evaluation. The image analyses provided complementary information as well as the necessary descriptive characters. An increase of the sample size would have been desired, however, the realistic situation of the current AMS did not made it possible.

In the present study, the results obtained from analysing the cariogenic biofilm (via CFU, CLSM, SEM) were consistent. Compared to the control group, the artificial cariogenic biofilm development was significantly suppressed in the AgF and $AgNO_3$ groups, whereas bacterial viability in the KF group was not significantly affected; which suggests that silver ions provide a significant bactericidal effect against the cariogenic biofilm while fluoride ions per se, do not. The bactericidal effect of AgF was significantly greater than that of $AgNO_3$, indicating that fluoride provides a significant synergistic effect to the silver ions in inhibiting biofilm formation. Although $AgNO_3$ exhibited a strong bactericidal effect on the cariogenic biofilm, it did not prevent the development of dentine caries, suggesting that the damaging effect of $AgNO_3$ to the hard tissue significantly outperforms its bactericidal effect against the cariogenic biofilm.

The artificial caries lesion formed in the AgF group was significantly shallower than lesions formed in the remaining three groups. No other statistically significant differences were found. These results indicate that the use of AgF alone, was significantly effective in preventing the development of dentine caries. This may be due to the synergistic effect of both Ag^+ and F^- ions in the solution. The combination of Ag^+ and F^- also overcame the damaging characteristics of the NO_3^- and Ag^+ combination, as above mentioned.

Consistent with previous studies[4-5], the lesion depth in the KF group was not affected by the presence of a high concentration of fluoride ions compared with the control group. Despite this, a lesion with a thick mineralised surface layer was detected in the KF group via the micro-CT images. Thus, KF prevented mineral loss in the artificial caries lesions, maintained the hard tissue integrity, enabled a possible remineralisation mechanism and provided more time before the lesions got effective treatment. The mineralised surface layer may have been formed due to the action of the fluoride ions in the KF solution, as aforementioned.

The particle size of the metallic silver depositions formed after the AgF and $AgNO_3$ solutions were applied was < 0.5 μm and < 10 nm, respectively[21]. The nanoscale particles of the metallic silver was well below the diameter of normal dentinal tubules, which is around 3 μm[22]; this allowed the particles to penetrate into sound dentine and tiny porosities created during the acid attack, which might have affected the lesion depth evaluation in the AgF and $AgNO_3$ groups, and might have exaggerated the true lesion depth, and thus, might have induced error. The true differences in the comparison between lesion depth of the AgF group versus control group, as well as the $AgNO_3$ group versus the control group, might have been underestimated, which has lead to a higher chance of having type 2 errors (accepting the null hypothesis when it should have been rejected). This would not have greatly affected the comparison between the AgF group versus the control group because the null hypothesis was rejected in this case. However, it was not possible to estimate how much it affected the comparison between the $AgNO_3$ versus the control group. The artefacts of silver metal ions generated during the mineral density evaluation could have hindered the calculation of true mineral loss in the artificial caries lesions in these two groups. Therefore, a quantitative mineral density evaluation was not performed in the present study. The micro-CT provided a resolution of 8 μm, which was considered precise enough to evaluate lesion depths of > 130 μm. Certainly, there are other methods, such as microradiography[23] and microhardness testing[24] that could have been used here for the evaluation of the artificial caries lesions. However, they are all highly technique-dependent and somewhat destructive to the artificial caries lesions.

In summary, the presence of both silver and fluoride ions in the AgF solution provided a significantly greater protection against dental caries compared with the presence of silver ions alone in the $AgNO_3$ solution, or fluoride ions alone in the KF solution. Recent Brazilian studies claimed that the preparation of nano-silver fluoride yielded no stains to the arrested caries in primary teeth[25-26]. A dose-effectiveness study of SDF in dental caries prevention should be conducted in the future to clarify as to the necessity of using a high concentration of the currently available product in the market. The change in the bacterial profile after topical application of SDF in a real clinical situation should be monitored for a better understanding of its clinical effect.

Conclusion

Silver ions have a significant bactericidal effect against cariogenic bacteria and suppress the cariogenic biofilm development on the dentine surface. Fluoride ions per se do not have a significant bactericidal effect against cariogenic bacteria but are synergistic to silver ions in inhibiting the biofilm formation. The use of silver ions only in the $AgNO_3$ solution provided no protection against the development of dentine caries, while the use of fluoride ions only, in a KF solution prevented mineral loss from the dentine tissue. The combined use of silver and fluoride ions in one solution (AgF) may provide a

significant protection to dentine against the development of caries lesions.

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Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Bao Ying LIU conceived, designed and performed the experiments and drafted the manuscript. Lei MEI, Chun Hung CHU and Edward Chin Man LO contributed greatly in the study design and results interpretation.

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