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# Sodium fluoride and silver diamine fluoride-coated tooth surfaces inhibit bacterial acid production at the bacteria/tooth interface

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ABSTRACT

*Objectives:* This study aimed to evaluate whether coating tooth surfaces with sodium fluoride (NaF) or silver diamine fluoride (SDF) inhibits bacteria-induced pH reductions at the bacteria/tooth interface.
*Methods:* Specimens of coronal enamel (CE) or root dentin (RD) were prepared. The surfaces of the specimens were treated with 2% NaF or 38% SDF solution. Some specimens were aged for 1 week after being treated. A tooth specimen was fixed to the bottom of the well of the experimental apparatus. A miniature pH electrode was placed on the specimen and the well was filled with *Streptococcus mutans* (SM) cells. The pH was monitored after the addition of 0.5% glucose. SM cells were recovered from the wells, and the amounts of lactate, calcium, fluoride, and silver were measured.
*Results:* The fluoride-treated tooth specimens exhibited significantly higher pH values than the untreated controls, irrespective of the tooth substrate at 120 min (CE: NaF 4.62 ± 0.06 vs 4.34 ± 0.10 and SDF 5.23 ± 0.29 vs 4.44 ± 0.16, RD: NaF 5.10 ± 0.11 vs 4.54 ± 0.33 and SDF 6.65 ± 0.47 vs 4.64 ± 0.39). The SDF-coated RD specimens released the greatest amounts of fluoride (103.3 ± 48.1 nmol/well) and silver (70.4 ± 36.9 nmol/well), while they exhibited significantly lower lactate production and decalcification (calcium release) than the control samples (lactate: 4.0 ± 0.7 vs 7.4 ± 0.3 mmol/l, calcium: 2.2 ± 0.4 vs 3.7 ± 0.5 μg/ml). This antimicrobial effect was weakened by 1 week's aging, while the acid resistance of the fluoride-treated surfaces seemed to increase with aging.
*Conclusions:* Fluoride-treated tooth surfaces inhibit bacterial acid production at the bacteria/tooth interface. The SDF-coated RD had the strongest inhibitory effect.
*Clinical significance:* Coating RD with SDF could help to prevent root caries.

## 1. Introduction

The application of fluoride to tooth surfaces is widely used to prevent caries because fluorides reduce demineralization and promote remineralization on tooth surfaces [1]. Sodium fluoride (NaF) is mainly applied to enamel to prevent caries in children and adolescents [2–4]. Recent systematic reviews have evaluated whether silver diamine fluoride (SDF) is effective at arresting dentin caries in children [5,6], and SDF has been shown to effectively prevent and arrest root caries in older adults [7]. Nevertheless, the mechanism by which the application of fluoride to tooth surfaces, especially to root surfaces, prevents caries has not been elucidated.

Besides reducing tooth demineralization and promoting tooth remineralization, fluorides are known to inhibit bacterial acid production [8–14]. However, it is unclear whether applying fluoride to tooth surfaces inhibits bacteria-induced pH reductions at the interface between cariogenic bacteria and the tooth surface. Recently, we developed an experimental apparatus with a miniature ion-sensitive field-effect transistor (ISFET) pH electrode and used it to monitor the pH and quantify the amounts of lactate and calcium at the bacteria/tooth surface interface [15]. In the present study, we used this model to evaluate the pH and amounts of lactate, calcium, fluoride, and silver at the

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interfaces between *Streptococcus mutans* cells and fluoride (NaF and SDF)-treated tooth surfaces by simulating an oral environment in which acid-producing bacteria were present on tooth surfaces.

The hypotheses tested in the present study were that examined coating tooth surfaces with fluoride inhibits bacteria-induced acid production at the *S. mutans* cell/tooth interface. We also evaluated these inhibitory effects of fluoride treatment on non-aged and aged coronal enamel (CE) and root dentin (RD) specimens.

## 2. Materials and methods

### 2.1. Bacterial growth conditions

*S. mutans* NCTC 10449 cells were cultured in medium containing 1.7% tryptone (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), 0.3% yeast extract (Becton, Dickinson and Company), 0.5% NaCl, and 0.5% glucose in 50 mM potassium phosphate buffer (PPB, pH 7.0). The *S. mutans* cells were cultured in the abovementioned medium at 37 °C, transferred to new medium (5% inoculum size), and incubated further at 37 °C until the logarithmic growth phase (an optical density of about 0.5 at 660 nm). Then, the *S. mutans* cells were harvested by centrifugation (21,000 × g for 15 min at 4 °C), washed with 2 mM PPB (pH 7.0), and suspended in the same buffer. Next, the *S. mutans* cells were incubated in air at 37 °C for 1 h to exhaust their intracellular polysaccharide supplies, before being washed with 2 mM PPB (pH 7.0). The cell suspension was then distributed into 1.5-mL tubes, centrifuged (16,000 × g for 7 min at 4 °C), and kept at 4 °C until use.

### 2.2. Tooth specimens

Bovine incisors were cut into crown and root specimens. Specimens of CE and RD were polished using sandpaper (#240, 600, and 2000 grit) to produce flat tooth surfaces. The CE and RD specimens were both divided into 3 groups, and the flat surfaces of the specimens were then immersed in 2% NaF solution, 38% SDF (Saforide®, Toyo Seiyaku Kasei, Japan) solution, or deionized water (control) for 10 min. The excess fluoride solution or water on each specimen was removed with filter paper. Three tooth specimens from each group were immersed in 20 mL of 2 mM PPB at pH 7.0 in polyethylene tubes. These specimens were artificially aged by being stored in a thermostatic chamber at 37 °C for 1 week. The non-aged and aged tooth specimens were used in the following experiments.

### 2.3. Experimental apparatus and measurement of pH at the S. mutans cell/tooth surface interface

pH measurements were obtained as described by Mayanagi et al. [12,14–16]. The pH at the interface between the *S. mutans* cells and fluoride-treated tooth surfaces was monitored using a specially designed experimental apparatus (Fig. 1). The experimental apparatus, which contained a well (4.0 mm in diameter and 2.0 mm deep), was made of polymethyl methacrylate. In each experiment, a tooth specimen was fixed to the bottom of the well, an ISFET miniature pH electrode ($H^+$ ion-sensitive area: 2.0 mm long, 1.0 mm wide, and 0.2 mm thick; model PH-60T1; Nihon Kohden, Tokyo, Japan) was placed on the fluoride-treated or control tooth surface, and the well was filled with *S. mutans* cells using a syringe and packed with a spatula. After preincubation at 37 °C for 10 min, 500 µL of 0.5% glucose was added to the *S. mutans* cells, and the pH at the *S. mutans* cell/tooth surface interface was monitored continuously at 37 °C for 120 min. At 120 min after the addition of glucose, the remaining glucose was removed using filter paper, and all the clamped *S. mutans* cells were collected by adding 500 µL of 2 mM PPB (pH 7.0) to the well and pipetting carefully for 3 times and then transferring to 1.5-mL tubes. The *S. mutans* cells were preserved at −80 °C until use.

### 2.4. Measurement of the amount of lactate produced by the S. mutans cells and the amount of calcium that dissolved from the tooth surfaces

The amount of lactate produced by the *S. mutans* cells and the amount of calcium that dissolved from the tooth surfaces were measured using the method described by Mayanagi et al. [15]. The preserved *S. mutans* cells were resuspended in the PPB and centrifuged to obtain supernatants. The lactate levels in the *S. mutans* cell supernatants were measured with a portable lactate meter (Lactate Pro 2, Arkray, Japan). Each supernatant sample (5 µL) was added to the sensor chip attached to the lactate meter. The amount of lactate was used as a measure of lactic acid production by the *S. mutans* cells.

The calcium levels in the *S. mutans* cell supernatants were measured using a calcium-specific fluorescent dye (Fluo-3, Dojindo, Japan). The supernatant (10 µL) was mixed with Fluo-3 solution (1 µL of Fluo-3 and 89 µL of 0.1 M KOH). The resultant fluorescence was measured with a plate reader (Varioskan Flash, Thermo Fisher Scientific, Inc., Rockford, IL, USA) at an excitation wavelength of 508 nm and an emission wavelength of 527 nm. The calcium levels of the supernatants were determined from standard curves.

### 2.5. Measurement of the amounts of fluoride and silver released from fluoride-treated tooth surfaces

A mixed solution of sodium acetate buffer and perchloric acid (pH 5.0) was added to 50 µL of a suspension of preserved *S. mutans* cells at a final concentration of 1 M. The cell mixtures were kept at 4 °C overnight to ensure the complete destruction of the *S. mutans* cells. The cell extracts were collected by centrifugation, and the fluoride concentrations of the extracts were measured by a fluoride-ion-specific electrode (Model 9409 BN, Orion, Cambridge, MA, USA) and a comparison electrode (Model 900100, Orion), based on the method of Hallsworth et al. [17].

The preserved *S. mutans* cell suspension (50 µL) was diluted with 450 µL of 2 mM PPB (pH 7.0), mixed with 2 mL of 61% nitric acid and 30% hydrogen peroxide, and heated (140 °C, 3 h) to induce decomposition. Samples of the bacterial suspension were then diluted to a total volume of 10 mL with ultrapure water. The amount of silver released into the cells from each fluoride-treated tooth surface was measured using inductively coupled plasma mass spectrometry (ICP-MS; 8800, Agilent, Tokyo, Japan) [18].

### 2.6. Statistical analysis

The Student's *t*-test was used to assess the significance of differences for comparisons between two groups. For multi-group comparisons, Tukey's multiple comparisons test was performed after one-way analysis of variance (ANOVA). p-values of $< 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Curves of the pH reductions induced at the S. mutans cell/tooth surface interface by bacterial sugar fermentation after the addition of glucose

Curves showing the reductions in pH induced at the interface between the *S. mutans* cells and the non-aged fluoride-treated tooth surfaces after the addition of glucose are shown in Fig. 2. At 120 min after the addition of glucose, significantly higher pH values were detected at the *S. mutans* cell/fluoride-treated tooth surface interfaces than at the control interfaces ($p < 0.05$) (Fig. 2). The pH values detected at the *S. mutans* cell/SDF-coated tooth interfaces were significantly higher than those detected at the *S. mutans* cell/NaF-coated tooth interfaces, irrespective of the tooth substrate (Fig. 2). Moreover, the pH values detected at the *S. mutans* cell/SDF-coated RD interfaces were significantly higher than those detected at the *S. mutans* cell/SDF-coated CE

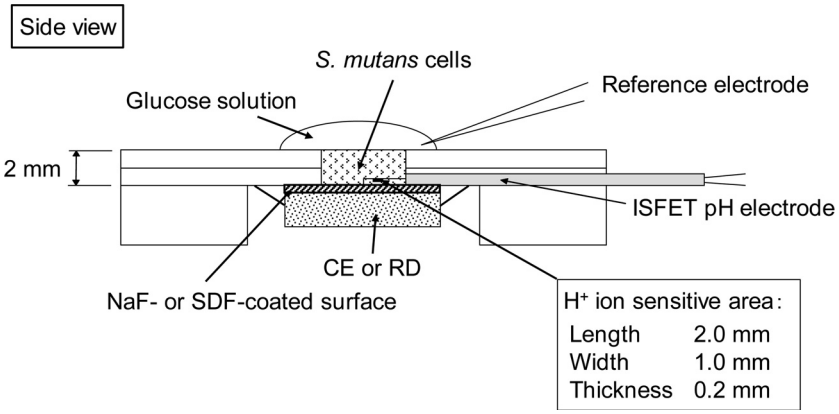


**Fig. 1.** Schematic drawing of the experimental apparatus used in the present study.
NaF, sodium fluoride; SDF, silver diamine fluoride; CE, coronal enamel; RD, root dentin.

interfaces (Fig. 2).

Curves of the reductions in pH seen at the interfaces between *S. mutans* cells and fluoride-treated tooth surfaces that had been subjected to aging in PPB for 1 week are shown in Fig. 3. The aged fluoride-treated CE specimens exhibited similar pH reduction curves to the controls (Fig. 3A). On the other hand, the aged SDF-coated RD specimens displayed higher pH values than the control samples, although there were no significant differences among the NaF-treated, SDF-treated, and control samples (Fig. 3B).

### *3.2. The amounts of lactate produced by S. mutans cells and calcium that dissolved from teeth after the addition of glucose*

Fig. 4A shows the amounts of lactate that had been produced by the *S. mutans* cells at 120 min after the addition of glucose. The *S. mutans* cells around the non-aged fluoride-treated teeth produced lower amounts of lactate than the *S. mutans* cells around the control teeth, irrespective of the tooth substrate (Fig. 4A). Significant differences in lactate production were observed between the *S. mutans* cells around the SDF-coated and control teeth. The amounts of lactate produced by the *S. mutans* cells around the aged fluoride-treated CE specimens were similar to those around the control teeth (Fig. 4A). On the other hand,

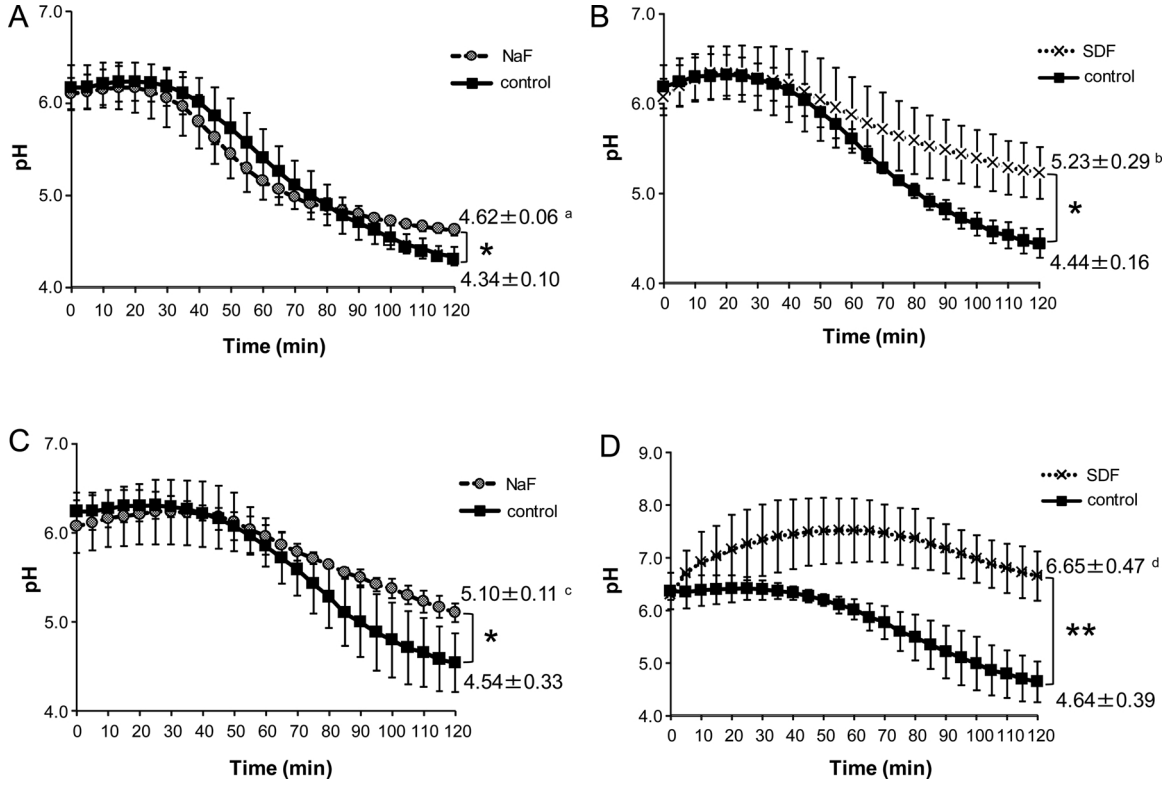


**Fig. 2.** Curves of the reductions in pH induced by bacterial acid production from 0.5% glucose at the interface between *S. mutans* cells and non-aged fluoride-treated tooth surfaces.
(A) NaF-coated CE, (B) SDF-coated CE, (C) NaF-coated RD, (D) SDF-coated RD.
CE, coronal enamel; RD, root dentin; NaF, sodium fluoride; SDF, silver diamine fluoride. The data represent the means of three independent experiments. The vertical bars indicate standard deviation. $p^* < 0.05$, $p^{**} < 0.01$ (significantly different from the control at 120 min after the addition of glucose). Numerical values indicate the pH at 120 min after the addition of glucose. a and b: $p < 0.05$; c and d, b and d: $p < 0.01$ (significantly different between groups).

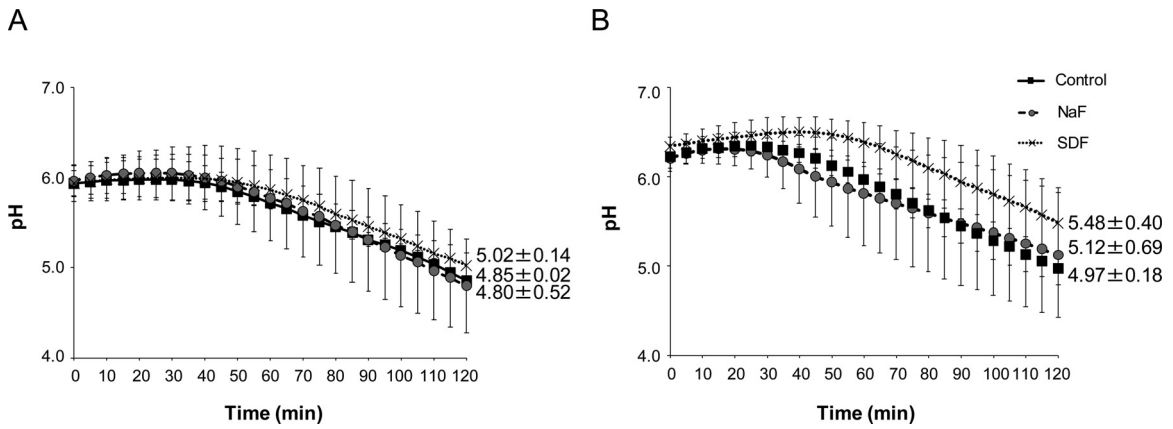


**Fig. 3.** Curves of the reductions in pH induced by bacterial acid production from 0.5% glucose at the interface between *S. mutans* cells and fluoride-treated tooth surfaces after 1 week's aging in potassium phosphate buffer. (A) CE, (B) RD. CE, coronal enamel; RD, root dentin; NaF, sodium fluoride; SDF, silver diamine fluoride. The data represent the means of three independent experiments. The vertical bars indicate standard deviation. The numerical values indicate the pH at 120 min after the addition of glucose.

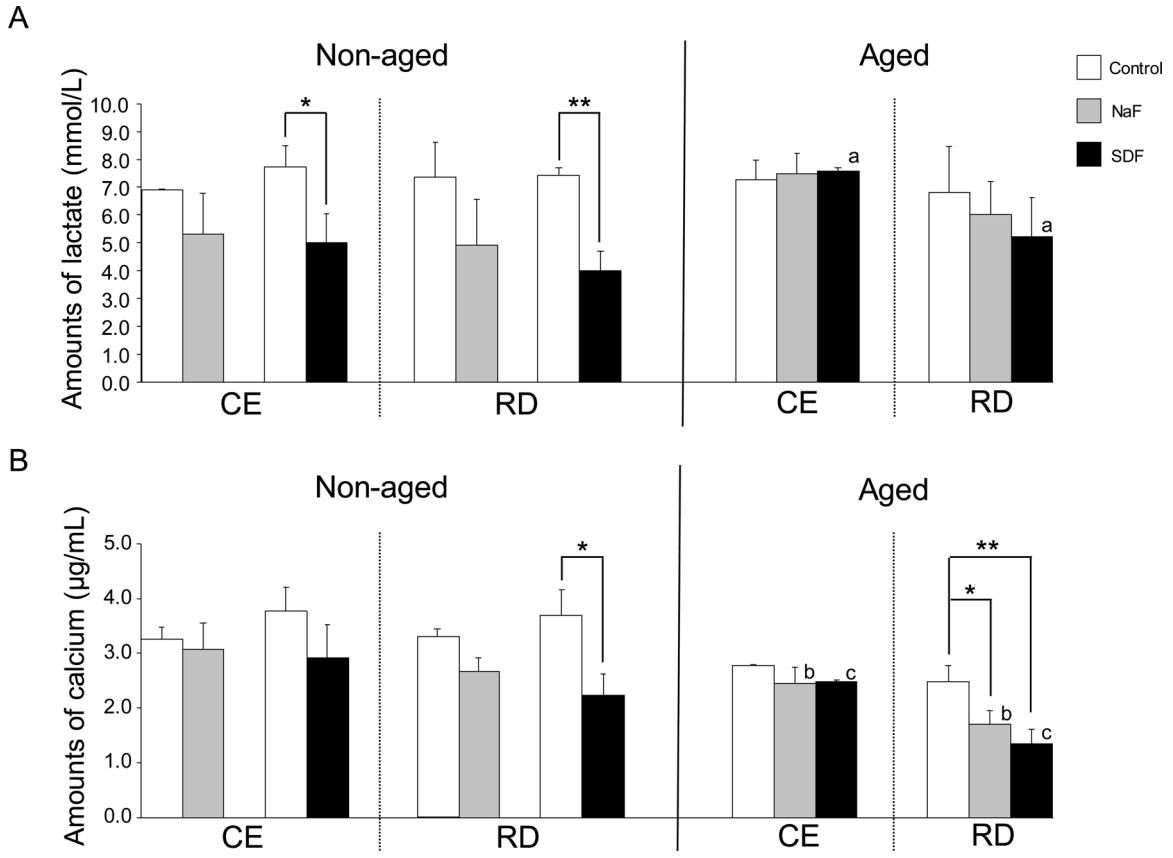


**Fig. 4.** (A) The amounts of lactate produced by *S. mutans* cells during the 120 min after the addition of 0.5% glucose. (B) The amounts of tooth surface calcium that dissolved during the same period. CE, coronal enamel; RD, root dentin; NaF, sodium fluoride; SDF, silver diamine fluoride. The data represent the means of three independent experiments. The vertical bars indicate standard deviation. $p^* < 0.05$, $p^{**} < 0.01$ (significantly different from the control at 120 min after the addition of glucose). The same letters indicate significant differences between tooth substrates ($p < 0.05$).

the *S. mutans* cells around the aged fluoride-treated RD specimens produced lower amounts of lactate than those around the control teeth, but there were no significant differences among the *S. mutans* cells around the NaF-treated, SDF-treated, and control teeth (Fig. 4A). The *S. mutans* cells around the aged SDF-coated RD specimens produced significantly lower amounts of lactate than those around the aged SDF-coated CE specimens (Fig. 4A).

Fig. 4B shows the amounts of calcium that had dissolved from the tooth surfaces at 120 min after the addition of glucose. Lower amounts of calcium dissolved from the non-aged fluoride-treated teeth than from the control teeth, irrespective of the tooth substrate (Fig. 4B). Significant differences in the amounts of dissolved calcium were observed between the SDF-coated RD and control specimens. Slightly lower amounts of calcium dissolved from the aged fluoride-treated CE specimens than from the control samples, whereas significantly lower amounts of calcium dissolved from the aged fluoride-treated RD specimens than from the control samples (Fig. 4B). Significantly lower amounts of calcium dissolved from the aged NaF- and SDF-coated RD

**Table 1**
The amounts of fluoride (F) and silver (Ag) detected in the *S. mutans* cells at 120 min after the addition of glucose (nmo l /well).

| | | F | | Ag |
|---|---|---|---|---|
| | | NaF | SDF | |
| Non-aged | CE | 6.5 ± 2.8 | 38.0 ± 19.2 | 19.5 ± 16.3[a] |
| | RD | 16.5 ± 3.9[b] | 103.3 ± 48.1[c] | 70.4 ± 36.9[d] |
| Aged | CE | 1.1 ± 0.3 | 1.1 ± 0.1 | 0.1 ± 0.1 |
| | RD | 1.5 ± 0.1 | 1.2 ± 0.6 | 0.6 ± 0.2 |

The data are the means of three independent experiments.
b and c, a and d: $p < 0.05$ (significantly different between groups).

samples than from the NaF- and SDF-coated CE samples, respectively (Fig. 4B).

### 3.3. The amounts of fluoride and silver detected in the S. mutans cells

Table 1 shows the amounts of fluoride and silver detected in the *S. mutans* cells at 120 min after the addition of glucose. The non-aged SDF-coated tooth surfaces released larger amounts of fluoride into the *S. mutans* cells than the non-aged NaF-coated tooth surfaces, and the difference was statistically significant for the RD specimens ($p < 0.05$) (Table 1). Among the non-aged fluoride-treated samples, the RD samples released larger amounts of fluoride than the CE samples although the differences were not statistically significant (Table 1). As for the non-aged SDF-treated samples, the RD samples released greater amounts of silver into the *S. mutans* cells than the CE samples ($p < 0.05$) (Table 1). Lower amounts of fluoride and silver were detected in the *S. mutans* cells surrounding the aged fluoride-treated specimens than in the *S. mutans* cells surrounding the non-aged fluoride-treated specimens (Table 1).

## 4. Discussion

The coating of non-aged tooth surfaces with NaF or SDF inhibited the reductions in pH induced by bacteria, as well as lactate production and decalcification, at the bacteria/tooth surface interface (Figs. 2 and 4). In addition, the coating of tooth surfaces with SDF inhibited these processes more efficiently than coating them with NaF (Figs. 2 and 4). Fluoride was detected in the *S. mutans* cells surrounding the NaF- and SDF-coated tooth surfaces, while silver was detected in the *S. mutans* cells surrounding the SDF-coated tooth surfaces (Table 1). These results clearly indicate that the fluoride and silver released from the fluoride-treated tooth surfaces inhibited the metabolic activity (acid production) of *S. mutans* cells. The mechanisms by which fluoride inhibits bacterial acid production are considered to involve the inhibition of enolase, a glycolytic enzyme [19–21], and a proton-translocating ATPase; i.e., a proton-expelling enzyme [22–24], and the enhancement of intracellular acidification due to the proton production associated with the dissociation of HF within bacterial cells [25,26]. Silver can also inhibit bacterial metabolism by binding to bacterial proteins. Bacteria possess cell membrane-bound enzymatic proteins, such as the phosphoenolpyruvate phosphotransferase system, which transports sugars across the cell membrane [27,28]. Thus, bacterial metabolism can be inhibited by inactivating such enzymes through silver-induced protein coagulation.

The finding that the non-aged SDF-coated tooth surfaces released more fluoride than the non-aged NaF-coated tooth surfaces, together with its silver release (Table 1), suggests that the fluoride and silver in SDF have additional or synergistic inhibitory effects on bacterial acid production at the bacteria/tooth surface interface (Fig. 2). It has been reported that SDF had antibacterial effects against cariogenic biofilms composed of *S. mutans*, *Streptococcus sobrinus*, *Lactobacillus,* and *Actinomyces*, which formed on dentin surfaces [29–31]. In addition to these previous findings, the present study demonstrated that the fluoride and silver released from non-aged SDF-coated tooth surfaces inhibited acid production by caries-associated bacteria; i.e., *S. mutans,* at the bacteria/tooth surface interface. Furthermore, the RD specimens always released more fluoride and silver than the CE specimens (Table 1), suggesting that RD retains and releases significant amounts of both fluoride and silver.

This study also evaluated whether treating teeth with fluoride inhibited the metabolic activity of *S. mutans* cells and/or influenced the reductions in pH seen at the *S. mutans* cell/tooth surface interface using tooth samples that had been aged for 1 week. The aged fluoride-treated CE specimens exhibited similar pH reduction curves, as well as similar lactate production and dissolved calcium levels, to the control samples (Figs. 3 A and 4), suggesting that the inhibitory effect of coating CE with fluoride on *S. mutans* metabolic activity disappeared after 1 week's aging. On the other hand, SDF still inhibited *S. mutans* acid production and the resultant decalcification in the aged RD samples (Figs. 3B and 4). However, the amounts of fluoride and silver detected in the *S. mutans* cells around the aged SDF-coated RD samples were much lower than those detected in the *S. mutans* cells around the non-aged SDF-coated RD samples, and were as low as those detected in the *S. mutans* cells around the aged SDF-coated CE samples (Table 1). These results suggest that a mechanism other than metabolic inhibition caused by SDF-derived fluoride and silver might have contributed to the observed effects, as discussed below.

Since proteins are negatively charged under physiological conditions, the proteins contained in teeth become coagulated by binding with silver ions ($Ag^+$) [32]. Thus, $Ag^+$ derived from SDF can bind to RD, which contains a greater amount of protein than CE, more efficiently. Although the amount of silver bound to each RD specimen was not measured in the present study, the fact that the aged SDF-coated RD specimens remained black (data not shown) and only released small amounts of silver (0.6 ± 0.2 nmol/well) (Table 1) suggests that after the rapid release of silver in the early stages after the application of SDF, silver binds to RD proteins tightly and stably. In addition, it is questionable whether the small amount of silver released from the aged SDF-coated RD specimens was sufficient to inhibit *S. mutans* acid production (Figs. 3B and 4 A, Table 1). These findings suggest that protein-bound silver might inhibit bacterial metabolism directly at the interface between bacteria and aged SDF-coated RD surfaces, but further study is necessary to confirm this.

Calcium release from the aged NaF- and SDF-coated RD specimens was inhibited (Fig. 4B), although the acid production of the *S. mutans* cells around the NaF-coated surfaces did not seem to be inhibited and that of the *S. mutans* cells around the SDF-coated surfaces was only slightly inhibited. These results indicate that the acid resistance of tooth surfaces treated with fluoride might increase with age. NaF that has been applied to hydroxyapatite reacts with calcium ions to form calcium fluoride ($CaF_2$). The $CaF_2$ that forms on hydroxyapatite surfaces is gradually converted into fluoroapatite [32–34], which exhibits greater acid resistance than hydroxyapatite [32]. On the other hand, when tooth surfaces are treated with SDF, the acid resistance of hydroxyapatite improves via the following reaction: $Ca_{10}(PO_4)_6(OH)_2 + Ag(NH_3)_2F \rightarrow CaF_2 + Ag_3PO_4 + NH_4OH$ [32,35,36]. Yamaga et al. [32] suggested that the formation of $CaF_2$ and $Ag_3PO_4$ could be responsible for the prevention of dental caries. Moreover, a recent study found that SDF reacts with calcium and phosphate ions and of hydroxyapatite and produces fluorohydroxyapatite with reduced solubility, which fluoride ions partially substitute hydroxyl ions of hydroxyapatite [37,38]. However, the modes of action of NaF and SDF on tooth surfaces have not been fully elucidated.

The model employed in the present study could be useful for evaluating caries-preventing treatments. However, it has some limitations, e.g., the manual packing of a single species of *S. mutans* cells did not allow the effects of biofilm architecture, the biofilm matrix, or saliva to be considered. Moreover, the amounts of lactate, calcium, fluoride, and

silver were not measured directly at the interface. Since the experimental conditions, such as the aging process employed, in the current study could differ from those found in the oral cavity, further development of the model is needed.

In conclusion, the present study demonstrated that applying NaF or SDF to tooth surfaces inhibited bacterial acid production at the bacteria/tooth surface interface, although these effects disappeared in fluoride-treated CE and NaF-coated RD and were weakened in SDF-coated RD after 1 week's aging. Moreover, the acid resistance of the fluoride-treated tooth surfaces might have been increased by aging. These results indicate that the application of fluoride to tooth surfaces is effective at preventing caries. Applying SDF to RD had the strongest inhibitory effects on bacterial acid production, suggesting that SDF is a promising tool for root-surface caries prevention.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Declaration of interests

None.

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