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Antimicrobial Persistence of Silver Diamine Fluoride and Silver Fluoride against *Streptococcus mutans*

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Purpose: To evaluate the antimicrobial persistence of silver diamine fluoride (SDF) and silver fluoride (AgF) on *Streptococcus mutans*.

Materials and Methods: An *in vitro* experiment was conducted to observe changes in the diameter of the inhibition zone of various materials, including AgF (Riva Star Aqua™ step 1; SDI), potassium iodine (Riva star aqua™ step 2; SDI), Fluor protector® (FP, Ivoclar Vivadent), SDF (Riva star™ step 1; SDI), Ampicillin (Sigma-Aldrich), Amphotericin B (Nexstar) and negative control on *S. mutans*.

Result: SDF, AgF and FP exhibited significant antimicrobial persistence over the 4 weeks period ($P<0.05$). At day 28, the diameter of inhibition zone was larger in SDF than in AgF.

Conclusion: SDF and AgF have significant antibacterial durability against bacteria commonly associated with dental caries, with the antimicrobial effect lasting for at least 4 weeks. Further clinical studies are needed to validate these findings *in vivo*.

Key Words: Anti-bacterial agents; Dental caries; Fluorides

Introduction

Dental caries is a typical bacterial disease, and its metabolites, acids, dissolve minerals in the tooth structure and penetrate into the enamel and dentin. This process is thought to be a dynamic one that involves both demineralization and remineralization, provided the superficial layer of enamel is intact, and remineralization can be promoted by fluoride ions[1].

Streptococcus mutans, the predominant cariogenic bacterium, causes demineralization within the gingival biofilm and produces acid, which changes the surrounding environment to an acidic one. This creates opportunities for other acid-producing and acid-

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resistant species to thrive[2,3].

In the hard tissues of the tooth, fluoride has a caries-preventive effect and can reduce the acid-producing capacity of some microbial species. In dental practice, fluoride is used in various forms, most commonly in the form of gels and varnishes[3].

Fluoride varnishes are divided into two main categories: 5% NaF products, which contain 22,600 ppm of fluoride, and Fluor Protector® (FP; Ivoclar Vivadent, Schaan, Liechtenstein), which contains 1,000 ppm of fluoride and 0.9% difluorosilane. FP is an ethanol-based liquid varnish with a lower fluoride concentration than 5% NaF varnish, reducing the potential for fluoride overdose[4,5].

Silver diamine fluoride (SDF), another liquid fluoride material, is an ammonia-added formulation that improves the instability of silver fluoride (AgF) and maintains a constant concentration over a period of time. In 2014, 38% of SDF (SDI, Bayswater, Australia) products were commercialized, and it is used for the purpose of treating tooth hypersensitivity, inhibiting caries progression, and remineralization, but discoloration is considered to be the biggest disadvantage[6,7].

The antimicrobial effect of fluoride varnish on *S. mutans* has been confirmed in previous studies[8,9]. The antimicrobial effect can be achieved not only by inhibition of demineralization and promotion of remineralization, but also by reducing the population of *S. mutans* itself.

Despite this effectiveness, clinical applications of SDFs are hampered by unpleasant taste and odor and gingival irritation. To improve this, an ammonia-free water-based AgF (Riva Star Aqua™ step 1; SDI) was introduced. There are domestic studies confirming the antibacterial effect of AgF on *S. mutans*, but studies on antibacterial persistence are thought to be lacking[10,11].

The purpose of this study was to determine the antimicrobial persistence of AgF and SDF against *S. mutans*.

Materials and Methods

This experimental study was exempt from IRB review because it didn't collect or record any personal identifiable information and used only simple measurement devices.

1. Bacterial Culture and Smear

Cryopreserved *S. mutans* (ATCC 25175) was incubated for 18 hours at 37°C and 5% CO_2 under atmospheric conditions. The medium used was Brain Heart Infusion broth (Becton, Dickinson and Company, Sparks, MD, USA) supplemented with 1.5% agar (BD Bacto™; Becton, Dickinson and Company) and autoclaved at 121°C for 15 minutes. In the concussion incubator, the OD_{600} value was maintained at 0.5, and flat coating was performed on a flat agar medium. The flat smearing method was performed according to the European committee on Antimicrobial Susceptibility Testing (EUCAST) version 10.0[11].

2. Experimental Materials

Three liquid fluoride materials, Riva Star Aqua™, Riva Star™ and FP were used. Riva Star Aqua™ was used with both AgF (step 1) and potassium iodine (KI, step 2), and all materials were new, unopened, and used immediately before use.

3. Experimental Design

Four samples were used as experimental groups: AgF alone, KI alone, SDF and FP. Ampicillin (Sigma-Aldrich, Saint Louis, MO, USA) was used as a positive control (PC) to compare the antibacterial effect. The negative control (NC) received no additional treatment beyond the medium. And Amphotericin B (AptB; Nexstar, San Dimas, CA, USA), an antifungal agent, was also used as a NC. Five agar plates were prepared, each containing seven paper disks (8 mm, Whatman, Edgewood, NM, USA) with 30 μl of each material applied (Fig. 1).

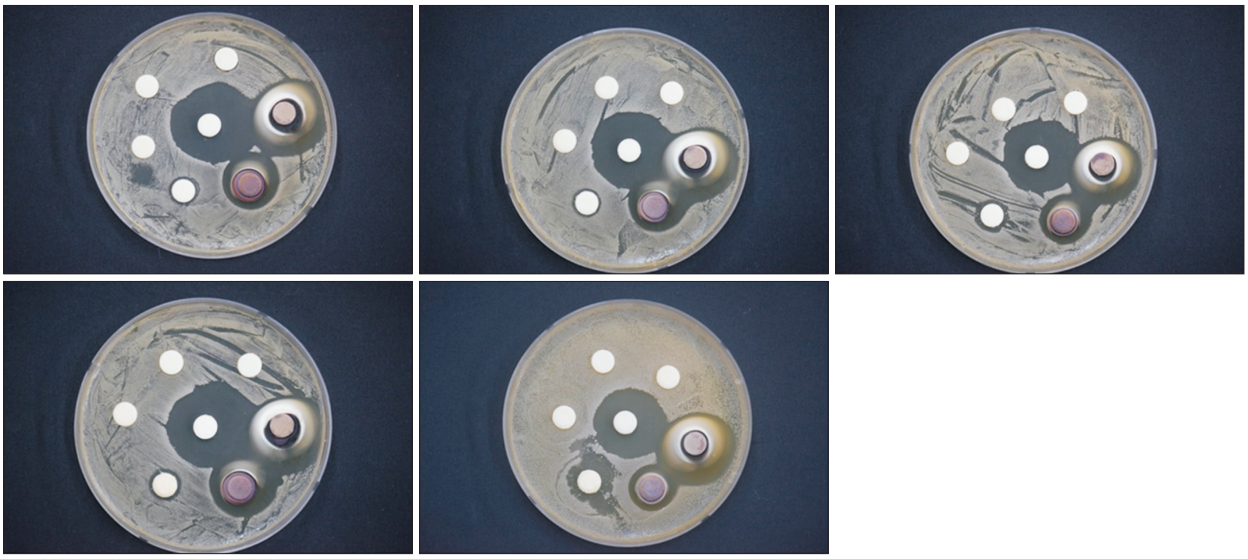

Fig. 1. Inhibition zone of each materials in the five badges.

4. Evaluation of Antimicrobial Persistence

Every day for 8 days, two observers independently measured the diameter of the inhibitory zone of each material formed around the paper disc in mm using a micrometer (Mitutoyo, Kawasaki, Japan), and then an additional observer and one original observer performed independent measurements at one-week intervals for one month (day 1, week 1, week 2, week 3, week 4). Repeated measurements were performed on five plates, and the mean and standard deviation were calculated for each experimental and control group.

5. SDF Component Analysis

Since the component analysis of AGF was performed in the previous paper of this study, we performed component analysis of SDF to compare the components with AgF[11]. SDF was analyzed by combustion ion chromatography using a Dionex Aquion™ IC (Thermo Fisher Scientific, Waltham, MA USA) and inductively coupled plasma spectrometry using a 5100 ICP-OES (Agilent Technologies, Santa Clara, CA, USA).

6. Statistical Analysis

Intra-class correlation (ICC) was evaluated to analyze inter-observer agreement, and repeated-measures ANOVA was performed to compare the effects of the materials over time, followed by Bonferroni post hoc tests. All statistical analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA).

Result

1. ICC

The inter-rater reliability of the two observers who measured the diameter of the inhibition zone of the materials in the five media daily for eight days was 0.999 (P<0.001), and the agreement between one of the original observers and another observer who took additional measurements was also very high at 0.999 (P<0.001).

2. Diameter of the Inhibition Zone Over Time

Analyses were performed using the mean of daily measurements taken by two observers over an 8-day period (Table 1) and the mean of measurements at weekly intervals for a month (Table 2).

For 8 days, PC showed the greatest increase in inhibition zone diameter, while AptB, KI, and NC showed no significant change. SDF showed a significant increase on day 2 (P<0.001), followed by a

Table 1. The diameter of inhibition zone from day 1 to day 8

Time	Diameter (mm)						
(day)	PC	AptB	SDF	AgF	KI	FP	NC
1	26.945±1.662	8.000±0.000**	19.567±2.645**	25.954±1.253	8.153±0.168**	11.743±2.567**	8.000±0.000**
2	26.728±2.045	8.000±0.000**	26.251±1.574	19.428±2.826**	8.127±0.203**	11.809±2.608**	8.000±0.000**
3	27.576±2.185	8.000±0.000**	25.862±2.029	20.368±2.321**	8.018±0.040**	11.587±2.930**	8.000±0.000**
4	26.996±2.137	8.000±0.000**	24.173±1.388*	18.660±3.118**	8.018±0.040**	11.741±3.167**	8.000±0.000**
5	26.522±1.786	8.000±0.000**	24.758±0.823	18.649±2.545**	8.018±0.040**	11.796±2.354**	8.000±0.000**
6	27.102±1.663	8.000±0.000**	25.186±0.840*	19.212±2.571**	8.038±0.085**	11.978±2.495**	8.000±0.000**
7	27.486±1.656	8.000±0.000**	25.525±0.961*	19.431±2.591**	8.040±0.089**	12.190±2.441**	8.000±0.000**
8	27.557±1.668	8.000±0.000**	25.698±1.030*	19.622±2.596**	8.047±0.105**	12.214±2.382**	8.000±0.000**

PC: positive control, AptB: Amphotericin B, SDF: silver diamine fluoride, AgF: silver fluoride, KI: potassium iodine, FP: Fluoro Protector®, NC: negative control.
Values are presented as mean±standard deviation.
P-values from repeated-measured ANOVA, the Bonferroni correction (α=0.05).
*P<0.05, **P<0.001.

Table 2. The diameter of inhibition zone by day 1, 7, 14, 21, 28

Time	Diameter (mm)						
(day)	PC	AptB	SDF	AgF	KI	FP	NC
1	26.724±1.476	8.000±0.000**	19.600±2.775**	26.040±1.568	8.134±0.126**	11.644±2.688**	8.000±0.000**
7	27.190±1.487	8.000±0.000**	25.771±1.151	19.583±2.227**	8.021±0.047**	12.285±2.836**	8.000±0.000**
14	27.457±1.350	8.000±0.000**	25.340±1.142*	19.224±2.037**	8.000±0.000**	11.532±1.813**	8.000±0.000**
21	27.650±1.347	8.000±0.000**	25.011±1.247**	19.111±1.995**	8.000±0.000**	11.266±1.839**	8.000±0.000**
28	27.754±1.283	8.000±0.000**	24.689±1.290**	18.963±2.001**	8.000±0.000**	11.059±1.748**	8.000±0.000**

PC: positive control, AptB: Amphotericin B, SDF: silver diamine fluoride, AgF: silver fluoride, KI: potassium iodine, FP: Fluoro Protector®, NC: negative control.
Values are presented as mean±standard deviation.
P-values from repeated-measured ANOVA, the Bonferroni correction (α=0.05).
*P<0.05, **P<0.001.

decrease, and then an increase in inhibition zone diameter on day 4 (P<0.05), which was not statistically different from the PC (P>0.05). AgF showed a significant decrease on day 2 (P<0.001), followed by an increase from day 5 (P<0.001). On day 8, AgF showed significantly smaller inhibition zone diameter than SDF (P<0.001) and significantly larger inhibition zone diameter than FP (P<0.001), indicating antimicrobial activity (Fig. 2).

The 4-week results showed a similar pattern of results. After week 1, PC showed a continuous increase, while the inhibition zone diameters of SDF, AgF, and FP gradually decreased (Fig. 3).

Until week 2, the difference between the inhibition zone diameter of SDF and the PC was not statistically significant (P>0.05), but from week 3, the size of the SDF inhibition zone diameter was significantly smaller than that of the PC (P<0.05).

*3. Trend of Effect by Material (Type * Time)*

The difference in the effect of each materials over the course of one month was also statistically significant (P<0.001).

In Fig. 3, the PC group showed no significant difference between day 1 and week 1, and the effect of the material increased significantly in weeks 3 and 4 compared to week 2, and SDF decreased after weeks 1 and 2. AgF also showed a gradual decrease in ma-

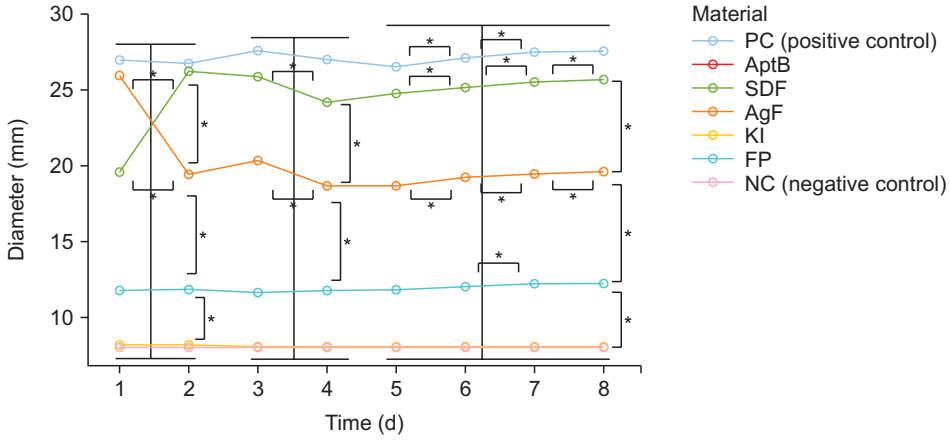

Fig. 2. Changes in the diameter of the zone of inhibition by materials (from day 1 to day 8). AptB: Amphotericin B, SDF: silver diamine fluoride, AgF: silver fluoride, KI: potassium iodide, FP: Fluoro Protector®. P-values from repeated-measured ANOVA. *Statistically significant (P<0.05).

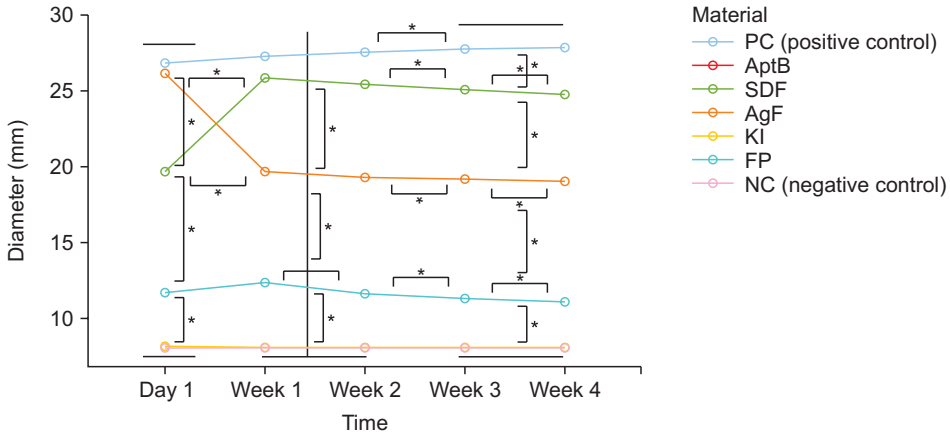

Fig. 3. Changes in the diameter of the zone of inhibition by materials (day 1, from week 1 to week 4). AptB: Amphotericin B, SDF: silver diamine fluoride, AgF: silver fluoride, KI: potassium iodide, FP: Fluoro Protector®. P-values from repeated-measured ANOVA. *Statistically significant (P<0.05).

terial effect after week 2, and FP also showed a decrease from week 1. In conclusion, SDF, AgF and FP showed antimicrobial persistence for 4 weeks, with AgF showing significantly less antimicrobial effect than SDF but significantly more than FP, and there were no significant differences in AptB, KI, and NC by time.

4. SDF Composition Analysis

After combustion ion chromatography analysis, the identified anion was F^- and was 49,303 ppm. Inductively coupled plasma spectrometry revealed 305,983 ppm of Ag in SDF, which is 30.60% of the total content. Therefore, approximately 35.53% of AgF was identified.

Discussion

In the treatment of dental caries, it can be challenging to provide traditional restorative treatment to children or patients with disabilities who require sedation and general anesthesia due to difficulties in behavioral control. Therefore, SDF may be a more effective, safe, and accessible option[12].

Due to its antibacterial properties and low cytotoxicity, SDF has been used in a wide range of applications including dental caries, dental corrosion, gingivitis, dentin hypersensitivity, remineralization, and recently, its use as an endodontic agent has been actively researched. Therefore, various applications of AgF that improve the disadvantages of SDF can be expected[13].

To date, there are two domestic studies on the antibacterial effect of AgF, one of which is a preceding

study of this study, which confirmed the antibacterial power of AgF against *S. mutans* by measuring the diameter of the inhibitory zone. Another study also confirmed that SDF and AgF effectively inhibited the initial biofilm formation of *S. mutans*. It was also found that particles containing silver and fluorine may contribute to the inhibitory effect of biofilm formation by sealing dentinal tubules[10].

Silver ions and fluoride ions contained in SDF play a role in preventing the formation of bacterial membranes on cariogenic teeth. In particular, silver ions have an antimicrobial effect by directly acting on bacteria to destroy bacterial membranes and interfere with metabolism, synthesis, and DNA replication, and fluoride ions at high concentrations also exert their effect by binding to bacterial components and affecting enzyme activity[14,15].

The antimicrobial persistence of SDF against *S. mutans* has been studied. In one study, colony forming unit (CFU)/ml counts of *S. mutans* in saliva following a 30% SDF application in children were assessed at baseline, 24 hours, 30 days, and 90 days, with the lowest CFU counts occurring at 30 days and increasing again at 90 days, but at significantly lower values compared to baseline[16]. A systematic review of the longevity of the antimicrobial effect of SDF on caries-causing bacteria also found that SDF has a maximum antimicrobial effect during the period from 3 to 30 days after application[17].

To our knowledge, there is still a lack of research on the antimicrobial longevity of AgF, which is why this study was conducted.

In our experiments, SDF showed antibacterial activity that was not statistically different from the PC up to 2 weeks ($P>0.05$), and showed significant antibacterial activity from week 3 ($P<0.05$).

SDF and AgF showed a significant difference in antimicrobial activity at all measurement periods ($P<0.05$). The difference in antibacterial activity between SDF and AgF was thought to be due to the difference in the content of silver and fluoride ions, so we further analyzed the composition. In the previous study of this study, silver ions in AgF of Riva star aqua™ were 266,477 ppm (26,65%) and fluoride ions were 42,429 ppm (4.24%), resulting in 30.89% AgF[11], and in the composition analysis of SDF in this experiment, the content of silver ions and fluoride ions in SDF was confirmed to be 35.53%, showing difference in amount.

FP showed a marginal difference compared to KI on day 1 ($P=0.052$), but a significant difference for NC and AptB ($P<0.05$) and a significant difference in antimicrobial activity at all subsequent measurement periods ($P<0.005$).

AptB is an antifungal agent, and some studies have shown that some antifungal agents can inhibit the growth and biofilm formation of *S. mutans* and block virulence factors, so it was placed in the medium to check[18], but it did not show antibacterial power and was classified as a NC. KI, the second agent of Riva star™ and Riva star aqua™, which is used to improve the coloring of SDF and AgF, also did not show antibacterial activity.

These results confirm that the two liquid fluoride varnishes have antimicrobial persistence against *S. mutans* and suggest that AgF may be an effective treatment option for early caries, but further studies are needed to determine the minimum inhibitory concentration and minimum bactericidal concentration for actual clinical application.

Limitations of this study include the fact that it was not conducted under conditions similar to the actual oral environment where a variety of bacterial strains are present, and SDF also requires a longer-term study to determine the frequency of application, as previous clinical studies have shown that annual application of SDF is more effective in preventing remineralization and caries progression compared to 3-month application of fluoride varnish[19].

Recently, studies on the antibacterial effect of adding nano silver particles to the denture base have been actively conducted, and one study confirmed

the antibacterial effect against *S. mutans*, *Candida albicans*, and *Escherichia coli*[20]. In addition, many studies have been conducted on the association between *C. albicans*, the main causative agent of candidiasis, and early childhood caries (ECC). It has been reported that *C. albicans* is found in children with ECC and that the incidence of caries is higher in children with *C. albicans* than in children without[21,22]. Therefore, based on this experimental study, we will confirm the antibacterial activity and antibacterial persistence of SDF and AgF against *C. albicans*, and conduct further studies on various strains.

Conclusion

In this study, SDF and AgF showed antimicrobial activity against *S. mutans* that lasted for 4 weeks, and the antimicrobial activity of AgF was less than that of SDF but significantly greater than that of FP. This was attributed to the difference in fluoride and silver ion content of SDF and AgF.

The above results confirmed that the two liquid fluoride varnishes showed antibacterial persistence against *S. mutans*, but further studies are needed for actual clinical application.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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