A Concise Review of Silver Diamine Fluoride on Oral Biofilm

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Review

A Concise Review of Silver Diamine Fluoride on Oral Biofilm

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Abstract: Studies have shown that silver diamine fluoride (SDF) is an effective agent to arrest and prevent dental caries due to its mineralizing and antibacterial properties. While plenty of studies have investigated the mineralizing properties, there are few papers that have examined its antibacterial effect on oral biofilm. The objective of this study was to identify the effect of silver diamine fluoride on oral biofilm. Method: The keywords used were (silver diamine fluoride OR silver diammine fluoride OR SDF OR silver fluoride OR AgF AND biofilm OR plaque). Two reviewers screened the titles and abstracts and then retrieved the full text of the potentially eligible publications. Publications of original research investigating the effect of SDF on oral biofilm were selected for this review. Results: This review included 15 laboratory studies and six clinical studies among the 540 papers identified. The laboratory studies found that SDF could prevent bacterial adhesion to the tooth surface. SDF also inhibited the growth of cariogenic bacteria, including *Streptococcus mutans*, *Lactobacillus acidophilus*, *Streptococcus sobrinus*, *Lactobacillus rhamnosus*, *Actinomyces naeslundii*, and *Enterococcus faecalis*, thus contributing to its success in caries arrest. One clinical study reported a decrease in *Streptococcus mutans* and *Lactobacillus* sp. in arrested caries after SDF treatment, and another clinical study found that SDF inhibited the growth of periodontitis microbiota, including *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Prevotella intermedia/nigrescens*. However, three clinical studies reported no significant change in the microbial diversity of the plaque on the tooth after SDF treatment. Moreover, one laboratory study and one clinical research study reported that SDF inhibited the growth of *Candida albicans*. Conclusion: Not many research studies have investigated the effects of SDF on oral biofilm, although SDF has been used as a caries-arresting agent with antibacterial properties. However, a few publications have reported that SDF prevented bacterial adhesion to the teeth, inhibited the growth of cariogenic and periodontal bacteria, and possessed antifungal properties.

Keywords: silver diamine fluoride; SDF; silver fluoride; AgF; biofilm; plaque

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1. Introduction

The oral microbiota colonizes oral biofilm on the surface of the tooth or on the mucosa within the mouth. The accumulation of oral biofilm can lead to oral diseases such as dental caries or periodontitis [1]. Oral biofilm can lead to dental caries in two ways. First, bacteria within the oral biofilm produce acid through sugar metabolism. Second, the acid causes a subsequent decrease in the environmental pH value. Both of the above are responsible for the demineralization of the tooth surface and the formation of dental caries [2]. The amount of tooth mineral and other calcium phosphates in the plaque fluid decreases rapidly after exposure to fermentable carbohydrates. Lactic acid production and a reduction in the plaque fluid volume can result in the formation of caries [3].

Acid-base-producing bacteria, in varying numbers and proportions, are the key pathogens associated with dental caries [4]. Among the suspected acid-producing bacteria, *Mutans streptococci* (MS) has been confirmed, in a systematic literature review, to play a central role in the initiation of dental caries on both enamel and root surfaces [5]. This is the case for several reasons: first, MS is the most frequently isolated species from a caries

lesion; second, MS is highly acidogenic and aciduric [6,7]; and third, MS can produce surface antigens I/II and water-insoluble glucan, which promote bacterial adhesion to the tooth surface and to other bacteria [6]. For the purpose of halting the closed circle of the process, an intervention that can inhibit the bacteria that produce acid is needed.

Silver diamine fluoride (SDF) has been widely used to prevent and arrest caries, and "silver diamine fluoride" is the most common spelling/keyword for this compound in the dental literature. SDF was approved for use as a therapeutic agent in Japan over 50 years ago [8]. It has also been used for dental caries treatment in Australia and in some countries in Latin America such as Argentina and Brazil for many years [9]. The Food and Drug Administration approved the marketing of SDF in the United States for the treatment of human dentinal hypersensitivity in adults in 2014 [10].

SDF contains silver and fluoride, which form a complex with ammonia in a colorless alkaline solution [9]. It is not only a combination of silver salt, ammonium, and fluoride ions but also a mixed heavy-metal halide co-ordination complex. Ammonia can provide an alkaline environment, thus keeping the solution constant for a certain period of time [11]. In addition, silver compounds have been reported to have antimicrobial properties in the application of medicine and dentistry for decades [12]. Fluoride is also used in various forms to prevent and arrest caries due to its remineralization function [9]. Therefore, SDF has been hypothesized to contain the combined effects of silver and fluorides.

Fluoride can inhibit the production and metabolic activity of certain strains of bacteria within biofilm at low concentrations [13]. At high concentrations, fluoride inhibits the growth of cariogenic bacteria in dental plaque [14]. One mechanism of biofilm inhibition is to bind its ions to the bacterial cell constituents and to influence enzymes such as enolase and proton-extruding adenosine triphosphatase [15]. Another mechanism is the inhibition of the carbohydrate metabolism of acidogenic oral bacteria [16,17]. However, this may contribute to the development of bacterial tolerance and shorten the duration of the antibacterial effect [18]. Nonetheless, the dominant advantage of fluoride is that the re-mineralizing effect, particularly for the SDF solution, effectively arrests dental caries [19].

Several reviews have been conducted regarding the clinical use of SDF for preventing and arresting dental caries with significant effect [20,21]. However, the rationale behind the impact of SDF on biofilm remains unclear. The aim of this systematic review was to identify the effect of SDF on biofilm. The study question was as follows: What is the effect of SDF on biofilm?

2. Materials and Methods

Two independent reviewers (Zhang and Got) conducted a literature search using the four most commonly used databases: Medline, PubMed, Embase, and Scopus. Only papers published in English were included. The keywords used were (silver diamine fluoride OR silver diamine fluoride OR SDF OR silver fluoride OR AgF AND biofilm OR plaque). No publication date limitation was established during the search. The latest search was conducted on 30 October 2020.

The inclusion criteria were as follows: (1) papers investigating biofilm; (2) SDF solution at any concentration adopted as an intervention. The exclusion criteria were (1) studies with no information concerning biofilm and (2) reviews, case reports, and conference papers. In addition, a manual search of the reference lists of the selected papers and review articles was conducted. The two reviewers independently screened the papers and identified the relevant studies. When a disagreement occurred, another researcher (Chu) was consulted to achieve a consensus.

The results were summarized into three tables classified by the aim of the studies: Table 1 is for the studies investigating the effect of SDF on biofilm formed via microbiota, Table 2 is for the studies on the diversity and microbial change resulting from the application of SDF, and Table 3 is for the studies focused on the effect of SDF on fungus. Information regarding the following aspects of the background of the included studies was extracted and is presented in the summary tables: (1) authors and year; (2) materials studied;

(3) setting of the study; (4) block (or sample) adopted in the study; (5) microbiota studied; (6) assessing method for the biofilm; (7) duration of the study; and (8) findings of the study.

Table 1. Studies on the antibacterial effect of silver diamine fluoride (SDF).

Author, Year	Setting; Substrate	Microbiota	Period; Assessment	Intervention	Antibacterial Effect
Hiraishi et al., 2010	In vitro; human dentin	*E. faecalis*	15 min, 60 min; CFU	Gp1: $Ca(OH)_2$ Gp2: SDF Gp3: NaOCl Gp4: NaCl	CFU 15 min Gp2, Gp3 < Gp1, Gp4 CFU 60 min: G2, Gp3 had no *E. faecalis*
Chu et al., 2012	In vitro; human dentin	*S. mutans*, *A. naeslundii*	7 days; CFU, CLSM	Gp1: SDF Gp2: Water	CFU: Gp1 < Gp2 CLSM: Gp2 < Gp1
Mei et al., 2013a	In vitro; human dentin	*S. mutans* *L. acidophilus*	7 days; CFU, CLSM	Gp1: SDF Gp2: Water	CFU: Gp1 < Gp2 CLSM: Gp2 < Gp1
Mei et al., 2013b	In vitro; human dentin	*Consortium of S. mutans, S. sobrinus, L. acidophilus, L. rhamnosus, A. naeslund*	7 days, 14 days, 21 days; CFU, CLSM	Gp1: SDF Gp2: Water	CFU: Gp1 < Gp2 CLSM: Gp2 < Gp1
Shah et al., 2013	In vivo	*S. mutans*	BL, 3 days, 6 months, 12 months, 18 months; CFU	Gp1: SDF Gp2: NaF + CaF Gp3: APF gel	CFU: Gp1 < Gp2 Gp1 = Gp3
Savas et al., 2015	In vitro; bovine enamel	*S. mutans*	7 days; TBC, pH	Gp1: Water Gp2: SDF	Antibacterial activity: Gp2 > Gp1
Göstemeyer et al., 2017	In vitro; bovine dentine	*L. rhamnosus*	6 days; CFU	Gp1: Water Gp2: SDF	CFU: Gp2 < Gp1
Soekanto et al., 2017	In vitro	*S. mutans*, *E. faecalis*	1 day; MIC, MBC	Gp1: SDF Gp2: NSF Gp3: PPF	MIC: Gp2: 3%-*S.mutans*, 3%-*E.faecalis*; Gp3: 3%-*S.mutans*, 6%-*E.faecalis*; MBC: Gp2: 4%-*S.mutans*, 4%-*E.faecalis*; Gp3: 10%-*S.mutans*
Göstemeyer et al., 2018	In vitro; bovine dentin	*L. rhamnosus*	12 days; CFU	Gp1: SDF Gp2: CHX Gp3: N/T	CFU: No significant difference
Vinson et al., 2018	In vitro	*S. mutans*	1 day; CFU	Gp1: SDF Gp2: SDF + KI Gp3: KI	CFU: Gp3 > Gp2 > Gp1
Yu et al., 2018	In vitro; human dentin	*S. mutans*	7 days; CFU, CLSM	Gp1: SDF + NaF Gp2: SDF Gp3: NaF Gp4: Water	CFU: Gp2 < Gp1 < Gp4 < Gp3 CLSM: Gp2 > Gp1 > Gp3,Gp4
Al-Madi et al., 2019	In vitro; human dentin	*E. faecalis*	21 days; CLSM	Gp1: SDF Gp2: CHX Gp3: NaOCl	CLSM: Gp3 >Gp1 > Gp2
Wu et al., 2019	In vitro	*S. mutans*	4 days: inhibition zone; 7 days: biofilm assay	Gp1: SDF Gp2: No silver Gp3: $AgNO_3$	Inhibition zone: Gp1,Gp3 > Gp2, Antibacterial activity: Gp1 > Gp2

Table 1. *Cont.*

Author, Year	Setting; Substrate	Microbiota	Period; Assessment	Intervention	Antibacterial Effect
Sorkhdini et al., 2020	In vitro; human enamel	*S. mutans*	3 days; CFU	Gp1: SDF Gp2: SDF + KI Gp3: $AgNO_3$ Gp4: Water	CFU:Gp1 < Gp2 < Gp3 < Gp4
Rams et al., 2020	In vitro	Plaque from adults with SP	7 days; CFU, MALDI-TOF	Gp1: SDF Gp2: N/T	TVC: Gp1 < Gp 2 PLPP: Gp1 < Gp 2

Abbreviations: Gp, group; CFU, colony forming unit; CLSM, confocal laser scanning microscopy (dead-to-live ratio); NaCl, sodium chloride; BL, baseline; $Ca(OH)_2$, calcium hydroxide; NaOCl, sodium hypochlorite; TBC, total bacteria count; APF, acidulated phosphate fluoride; AHF, ammonium hexafluorosilicate; CPC, cetylpyridinium chloride; CHX, chlorhexidine; NaF, sodium fluoride; N/T, no treatment; NSF, nano silver fluoride; PPF, propolis fluoride; KI, potassium iodide; $AgNO_3$,silver nitrate; SP, severe periodontitis; TVC, total viable counts; PLPP, proportional levels of periodontal pathogens; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.

Table 2. Microbiota change within biofilm after silver diamine fluoride treatment.

Authors, Year	Setting; Plaque Sample	Microbiome Detecting Method; Time Points	Diversity Assessing Method or Index	Main Findings
Milgrom et al., 2018	In vivo; plaque from children	RNA sequencing; BL, follow-up after 14 to 21 days	LME4 (R core team)	*S. mutans* and *lactobacilli*: in all samples except one; Cariogenic bacteria: no significant changes; Diversity analyses: no significant differences
Mitwalli et al., 2019	In vivo; plaque from adults	16S rDNA PCR; BL, 1 month	Shannon–Weaver index, Fisher's alpha	Reduced: *Actinomyce sp.*, *P. acidifaciens*, *S. inopinata*, *Propionibacterium sp.*, *T. denticola*, *B. dentium*, *P. denticolens*, *A. israelii*; Diversity analyses: no significant difference pre- and post-intervention
Mei et al., 2020	In vivo; plaque from children	16S rRNA gene sequencing; BL, 2 weeks, 12 weeks	Shannon index	Active caries: reduced diversity 12 weeks after SDF; *S. mutans*, *Lactobacillus* sp. increased; Arrested caries: *S. mutans*, *Lactobacillus* sp. reduced; Diversity analyses: no significant change before and after 2 or 12 weeks in active caries
Liu et al., 2020	In vitro; plaque from children	16S rRNA gene sequencing; BL, 1 day, 7 days	Shannon index	Diversity analyses: no significant change in plaque; Reduction in carbohydrate transportation and metabolic functions in plaque at 1 day and 7 days post-intervention

Abbreviations: BL, baseline; LMER, linear mixed effects regression; OTU, operational taxonomic unit.

Table 3. Studies on the antifungal effect of silver diamine fluoride.

Authors, Year	Setting; Substrate	*Candida* Species	Period; Assessment	Intervention Group	Main Findings
Alshahni et al., 2020	In vitro; human dentin	*C. albicans*	3 days; CFU, CQ, Rt-PCR, SEM	Gp1: 3.8% SDF Gp2: 38% SDF Gp3: No treatment	CFU: Gp1,Gp2 < Gp3 CQ: Gp1,Gp2 < Gp3 Rt-PCR: Gp1,Gp2 < Gp3 SEM: Cell wall damage in Gp1, Gp2
Fakhruddin et al., 2020	In vitro; paper disc	*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. dubliniensis*	2 days; ZGI, Multi-PCR, MIC	Gp1: Amphotericin B Gp2: Fluoconazole Gp3: SDF complex	Gp3: anti-candidal potency; UI: Cell wall damage in Gp3; ZGI: *C. tropicalis:* Gp3 < *Gp1,Gp2*; *C. krusei, C. glabrata, C. albicans: Gp3 > Gp1,Gp2*

Abbreviations: CQ, colorimetric quantification; Rt-PCR, real-time PCR-based quantification; SEM, scanning electron microscopy; ZGI, zones of growth inhibition; Multi-PCR, multiplex PCR; MIC, minimal inhibitory concentration; UI, ultrastructural image.

3. Results

A total of 540 potentially relevant records were identified through the database search, and 194 were left after the removal of duplicates. A manual search of the references of these selected publications was conducted. One article that met the inclusion criteria was added to the list. After the titles and abstracts were screened, 133 records were excluded. The remaining 62 papers, all published in English, were retrieved for full-text reading. Among them, 20 articles did not report microbial analysis and 21 did not contain biofilm or microbiome information.

Among the 21 papers included, six were laboratory studies and 15 were clinical studies. The 21 included papers were published between 2010 and 2020. An SDF solution concentration of 38% was reported in 16 studies, followed by 3.8% SDF in three studies and 25% SDF, or 8.5 wt%, in the other studies. Figure 1 is a flowchart showing the search results.

3.1. Application of Silver Diamine Fluoride on Bacteria

3.1.1. Antibacterial Effect

A total of 15 studies reporting the antibacterial effects of SDF on bacteria are summarized in Table 1. Among them, 14 studies measured the antibacterial properties using monospecies bacteria such as *Lactobacillus acidophilus*, *Streptococcus sobrinus*, *Enterococcus faecalis*, and *Actinomyces naeslundii*. However, subgingival microbial biofilm specimens were used on 24 adults with severe periodontitis [22]. The majority of the included studies that explored bactericidal properties were operated in vitro on dentin or enamel samples, with no difference in the effect regarding the sample material. Only one research study was conducted in vivo [23]. The longest study duration was 21 days, whereas the shortest one was 14 min. They also revealed that biofilm treated with SDF had fewer bacteria compared with that treated with water or other interventions. However, one study did not detect any significant difference among the study groups [24]. Among the included studies, eight out of nine studies reported that SDF inhibited the growth of *Streptococcus mutans* through colony-forming unit counts. Two studies revealed that SDF also had an antibacterial function in *Lactobacillus acidophilus* and *Actinomyces naeslundii*. In addition, only one out of the two studies reported that SDF reduced the amount of *Enterococcus faecalis* [25] while the other reported reduced *Lactobacillus rhamnosus* [26]. One study reported that a 38% and a 19% SDF solution inhibited cultivable bacteria without any significant difference [27]. Only *P. micra* and *S. constellatus* belong to red- and orange-complex species recovered from SDF-treated specimens.

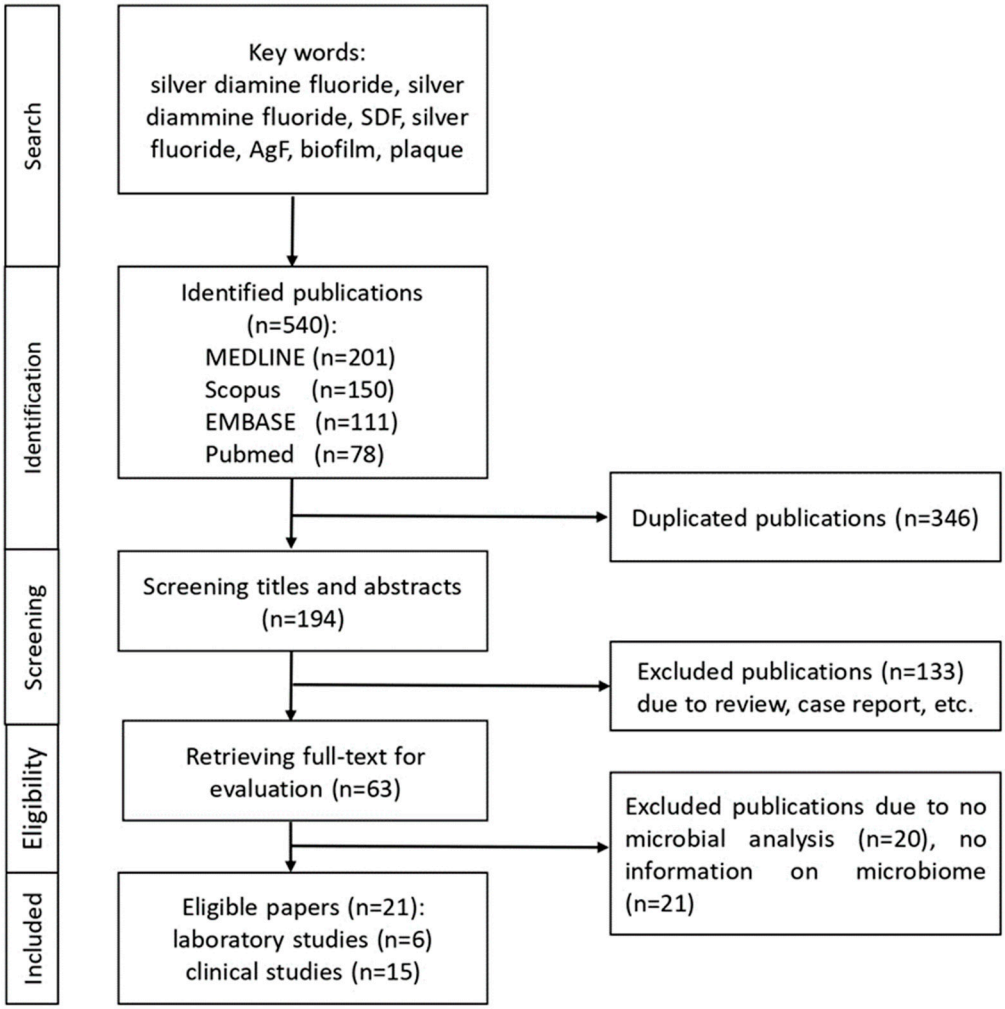

Figure 1. Flowchart of the study selection process: identification, selection, eligibility, included.

Furthermore, some studies reported that the live-to-dead ratios of *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Actinomyces naeslundii* in biofilm were significantly lower after the application of SDF [23–27]. Only Al-Madi and co-workers reported that a 5.25% sodium hypochlorite solution had a significantly higher live-to-dead ratio of *Enterococcus faecalis* compared with the study's SDF group [27]. Only one study reported that the antibacterial effects of nano silver fluoride varnish and the potency of propolis fluoride varnish were comparable with a 38% SDF varnish. Another study compared the minimum inhibitory concentration and the minimum bactericidal concentration of propolis fluoride and nano silver fluoride for the inhibition of biofilm formation [28].

3.1.2. Microbiota Change in Community Diversity and/or Composition within Biofilm

A total of four studies reported on the microbiota diversity before and after SDF treatment. In terms of intervention with SDF solution, only a 38% concentration was adopted in all four studies (Table 2). The majority of the included studies that explored bactericidal properties were operated in vivo, with one study conducted in vitro; it used extracted caries teeth in an artificial mouth model [29]. Both saliva and plaque were collected in two studies [29,30], whereas only plaque was collected in the other two studies [31,32]. Two studies were conducted on preschool children, whereas one was conducted on primary school students [29], and another was conducted on adults with a mean age of 47 years [32]. The shortest study duration was 24 h, whereas the longest was 12 weeks. Among the included studies, three out of the four used the polymerase chain reaction amplification of 16S ribosomal ribonucleic acid genes and MiSeq sequencing in diversity analyses. One

study used ribosomal ribonucleic acid sequencing and quantitative polymerase chain reaction quantification [31]. The Shannon index was the most frequently used index for diversity assessment.

All four studies reported no significant change in diversity. However, one study found a significant decrease in the diversity of oral bacteria plaque within active caries, but not in arrested caries, 12 weeks after SDF treatment [30]. Furthermore, the same paper reported an alteration in the species of the bacteria both before and after the SDF treatment. *Streptococcus mutans* and *Streptococcus sobrinus* increased significantly after two and 12 weeks of the SDF treatment in the plaque within active caries. Meanwhile, *Lactobacillus* sp. and *Rothia* sp. increased significantly after two weeks. Another study reported that the carbohydrate transportation and metabolic functions in plaque were significantly reduced at 24 h and one week post-intervention [29].

3.2. Application of Silver Diamine Fluoride on Fungus

Two studies explored the anti-candidal effect of SDF [16,33] (Table 3). One study of *Candida albicans* on a dentin block taken from human teeth reported that the anti-candidal effect was a dose-related property. No significant differences were found between 3.8% and 38% SDF solutions for the colony-forming unit counts, the colorimetric quantification of *Candida*, the real-time polymerase chain reaction-based quantification, and the scanning electron microscopy. The 38% SDF caused severe damage to candida cell walls, whereas the 3.8% SDF caused only partial damage. In another study, *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, and *Candida dubliniensis* were isolated from the carious lesions of preschool children. Zones of growth inhibition, a multiplex polymerase chain reaction, and minimal inhibitory concentrations (MIC50 and MIC90) were adopted for assessing the anti-candidal effect of SDF and the controls. *C. tropicalis* was more resistant to SDF based on the diameter of the inhibitory zone of the culture growth. Meanwhile, *C. krusei* and *C. glabrata* were more sensitive to SDF.

4. Discussion

This review is the first review concerning the effect of SDF applied directly to biofilm. It is based on the count of bacteria and fungi as well as the diversity within the biofilm. A large number of reviews have reported the effectiveness of SDF for preventing and arresting caries in clinical studies, and its effect was reported as extraordinary, mostly due to its antibacterial function and remineralization property [20].

Although no limitation was set regarding the publication year during the search, the earliest study found was published in 2010. This shows a new trend where research is being turned into laboratory work exploring the reason for SDF's effectiveness in preventing and arresting caries. The majority (15/21, 71%) of the included studies were published between 2016 and 2020. SDF solutions featuring different concentrations [22,34] or combined with different materials [17,35,36] were adopted to investigate their potential use for antibacterial purposes.

SDF has an antibacterial function because both the silver ions and the fluoride contained in SDF appear to have the ability to inhibit the formation of cariogenic biofilm [37]. A 38% SDF solution contains approximately 253,870 ppm silver and 44,800 ppm fluoride ions [38]. The microorganisms can be killed and silver ions can interfere with metabolic processes [12]. A three-pronged approach exists for silver ions to kill the microbiota: damage the cell wall structure of bacteria, influence metabolic processes and inhibit enzyme activities, and, finally, inhibit the replication of bacterial deoxyribonucleic acid [12]. In addition, it has been suggested that silver ions at a concentration of 20 ppm can inhibit the growth of *Streptococcus mutans* [39]. It has also been reported that the antimicrobial effect stems from the silver ions, especially at low concentrations [40]. Furthermore, the antimicrobial function of a high concentration of fluoride cannot be ignored [41]. The reason for this is that a high concentration of fluoride can influence enzymes' carbohydrate metabolism and sugar uptake and bind the bacterial cellular components, resulting in

the inhibition of biofilm formation [37]. However, only one study reported no significant difference in the count of *Lactobacillus rhamnosus* among the 38% SDF group and the other groups (35% chlorhexidine varnish, 5% sodium fluoride varnish, 500 ppm sodium fluoride solution + 0.1% chlorhexidine solution, and the blank control) [26]. The possible reason for this may be that the formation of the SDF adopted in the study was a varnish rather than a solution, which indicates that the reduction in bioactive silver ions was not the same as that in the 38% SDF solution.

According to this review, even though SDF inhibits some oral microbiota species, the diversity of the biofilm does not seem to change before and after SDF application. One possible reason for this is that SDF only reduces the number of carious species, for example, *Streptococcus mutans* and *Lactobacillus*, rather than inhibiting all of the microbiota species. In the caries process, *Streptococci*, *Lactobacilli*, and *Actinomycetes* are treated as the initial bacterial invasion species. Among them, *Streptococcus mutans* is one of the most important pathogens associated with the initiation and progression of caries [42]. *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* are routinely found in deep and superficial carious lesions, which indicates that they are the most abundant bacteria species [43,44]. *Actinomyces naeslundii* is related to root caries, which have the potential to invade dentinal tubules [15]. After the results of the studies were analyzed, it was found that the possible mode of action of SDF can be related to its antibacterial properties on cariogenic bacteria rather than a change in the diversity of the biofilm. The microbiota appeared to reach a new balance, but with fewer carious species [45].

According to this review, SDF also has antifungal properties. As discussed above, the bioactive form of silver in SDF in its ionized form is "Ag+". It has been reported that silver particles can inhibit the growth of *C. albicans* under high concentrations [46]. One of the possible reasons for this is that silver ions can also suppress extracellular phospholipase production, which plays a crucial role in the pathogenicity of *C. albicans* [47]. Phospholipase is associated with the development of hyphae, which plays an essential role in the biofilm's adherence and formation [48]. The blocking transformation from a yeast form to a hyphal form could stop colonization and initiation or pathogenesis. However, variations in inhibition still exist among different species in the yeast. Another possible explanation for this could be the avidity of the biological ligands of the yeast to SDF [49].

Based on the review, the most frequently used approach for assessing biofilm is still the colony-forming unit count and the live-to-dead ratio in the traditional culture method. New molecular biological technology is being adopted into assessments, usually combined with traditional ones. The majority of the studies included in this review were conducted in vitro. As the duration of the selected laboratory studies was relatively short, the long-term caries-arresting effect and the periodicity of the SDF application could not be evaluated. It was not the objective of this review to judge the quality of the studies or to discuss the limitations of each study. This should be taken into consideration when interpreting the results as well as the conclusions of this review.

This concise review was intended to provide the best evidence regarding SDF's antimicrobial effect, as presented in 21 selected papers, to obtain a definitive answer to a research question involving antibacterial function. It provides an overview and updated information about the antimicrobial effect of SDF on oral biofilm, as well as its known role in the inhibition of cariogenic bacteria. The major lines of investigation in this review involved 15 laboratory studies and six clinical studies. Some data are quite limited due to the lack of studies on certain topics such as the antibacterial effect of SDF on periodontal pathogens. As a whole, this field shows significant gaps, with only one study so far providing limited information [22]. In addition, only two published papers have shown promising results for SDF's antifungal effect against candida [16,33]. However, overall, this review provides strong evidence that SDF can, indeed, inhibit the growth of cariogenic bacteria and prevent bacterial adhesion to the tooth surface. This review—the first concise review of the effect of SDF applied directly to biofilm—can be used to guide future research in this area.

Even though SDF has been used for decades, its properties remain under-investigated. Thus far, most laboratory studies have focused on its remineralization properties rather than its antimicrobial functions within biofilm. It has been suggested that the concentrations of antibacterial agents required to inhibit biofilm are more than 100 times higher than those needed to inhibit planktonic bacteria, as biofilm is more resistant to antimicrobial agents than planktonic bacteria are [12]. More studies exploring the rationale behind these materials are still needed.

5. Conclusions

The number of publications exploring the effects of SDF on oral biofilm is limited, although SDF has been used as a caries-arresting agent with antibacterial properties. The limited publications reported that SDF prevented bacterial adhesion to teeth, inhibited the growth of cariogenic and periodontal bacteria, and possessed antifungal properties.

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References

1. Washio, J.; Mayanagi, G.; Takahashi, N. Challenge to metabolomics of oral biofilm. *J. Biosci.* **2010**, *52*, 225. [CrossRef]
2. Takahashi, N.; Nyvad, B. The role of bacteria in the caries process: Ecological perspectives. *J. Dent. Res.* **2011**, *90*, 294–303. [CrossRef]
3. Margolis, H.; Moreno, E. Composition and cariogenic potential of dental plaque fluid. *Crit. Rev. Oral Biol. Med.* **1994**, *5*, 1–25. [CrossRef]
4. Kleinberg, I. A mixed-bacteria ecological approach to understanding the role of the oral bacteria in dental caries causation: An alternative to Streptococcus mutans and the specific-plaque hypothesis. *Crit. Rev. Oral Biol. Med.* **2002**, *13*, 108–125. [CrossRef]
5. Tanzer, J.M.; Livingston, J.; Thompson, A.M. The microbiology of primary dental caries in humans. *J. Dent. Educ.* **2001**, *65*, 1028–1037. [CrossRef]
6. Hamada, S.; Slade, H.D. Biology, immunology, and cariogenicity of Streptococcus mutans. *Microbiol. Rev.* **1980**, *44*, 331–384. [CrossRef]
7. Loesche, W.J. Role of Streptococcus mutans in human dental decay. *Microbiol. Rev.* **1986**, *50*, 353–380. [CrossRef]
8. Yamaga, R.; Yokomizo, I. Arrestment of caries of deciduous teeth with diamine silver fluoride. *Dent. Outlook* **1969**, *33*, 1007–1013.
9. Chu, C.H.; Lo, E.C.M. Promoting caries arrest in children with silver diamine fluoride: A review. *Oral Health Prev. Dent.* **2008**, *6*, 315–321.
10. Horst, J.A. Silver fluoride as a treatment for dental caries. *Adv. Dent. Res.* **2018**, *29*, 135–140. [CrossRef]
11. Mei, M.L.; Lo, E.C.M.; Chu, C.H. Clinical use of silver diamine fluoride in dental treatment. *Compend. Contin. Educ. Dent.* **2016**, *37*, 93–98. [PubMed]
12. Peng, J.Y.; Botelho, M.; Matinlinna, J. Silver compounds used in dentistry for caries management: A review. *J. Dent.* **2012**, *40*, 531–541. [CrossRef]
13. Nassar, H.M.; Gregory, R.L. Biofilm sensitivity of seven Streptococcus mutans strains to different fluoride levels. *J. Oral Microbiol.* **2017**, *9*, 1328265. [CrossRef] [PubMed]
14. Pandit, S.; Kim, J.E.; Jung, K.H.; Chang, K.W.; Jeon, J.G. Effect of sodium fluoride on the virulence factors and composition of Streptococcus mutans biofilms. *Arch. Oral Biol.* **2011**, *56*, 643–649. [CrossRef] [PubMed]
15. Chu, C.H.; Mei, L.; Seneviratne, C.J.; Lo, E.C.M. Effects of silver diamine fluoride on dentine carious lesions induced by Streptococcus mutans and Actinomyces naeslundii biofilms. *Int. J. Paediatr. Dent.* **2012**, *22*, 2–10. [CrossRef]
16. Fakhruddin, K.S.; Egusa, H.; Ngo, H.C.; Panduwawala, C.; Pesee, S.; Venkatachalam, T.; Samaranayake, L.P. Silver diamine fluoride (SDF) used in childhood caries management has potent antifungal activity against oral Candida species. *BMC Microbiol.* **2020**, *20*, 95. [CrossRef]
17. Yu, O.Y.; Zhao, I.S.; Mei, M.L.; Lo, E.C.M.; Chu, C.H. Caries-arresting effects of silver diamine fluoride and sodium fluoride on dentine caries lesions. *J. Dent.* **2018**, *78*, 65–71. [CrossRef]
18. Lu, M.; Xiang, Z.; Gong, T.; Zhou, X.; Zhang, Z.; Tang, B.; Zeng, J.; Wang, L.; Cui, T.; Li, Y. Intrinsic fluoride tolerance regulated by a transcription factor. *J. Dent. Res.* **2020**, *99*, 1270–1278. [CrossRef]

19. Mei, M.L.; Lo, E.C.M.; Chu, C.H. Arresting dentine caries with silver diamine fluoride: What's behind it? *J. Dent. Res.* **2018**, *97*, 751–758. [CrossRef]
20. Gao, S.S.; Zhao, I.S.; Hiraishi, N.; Duangthip, D.; Mei, M.L.; Lo, E.C.M.; Chu, C.H. Clinical trials of silver diamine fluoride in arresting caries among children: A systematic review. *JDR Clin. Trans. Res.* **2016**, *1*, 201–210. [CrossRef]
21. Hendre, A.D.; Taylor, G.W.; Chávez, E.M.; Hyde, S. A systematic review of silver diamine fluoride: Effectiveness and application in older adults. *Gerodontology* **2017**, *34*, 411–419. [CrossRef] [PubMed]
22. Rams, T.E.; Sautter, J.D.; Ramírez-Martínez, G.J.; Whitaker, E.J. Antimicrobial activity of silver diamine fluoride on human periodontitis microbiota. *Gen Dent.* **2020**, *68*, 24–28.
23. Shah, S.; Bhaskar, V.; Venkataraghavan, K.; Choudhary, P.; Ganesh, M.; Trivedi, K. Efficacy of silver diamine fluoride as an antibacterial as well as antiplaque agent compared to fluoride varnish and acidulated phosphate fluoride gel: An in vivo study. *Indian J. Dent. Res.* **2013**, *24*, 575–581. [CrossRef] [PubMed]
24. Göstemeyer, G.; Kohls, A.; Paris, S.; Schwendicke, F. Root caries prevention via sodium fluoride, chlorhexidine and silver diamine fluoride in vitro. *Odontology* **2018**, *106*, 274–281. [CrossRef] [PubMed]
25. Hiraishi, N.; You, C.K.; King, N.M.; Tagami, J.; Tay, F.R. Antimicrobial efficacy of 3.8% silver diamine fluoride and its effect on root dentin. *J. Endod.* **2010**, *36*, 1026–1029. [CrossRef] [PubMed]
26. Göstemeyer, G.; Schulze, F.; Paris, S.; Schwendicke, F. Arrest of root carious lesions via sodium fluoride, chlorhexidine and silver diamine fluoride in vitro. *Materials* **2017**, *11*, 9. [CrossRef] [PubMed]
27. Al-Madi, E.M.; Al-Jamie, M.A.; Al-Owaid, N.M.; Almohaimede, A.A.; Al-Owid, A.M. Antibacterial efficacy of silver diamine fluoride as a root canal irrigant. *Clin. Exp. Dent. Res.* **2019**, *5*, 551–556. [CrossRef]
28. Soekanto, S.A.; Marpaung, L.J.; Himmatushohwah Djais, A.A.; Rina, R. Efficacy of propolis fluoride and nano silver fluoride for inhibition of streptococcus mutans and enterococcus faecalis biofilm formation. *Int. J. App. Pharm.* **2017**, *9*, 51–54. [CrossRef]
29. Liu, B.Y.; Mei, L.; Chu, C.H.; Lo, E.C.M. Effect of silver fluoride in preventing the formation of artificial dentinal caries lesions in vitro. *Chin. J. Dent. Res.* **2019**, *22*, 273–280.
30. Mei, M.L.; Yan, Z.; Duangthip, D.; Niu, J.Y.; Yu, O.Y.; You, M.; Lo, E.C.M.; Chu, C.H. Effect of silver diamine fluoride on plaque microbiome in children. *J. Dent.* **2020**, *102*, 103479. [CrossRef]
31. Milgrom, P.; Horst, J.A.; Ludwig, S.; Rothen, M.; Chaffee, B.W.; Lyalina, S.; Pollard, K.S.; DeRisi, J.L.; Mancl, L. Topical silver diamine fluoride for dental caries arrest in preschool children: A randomized controlled trial and microbiological analysis of caries associated microbes and resistance gene expression. *J. Dent.* **2018**, *68*, 72–78. [CrossRef]
32. Mitwalli, H.; Mourao, M.D.A.; Dennison, J.; Yaman, P.; Paster, B.J.; Fontana, M. Effect of silver diamine fluoride treatment on microbial profiles of plaque biofilms from root/cervical caries lesions. *Caries Res.* **2019**, *53*, 555–566. [CrossRef] [PubMed]
33. Alshahni, R.Z.; Alshahni, M.M.; Hiraishi, N.; Makimura, K.; Tagami, J. Effect of silver diamine fluoride on reducing candida albicans adhesion on dentine. *Mycopathologia* **2020**, *185*, 691–698. [PubMed]
34. Wu, L.; Gareiss, S.K.; Morrow, B.R.; Babu, J.P.; Hottel, T.; Garcia-Godoy, F.; Li, F.; Hong, L. Antibacterial properties of silver-loaded gelatin sponges prepared with silver diamine fluoride. *Am. J. Dent.* **2019**, *32*, 276–280.
35. Vinson, L.A.; Gilbert, P.R.; Sanders, B.J.; Moser, E.; Gregory, R.L. Silver diamine fluoride and potassium iodide disruption of in vitro streptococcus mutans biofilm. *J. Dent. Child.* **2018**, *85*, 120–124.
36. Sorkhdini, P.; Gregory, R.L.; Crystal, Y.O.; Tang, Q.; Lippert, F. Effectiveness of in vitro primary coronal caries prevention with silver diamine fluoride—Chemical vs biofilm models. *J Dent.* **2020**, *99*, 103418. [CrossRef]
37. Mei, M.L.; Li, Q.L.; Chu, C.H.; Lo, E.C.M.; Samaranayake, L.P. Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries. *Ann. Clin. Microbiol. Antimicrob.* **2013**, *12*, 4. [CrossRef] [PubMed]
38. Lou, Y.; Botelho, M.G.; Darvel, B.W. Reaction of silver diamine fluoride with hydroxyapatite and protein. *J. Dent.* **2011**, *39*, 612–618. [CrossRef] [PubMed]
39. Knight, G.M.; McIntyre, J.M.; Craig, G.G.; Mulyani Zilm, P.S.; Gully, N.J. Inability to form a biofilm of Streptococcus mutans on silver fluoride-and potassium iodide-treated demineralized dentin. *Quintessence Int.* **2009**, *40*, 155–161.
40. Lou, Y.; Darvell, B.W.; Botelho, M.G. Antibacterial effect of silver diamine fluoride on cariogenic organism. *J. Contemp. Dent. Pract.* **2018**, *19*, 591–598.
41. Pandit, S.; Kim, G.; Lee, M.; Jeon, J. Evaluation of streptococcus mutans biofilms formed on fluoride releasing and non fluoride releasing resin composites. *J. Dent.* **2011**, *39*, 780–787. [CrossRef] [PubMed]
42. Esberg, A.; Sheng, N.; Mårell, L.; Claesson, R.; Persson, K.; Borén, T.; Strömberg, N. Streptococcus mutans adhesin biotypes that match and predict individual caries development. *EBioMedicine* **2017**, *24*, 205–215. [CrossRef] [PubMed]
43. Ahmed, A.; Dachang, W.; Lei, Z.; Jianjun, L.; Juanjuan, Q.; Yi, X. Effect of Lactobacillus species on Streptococcus mutans biofilm formation. *Pak. J. Pharm. Sci.* **2014**, *27*, 1523–1528. [PubMed]
44. Zhu, B.; Li, J.Y.; Hao, Y.Q.; Zhou, X.D. Establishment and evaluation of the in vitro dynamic biofilm model. *Shanghai Kou Qiang Yi Xue.* **2010**, *19*, 60–65.
45. Takahashi, N.; Nyvad, B. Caries ecology revisited: Microbial dynamics and the caries process. *Caries Res.* **2008**, *42*, 409–418. [CrossRef] [PubMed]
46. Pereira, C.A.; Costa, A.C.; Silva, M.P.; Back-Brito, G.N.; Jorge, A.O. Candida albicans and virulence factors that increases it pathogenicity. The battle against microbial pathogens: Basic science, technological advances and educational programs; microbiology series, vol. 2. *Badajoz FORMATEX Res. Cent.* **2015**, *61*, 1.

47. Modrzewska, B.; Kurnatowski, P. Adherence of Candida sp. to host tissues and cells as one of its pathogenicity features. *Ann. Parasitol.* **2015**, *61*, 1.
48. Carradori, S.; Chimenti, P.; Fazaari, M.; Granese, A.; Angiolella, L. Antimicrobial activity, synergism and inhibition of germ tube formation by crocus sativus-derived compounds against Candida spp. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 189–193. [CrossRef]
49. Xiao, J.; Huang, X.; Alkhers, N.; Alzamil, H.; Alzoubi, S.; Wu, T.T.; Castillo, D.A.; Campbell, F.; Davis, J.; Hrzog, K. Candida albicans and early childhood caries: A systematic review and meta-analysis. *Caries Res.* **2018**, *52*, 102–112. [CrossRef]