CONCISE REVIEW

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Mechanisms of silver diamine fluoride on arresting caries: a literature review

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Abstract: Objective: To review the evidence regarding the mechanisms of silver diamine fluoride (SDF) for arresting caries. **Methods:** A literature search was conducted using the keywords silver diamine fluoride, and its alternative names, in seven databases: PubMed, Embase and Scopus (English); China National Knowledge Infrastructure (Chinese); Bilioteca Virtual em Saude (Portuguese); Biblioteca Virtual en Salud Espana (Spanish); and Ichushi-Web (Japanese). The titles and abstracts were screened. Full texts were retrieved for publications that studied mechanisms of actions of SDF, including its effects on remineralisation of carious lesions and on cariogenic bacteria. **Results:** A total of 1,123 publications were identified. Twenty-nine articles were included and they investigated the effect of SDF on cariogenic bacteria and dental hard tissues. Eleven studies investigated the antibacterial properties of SDF. They found that SDF was bactericidal to cariogenic bacteria, mainly *Streptococcus mutans*. It inhibited the growth of cariogenic biofilms on teeth. Twenty studies reported the remineralisation of demineralised enamel or dentine by SDF. They found that mineral loss of demineralised enamel and dentine was reduced after SDF treatment. A highly mineralised surface rich in calcium and phosphate was formed on arrested carious lesions. Four studies examined the effect of SDF on dentine collagen. They found that SDF inhibited collagenases (matrix metalloproteinases and cysteine cathepsins) and protected dentine collagen from destruction. **Conclusion:** SDF is a bactericidal agent and reduces the growth of cariogenic bacteria. It inhibits demineralisation and promotes the remineralisation of demineralised enamel and dentine. It also hampers degradation of the dentine collagen.

Key words: *Silver diamine fluoride, caries, mechanism, review*

INTRODUCTION

Dental caries is a localised chemical dissolution of dental hard tissues that is caused by acidic by-products of the metabolic processes of the biofilm (dental plaque) covering an affected tooth surface[1]. Margolis and Moreno[2] suggested that the dental plaque fluid is an important factor affecting caries development. Following exposure to fermentable carbohydrate, the amounts of tooth mineral and other calcium phosphates in plaque fluid decrease rapidly; this is primarily because of lactic acid production and reduction in the volume of plaque fluid, which can result in caries formation[2]. Caries progression in enamel and dentine is different. Enamel caries refers to the dissolution of highly mineralised tissue as a result of attack by bacterial acid[3], whereas that in dentine involves both mineral demineralisation and organic matrix degradation of the type I collagen fibre network[4].

Silver diamine fluoride (SDF) is used to prevent and arrest caries, and "silver diamine fluoride" is the most common spelling/keyword for this compound in the dental literature. There are a number of different nomenclatures for this dental product: 'silver diamine fluoride'[5–8]; 'diammine silver fluoride'[9]; 'silver diammine fluoride'[10]; 'diamine silver fluoride or silver fluoride'[11,12]; and 'silver ammonium fluoride'[13]. SDF is a colourless alkaline solution containing silver and fluoride, which forms a complex with ammonia[14]. SDF is not merely a simple salt of silver, ammonium and fluoride ions. Rather, it is a mixed heavy-metal halide coordination complex. Ammonia can keep the solution at a constant concentration for a certain period of time[15]. Silver compounds have a long history of use in both medicine and dentistry because of their antimicrobial properties[16]. Fluoride is used in various forms to prevent and arrest caries[14]. Hence, the combined effects of silver and fluorides have been hypothesised to have the

ability to halt caries progression and prevent the development of new caries simultaneously[17]. A review concluded that SDF is an effective, efficient, equitable and safe caries-preventive agent that appears to meet the standards of the US Institute of Medicine and the Millennium Goals of the World Health Organization[17].

SDF was approved for use as a therapeutic agent in Japan in the 1960s[18]. It has also been used in Argentina, Australia, Brazil and China for many years to treat dental caries[14]. In 2014, the US Food and Drug Administration (FDA) cleared the first SDF product for use in the USA[4]. Since 1969, SDF has been used to arrest caries of the primary teeth in children[18], prevent pit and fissure caries of the erupting permanent molars[9] and prevent root caries in elderly people[19]. Apart from caries management, SDF is also used to treat tooth hypersensitivity and to sterilise infected root canals[15]. It can be applied directly onto a carious lesion to arrest the caries or onto a caries-free surface for prevention. Clinical studies have demonstrated that SDF is effective in reducing enamel carious lesions in first permanent molars[20] and dentine caries in the primary anterior teeth[8].

Although studies have demonstrated that SDF is effective in arresting dental caries, the mechanism of action is unclear. Studies that investigated the mechanism of SDF vary markedly in terms of perspective, hypotheses, objectives, methodology, experimental conditions, model systems and conclusions. Past literature reviews were performed based on publications in the English language. As SDF has been widely used for dentistry in Argentina, Brazil, China and Japan for many years, a number of studies related to SDF were published in Spanish, Portuguese, Chinese and Japanese. A systematic review was performed on clinical studies of SDF, and a meta-analysis was carried out to evaluate the effectiveness of SDF in arresting dental caries[21]. To date, there has been no comprehensive review in the literature to evaluate studies, published in different languages, performed to investigate the mechanisms of actions of SDF. This paper is a literature review of SDF on publications published in Chinese, English, Japanese, Portuguese and Spanish. The aim of this paper was to review the evidence on the mechanisms of actions of SDF on dental caries in terms of its effect on carious lesions, including its action on cariogenic bacteria.

MATERIALS AND METHODS

Search strategy

A literature search was conducted to identify papers in seven databases that included references in five different languages. English publications in PubMed, Embase and Scopus were searched using the keywords 'silver diamine fluoride' OR 'silver diammine fluoride' OR 'silver fluoride' OR 'diamine silver fluoride' OR 'diammine silver fluoride'. A search of the Chinese literature in the China National Knowledge Infrastructure (CNKI) was conducted using the keywords '氟化氨銀' OR '氟化銀'. Spanish publications in Biblioteca Virtual en Salud Espana (BVSE) were searched using the keywords 'fluoruro diaminico del plata' OR 'fluoruro del plata'. A Portuguese literature search was conducted in Biblioteca Virtual em Saude (BVS) using the keywords 'diamino fluoreto de prata' OR 'fluoreto de prata'. Japanese papers in Ichushi-Web were searched using the keywords 'フッ化ジアンミン銀' OR 'サホライド'. No publication-year limit was set, and the last search was made in March 2016. A potentially eligible list of publications was developed including papers searched using the keywords (*Figure 1*).

Study selection and data extraction

Reference lists identified from searching the seven databases were checked for duplication. After eliminating the duplicate publications, the titles and abstracts of articles initially identified from the potentially eligible list were screened. Publications in which the mechanisms of SDF on caries or bacteria were not studied were excluded via the screening of titles and abstracts. Afterwards, the full texts of the remaining articles were retrieved. Manual screening was performed on the reference lists of all possible eligible papers. Studies were selected for this review in accordance with the following inclusion criterion: studies investigated the properties of SDF on carious lesions, including its action on cariogenic bacteria. If consensus about the inclusion of a study was not reached, the paper was discussed with an independent investigator until agreement was achieved.

Related information of non-English publications included in the final list was translated into English. For the studies included, the following information was recorded: publication details (authors and years); methods; outcome assessments (various criteria for evaluating the remineralisation of caries: lesion depth; mineral loss; calcium and phosphate absorption and release; microhardness of tooth surfaces; surface morphology; collagen degradation; and bacterial counts); and the main findings.

RESULTS

The initial literature search found 1,123 potentially eligible publications up to March 2016 (175 articles in PubMed, 161 articles in Embase, 206 articles in Scopus, 208 articles in CNKI, 249 articles in BVS, eight articles in BVSE and 116 articles in Ichushi-Web). Two-hundred and seventy-three duplicate

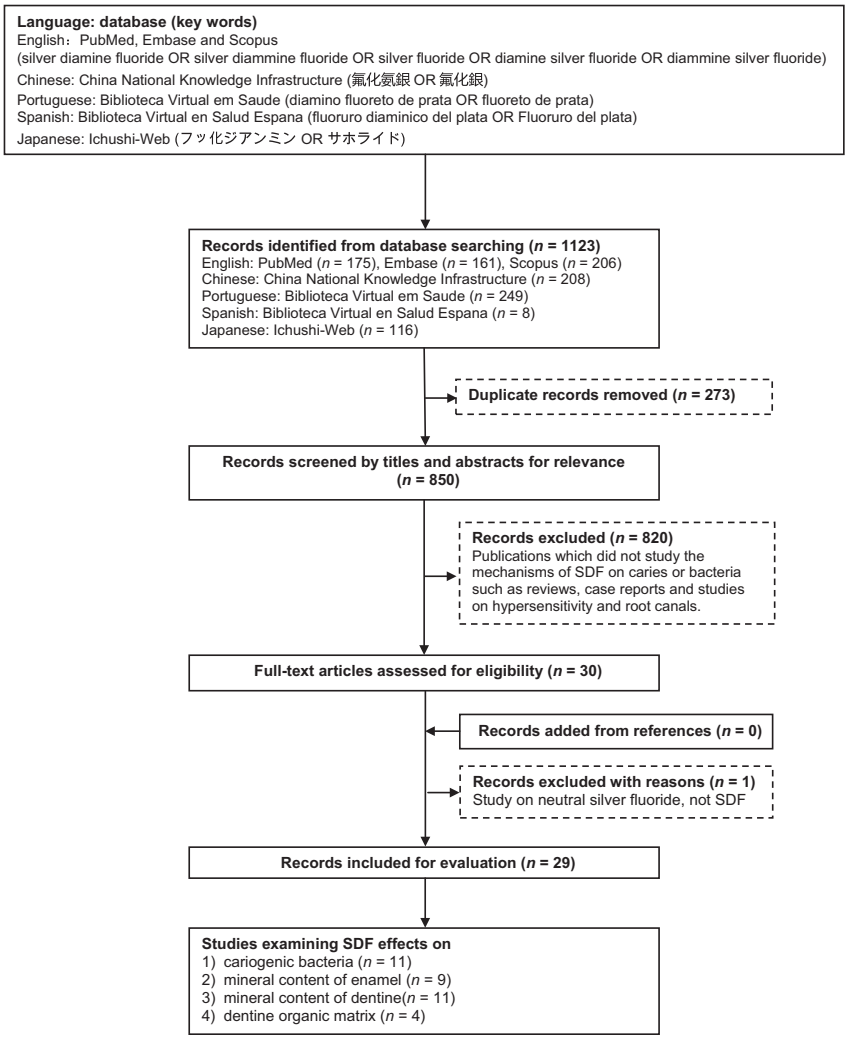

Figure 1. Flow chart of literature search. SDF, silver diamine fluoride.

records were removed (*Figure 1*). After screening the titles and abstracts, 820 articles that were classified as literature reviews, case reports, studies on hypersensitivity, root canal treatment, cytotoxicity and caries prevention, along with other irrelevant studies, were excluded. In this review, no clinical trial was found to study the mechanism of SDF. Therefore, the publications included were either *ex vivo* or *in vitro* studies. Full-text papers were obtained for the remaining 30 publications. Hand searches of the references of the selected papers did not identify any additional publications that met the inclusion criteria. One article was excluded from the final evaluation because it used neutral silver fluoride without ammonia[22]. The remaining 29 papers were found to meet the eligibility criteria and were included in this review. Among them, 11 studies examined the action of SDF on cariogenic bacteria (*Table 1*), nine studies investigated the effect of SDF on the mineral content of enamel (*Table 2*), 11 studies investigated the effect of SDF on the mineral content of dentine (*Table 3*) and four studies examined the effect of SDF on the dentine organic matrix (*Table 4*).

Actions of SDF on cariogenic bacteria

Dentine surfaces treated with SDF had significantly less growth of *Streptococcus mutans* than did those without SDF treatment[11]. Colony-forming unit (CFU) counts of monospecies strains of *S. mutans* and *Actinomyces naeslundii* were reduced after application of SDF, with very few bacteria found to be alive[7]. CFU counts of dual-species biofilms containing the cariogenic bacteria *S. mutans* and *Lactobacillus acidophilus* were significantly lower on demineralised dentine treated with SDF than when treated with water; the dead-to-live ratios of the bacteria were significantly higher after SDF application than after water application[6]. A further study used multispecies cariogenic biofilms consisting of *S. mutans, Streptococcus sobrinus, L. acidophilus, Lactobacillus rhamnosus* and *A. naeslundii*, with the results showing that CFU counts were reduced with SDF

Table 1 Summary of publications studying the action of silver diamine fluoride (SDF) on cariogenic bacteria

Authors, Year (Language)	Methods	Main findings
Suzuki *et al.* 1976 (English)[27]	The visual broth microdilution method was used to determine the MICs of SDF, $Ag(NH_3)_2NO_3$ and NaF against *S. mutans*	The MIC of SDF was 19.0 μg/ml, which was lower than the MICs of $Ag(NH_3)_2NO_3$ and NaF
Igarashi, 1978 (Japanese)[26]	The agar diffusion method was used to study the antibacterial activity of SDF, $AgNO_3$ and NaF against *S. mutans*	SDF was more effective than $AgNO_3$ and NaF at inhibiting the growth of *S. mutans*
Tsutsumi, 1981 (Japanese)[28]	SEM was used to study the adhesion of *S. mutans* on carious enamel treated with 7.6% SDF incubated in Trypticase Soy Broth	SDF inhibited adherence and growth of *S. mutans* on the carious enamel surface
Li *et al.*, 1984 (Chinese)[23]	The agar diffusion method was used to determine the MICs of SDF, $Ag(NH_3)_2NO_3$ and NaF against *S. mutans*	The MICs (%) of SDF, $Ag(NH_3)_2NO_3$ and NaF against *S. mutans* were $<3.3 \times 10^{-11}$, 3.3×10^{-11} and 5.4×10^{-7}, respectively
Knight *et al.*, 2005 (English)[11]	Spectrophotometry was used to determine the effect of 29% (1.8 mol/l) SDF on bacterial growth (optical density) of *S. mutans* supplied by the chemostat system	SDF was effective at inhibiting the growth of bacteria (SDF *vs.* control, $P < 0.05$)
de Almeida *et al.*, 2011 (English)[25]	The agar diffusion method was used to study the antibacterial activity (MID) of 12% and 30% SDF against *S. mutans*	The agar diffusion method showed that 12% and 30% SDF inhibited the growth of *S. mutans*
Targino *et al.*, 2014 (English)[29]	The spectrophotometric broth microdilution method and turbidity were used to determine the MICs of SDF and CHX against *S. mutans*. The MBC was evaluated in plates containing brain–heart infusion agar	The MICs of SDF and CHX were 33.3 μg/ml and 3.3 μg/ml, respectively. The MBCs of SDF and CHX were 50.0 μg/ml and 6.0 μg/ml, respectively
Chu *et al.*, 2012 (English)[7]	SEM, CFU and CLSM were used to study two biofilms (*S. mutans* and *A. naeslundii*) on carious dentine treated with 38% SDF	Silver particles were found on the dentine surface after SDF treatment. Compared with the control, the SDF-treated dentine had fewer CFUs of bacteria ($P < 0.001$) and more dead bacteria ($P < 0.05$)
Alves *et al.*, 2010 (Portuguese)[24]	The agar diffusion method was used to study the antibacterial activity (MID) of 12%, 16% and 30% SDF against *S. mutans*, *S. oralis* and *L. casei*	The agar diffusion method showed that 12%, 16% and 30% SDF inhibited the growth of the three species of bacteria
Mei *et al.*, 2013 (English)[6]	SEM, CFU and CLSM were used to study a dual-species biofilm (*S. mutans* and *L. acidophilus*) on carious dentine treated with 38% SDF	Silver particles were found on the dentine surface after treatment with SDF. Compared with the control, the SDF-treated dentine had fewer CFUs of bacteria ($P < 0.01$) and more dead bacteria ($P < 0.05$)
Mei *et al.*, 2013 (English)[5]	SEM, CFU and CLSM were used to study a multispecies biofilm (*S. mutans*, *S. sobrinus*, *L. acidophilus*, *L. rhamnosus* and *A. naeslundii*) on carious dentine treated with 38% SDF	Silver particles were found on the dentine surface after treatment with SDF. Compared with the control, the SDF-treated dentine had lower CFU counts of bacteria ($P < 0.01$) and more dead bacteria ($P < 0.01$)

$AgNO_3$, silver nitrate; $Ag(NH_3)_2NO_3$, silver ammonium nitrate; CFU, colony-forming unit; CHX, chlorhexidine; CLSM, confocal laser scanning microscopy; NaF, sodium fluoride; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MID, maximum inhibitory dilution; SDF, silver diamine fluoride; SEM, scanning electron microscopy.

treatment[5]. The growth of *S. mutans, Streptococcus oralis* and *Lactobacillus casei* was reduced after treatment with SDF[23–26]. SDF also inhibited the adherence of *S. mutans* to tooth surfaces[27,28]. The minimum inhibitory concentration and minimum bactericidal concentration of SDF for *S. mutans* were 33.3 μg/ml and 50.0 μg/ml, respectively[29], showing that SDF was more effective than silver ammonium nitrate and sodium fluoride[23,27].

Effects of SDF on mineral content of enamel and dentine

Demineralised tooth surfaces became black after application of SDF[23]. The lesion depth of a demineralised tooth surface decreased after the application of SDF[3,23,30,31] and it was also effective in slowing down the progression of lesions[10]. Carious lesions treated with SDF had significantly higher surface microhardness, to a depth of approximately 150 μm, compared with the control lesions treated with deionised water[5,7,23,32,33]. The concentration of calcium in the remineralisation solution was found to be reduced[23,34], which indicates that SDF promotes absorption of calcium. In addition, the concentration of calcium in the demineralisation solution was also decreased[35], which shows that SDF inhibited calcium dissolution from enamel. Using a polarised light technique with photo-microscopy, demineralised enamel surfaces treated with SDF had significantly lower mineral loss than did those without SDF treatment[13].

A study reported that silver chloride and metallic silver were formed after application of SDF[3]. In addition, SDF appeared to produce calcium fluoride and metallic

Table 2 Summary of publications studying the effects of silver diamine fluoride (SDF) on the mineral content of enamel

Authors, Year (Language)	Methods	Main findings
Suzuki *et al.*, 1974 (English)[37]	(1) Human enamel blocks treated with 38% SDF were immersed in artificial saliva for 1 week before EPMA. (2) Enamel powder treated with 38% SDF was immersed in artificial saliva containing thiocyanate for 20 weeks before XRD	(1) Fluoride and silver were detected within 20 μm and 10 μm from the enamel surface, respectively. (2) CaF_2 was formed but gradually disappeared within 10 weeks. Ag_3PO_4 was formed but disappeared after 1 week. AgSCN was retained for up to 20 weeks
Li *et al.*, 1984 (Chinese)[23]	Demineralised enamel blocks with internal control, treated with 38% SDF, were immersed in lactic acid for 2 days before MCR and MHT	SDF-treated blocks had less lesion depth and increased microhardness ($P < 0.05$) compared with the negative control. SDF stained demineralized, but not sound, enamel black
Klein *et al.*, 1999 (English)[10]	Demineralised enamel blocks treated with 38% SDF were subjected to challenge with cariogenic biofilm for 2, 4 and 6 weeks before PLM	SDF-treated enamel blocks had less lesion depth compared with control blocks up to 4 weeks of biofilm challenge
Li *et al.*, 2001 (Chinese)[32]	Demineralised enamel blocks were treated with SDF three times per week for 4 weeks before MHT	SDF-treated enamel blocks had increased microhardness compared with the control
Wu *et al.*, 2002 (Chinese)[34]	Demineralised enamel blocks, treated and untreated (control) with 38% SDF, were immersed in remineralising solution for 4 days before AAS	SDF-treated blocks took up more calcium than did control blocks from the remineralising solution ($P < 0.001$)
Wu *et al.*, 2002 (Chinese)[35]	Demineralised enamel blocks treated with 38% SDF were immersed in demineralising solution for 6 days before AAS	SDF-treated blocks released less calcium into the demineralising solution than did control blocks ($P < 0.01$)
Wang *et al.*, 2005 (Chinese)[40]	Demineralised enamel blocks treated with 38% SDF were subjected to pH cycling for 10 days before SEM	Precipitates were formed on SDF-treated surfaces but not on water-treated surfaces
Rosas *et al.*, 2014 (Spanish)[13]	Demineralised enamel blocks treated with 38% SDF were subjected to pH cycling for 5, 10 and 15 days before PLM	SDF-treated enamel blocks had less mineral loss than the control after 5 and 10 days ($P < 0.05$)
Lou *et al.*, 2011 (English)[36]	Hydroxyapatite powder and 10% gelatin were treated with 38% SDF. Treated materials were studied with SEM/TEM, EDX and ED before and after washing with water	CaF_2 was formed when SDF reacted with hydroxyapatite powder but disappeared after washing. Metallic silver was produced when SDF reacted with gelatin

AAS, atomic absorption spectrometry; AgSCN, silver thiocyanate; Ag_3PO_4, silver phosphate; CaF_2, calcium fluoride; ED, electron diffraction; EDX, energy-dispersive X-ray analysis; EPMA, electron probe microanalysis; MCR, micro-contact radiography; MHT, micro-hardness testing; PLM, polarized light microscopy; SEM, scanning electron microsopy; TEM, transmission electron microscopy; XRD, X-ray diffraction.

silver when reacted with hydroxyapatite[36]. Other studies discovered calcium fluoride and silver phosphate when enamel powder or dentine powder were mixed with SDF[37,38]. Elemental analysis revealed that the weight percentages of calcium and phosphorus in demineralised dentine treated with SDF were significantly higher than those of calcium and phosphorus in demineralised dentine without SDF treatment (control group)[5,7]. Moreover, demineralised dentine treated with SDF had less mineral loss than did demineralised dentine with no SDF treatment[5,7]. The levels of calcium and phosphorus increased from the surface to a depth of 300 μm[12]. There was also a significantly higher uptake of fluoride in the SDF-treated dentine samples than in the water-treated dentine samples[12]. An *ex vivo* study showed that a highly remineralised zone abundant in calcium and phosphate was detected on arrested dentine carious lesions treated with SDF[39]. Studies using scanning electron microscopy observed dense precipitates covering tooth surfaces after application of SDF[40,41]. However, the investigators did not mention the content of these precipitates. Cross-section scanning electron micrographs revealed that dense granular structures of spherical grains were found in the intertubular area of dentine after treatment with SDF [3]. X-ray diffraction found reduced loss of dentine crystallinity resulting from the dissolution of hydroxyapatite in dentine treated with SDF[6].

Effects of SDF on the organic content of dentine

A study using immunolabeling revealed that a larger amount of intact collagen remained on the dentine surface after treatment with SDF than after treatment with water (i.e. the control)[6]. Dentine treated with SDF showed significantly less liberation of hydroxyproline as a result of collagen degradation than did dentine treated with water[3]. SDF had an inhibitory effect on matrix metalloproteinases (MMPs), which play an important role in the enzymatic degradation of collagen, by inhibiting the proteolytic activities of MMP-2, MMP-8 and MMP-9[42]. The activities of cysteine cathepsins (or cathepsins), which are proteolytic enzymes that contribute to dentine collagen degradation, were also inhibited by SDF[43].

DISCUSSION

Rosenblatt *et al.*[17] performed a literature review of publications on SDF in three languages: English,

Table 3 Summary of publications studying the effects of silver diamine fluoride (SDF) on the mineral content of dentine

Authors, Year (Language)	Methods	Main findings
Li *et al.*, 1997 (Chinese)[38]	Human dentine powder was immersed in 38% SDF solution. The product after reaction was analysed by XRD	CaF_2 and Ag_3PO_4 were formed
Yang *et al.*, 2004 (Chinese)[30]	Demineralised human root surfaces treated with 38% SDF were subjected to challenge with cariogenic biofilm for 2 days before MCR	SDF-treated root surfaces had less lesion depth ($P < 0.05$) and mineral loss than control
Yao *et al.*, 2006 (Chinese)[31]	Demineralised human root surfaces treated with 38% SDF were immersed in remineralising solution for 7 days before SEM and MCR	Precipitates were formed on SDF-treated surfaces but not on water-treated surfaces. SDF-treated surfaces had less lesion depth ($P < 0.05$) and mineral loss ($P < 0.05$) than the control
Chu *et al.*, 2008 (English)[33]	Primary teeth with arrested dentine caries treated with 38% SDF were extracted and underwent KHN measurements	Within the outer 25–200 μm, the median KHN of arrested carious lesions were greater (no statistics presented) than those of soft carious lesions
Knight *et al.*, 2009 (English)[12]	Demineralised human dentine disks treated with 29% (1.8 mol/l) SDF were subjected to cariogenic biofilm challenge for 2 weeks before SEM and EPMA	SDF-treated dentine had less calcium ($P < 0.05$) and phosphorus ($P < 0.05$) loss and more fluoride uptake than the control
Guo *et al.*, 2011 (Chinese)[41]	Demineralised human root surfaces treated with 38% SDF were subjected to cariogenic biofilm challenge for 6 days before SEM. The calcium concentration was evaluated at day 2, 4 and 6 by AAS	SDF-treated root surfaces had less calcium release than control ($P < 0.05$) and precipitates were formed
Chu *et al.*, 2012 (English)[7]	Demineralised human dentine blocks treated with 38% SDF were subjected to cariogenic biofilm challenge for 7 days before MHT, EDX and FTIR	SDF-treated dentine blocks had increased microhardness and calcium/phosphate weight-percentage than the control ($P < 0.05$); the ratio of amide I to hydrogen phosphate was reduced ($P < 0.05$)
Mei *et al.*, 2013 (English)[6]	Demineralised human dentine blocks treated with 38% SDF were subjected to challenge with cariogenic biofilm for 7 days before XRD and FTIR	SDF-treated dentine blocks had reduced mineral loss and reduced ratio of amide I to hydrogen phosphate ($P < 0.05$)
Mei *et al.*, 2013 (English)[5]	Demineralised human dentine blocks treated with 38% SDF were incubated in artificial mouth for 21 days before MHT, EDX and FTIR	SDF-treated dentine blocks had increased microhardness and calcium/phosphate weight percentage ($P < 0.05$); the ratio of amide I to hydrogen phosphate was reduced ($P < 0.01$)
Mei *et al.*, 2013 (English)[3]	Demineralised human dentine blocks treated with 38% SDF were subjected to pH cycling for 8 days before SEM, micro-CT and XRD	SDF-treated dentine blocks had reduced lesion depth ($P < 0.01$). Silver chloride and metallic silver were formed
Mei *et al.*, 2014 (English)[39]	Primary teeth with arrested dentine caries, treated with 38% SDF, were extracted and underwent assessments of micro-CT, EDX, SEM and TEM	A highly remineralised surface zone (about 150 μm), rich in calcium and phosphate, was found on the arrested dentinal lesion. Collagens were protected and not exposed as a result of SDF treatment

AAS, atomic absorption spectrometry; Ag_3PO_4, silver phosphate; CaF_2, calcium fluoride; EDX, energy-dispersive X-ray analysis; EPMA, electron probe microanalysis; FTIR, Fourier transform infrared spectroscopy; KHN, Knoop hardness number; MCR, micro-contact radiography; micro-CT, micro-computed tomography; MHT, micro-hardness testing; SEM, scanning electron microsopy; TEM, transmission electron microscopy; XRD, X-ray diffraction.

Portuguese and Spanish. SDF has also been widely used in China and Japan for several decades[9,23]. Thus, a number of research publications on SDF, written in Chinese and Japanese, may have been included in the literature search, making the search comprehensive and providing a wider evidence base. The most common concentration of SDF used for caries management was 38%, but concentrations of SDF of 30% and 12% were also used. In this review, most laboratory studies used 38% SDF for their experiments; however, some older studies did not mention the concentrations of SDF used. A systematic review on clinical studies showed that effectiveness of SDF in caries arrest would be enhanced by increasing the concentration from 12% to 38%, and by increasing the frequency of application from annual to semi-annual[21]. The findings of the selected laboratory studies generally concur that an SDF concentration of 38% is more effective at inhibiting collagenase activity and preventing collagen degradation than low concentrations[42,43]. 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. A time limitation was not set for the literature search; there were studies published as early as the 1970s[26,27,37]. Some of these early laboratory studies did not present their results in a contemporary format. The methodology and outcome assessment varied between studies, making quantitative analysis difficult. Last, but not least,

Table 4 Summary of publications studying the effects of silver diamine fluoride (SDF) on the organic content of dentine

Authors, Year (Language)	Methods	Main findings
Mei *et al.*, 2012 (English)[42]	Fluorescent MMP kits (for MMP-2, MMP-8 and MMP-9) were used to study the inhibition of collagen degradation by $AgNO_3$, NaF and 12%, 30% and 38% SDF	Collagen was degraded less by MMPs in the presence of SDF than in the presence of $AgNO_3$ or NaF ($P < 0.001$). Collagen was degraded less in the presence of 38% SDF than in the presence of 30% SDF and 12% SDF
Mei *et al.*, 2013 (English)[6]	Human demineralised dentine blocks treated with 38% SDF were subjected to challenge with cariogenic biofilm. An immunolabelling method was used to detect intact collagen I in dentine	SDF-treated dentine blocks had more intact collagen I than did the control ($P < 0.05$)
Mei *et al.*, 2013 (English)[3]	Human demineralised dentine blocks treated with 38% SDF were subjected to pH cycling. The hydroxyproline assay was used to assess the amount of degraded collagen	SDF-treated dentine blocks had less collagen degradation ($P < 0.01$)
Mei *et al.*, 2014 (English)[43]	Fluorescent cathepsin kits (cysteine cathepsin B and cysteine cathepsin K) were used to study the inhibition of collagen degradation by $AgNO_3$, NaF and 12%, 30% and 38% SDF	Collagen degradation by cysteine cathepsins was lower in the presence of SDF than in the presence of $AgNO_3$ or NaF ($P < 0.001$). SDF at different concentrations had no significant difference on inhibiting proteolytic activity of cysteine cathepsins

$AgNO_3$, silver nitrate; MMP, matrix metalloproteinases; NaF, sodium fluoride; SDF, silver diamine fluoride.

this review summarised the relevant findings of the studies in peer-reviewed publications. It is not the objective of this review to judge the quality of the study or to discuss the limitations of each study. This should be taken into consideration when interpreting the results and in the conclusions of this review.

After analysing the results of the studies, it was found that the possible mode of action of SDF can be related to its antibacterial properties on cariogenic bacteria, its remineralisation effect on the inorganic content of the tooth and its inhibitory effect on the degradation of the organic matrix.

According to this review, it was found that SDF possessed antimicrobial action against cariogenic monospecies strains of *S. mutans* or *A. naeslundii*[7], dual-species cariogenic biofilms of *S. mutans* and *L. acidophilus*[6] and multispecies cariogenic biofilms formed on dentine surfaces[5]. In the caries process, bacterial invasion initially involves *Streptococci*, *Actinomycetes* and *Lactobacilli*. *Streptococcus mutans* is one of the most important pathogens associated with the initiation and progression of caries. *Lactobacillus acidophilus* and *L. rhamnosus* are the most abundant species of bacteria, routinely found in both deep and superficial carious lesions. *Actinomyces naeslundii* is linked to root caries that has the potential to invade dentinal tubules. It is suggested that the concentrations of antibacterial agents required for inhibiting biofilms are more than 100 times higher than those required for inhibiting planktonic bacteria because biofilms are more resistant to antimicrobial agents than are planktonic bacteria[16]. Both fluoride and silver ions contained in SDF appear to have the ability to inhibit the formation of cariogenic biofilms[5]. High-concentration fluorides can inhibit biofilm formation because fluorides can bind to bacterial cellular components and influence enzymes that are related to carbohydrate metabolism as well as to sugar uptake[5]. Ionised silver can either kill cariogenic microorganisms or interfere with their metabolic processes, depending on its concentration. It has been suggested that silver ions at a concentration of 20 p.p.m. can inhibit the growth of *S. mutans*[12]. A review concluded that silver ions have three antimicrobial effects[16]: first, silver ions can destroy the cell-wall structure of bacteria; second, they can inhibit enzyme activities and influence metabolic processes; and, third, they can inhibit the replication of bacterial DNA.

SDF at a concentration of 38% contains 44,800 p.p.m. fluoride. Its fluoride concentration is the highest among the fluoride agents available for dental use. Fluoride promotes the remineralisation of hydroxyapatite in enamel and dentine. One proposed chemical reaction between SDF and hydroxyapatite of teeth involves the formation of silver phosphate and calcium fluoride[3,38]. The subsequent dissolution of fluoride and calcium facilitates the formation of insoluble fluorapatite, which is a possible reaction product of fluoride ions with hydroxyapatite. It is difficult to confirm the formation of fluorapatite, primarily because its crystal structure is similar to that of hydroxyapatite. Calcium fluoride is less acid resistant than fluorapatite. The calcium fluoride formed after application of SDF is considered to be a pH-regulated slow-release reservoir of fluoride on the tooth surface. Nevertheless, calcium fluoride could be removed easily from a tooth surface by toothbrushing or mastication[37]. The solubility of silver phosphate (6.4×10^{-3} g/100 ml) is higher than that of silver chloride (8.9×10^{-4} g/100 ml). Therefore, silver phosphate could react with alkali chlorides in remineralisation solutions to form silver chloride. This

could explain why silver chloride was detected as the principal precipitate on the tooth surface after SDF treatment. The reaction between SDF and hydroxyapatite also led to the formation of nanoscopic metallic silver particles attached to hydroxyapatite crystals[3], while the production of metallic silver was accelerated by exposure to light and high temperature[36]. Silver nanoparticles have shown a great inhibitory effect on the growth of cariogenic bacteria, which might be an important reason why SDF can arrest caries even without removal of carious tooth structure[39]. The changes in the microhardness of a tooth are directly linked to its mineral content[33]. Laboratory studies reported that the microhardness of demineralised enamel and dentine increased after treatment with SDF[7,23]. Laboratory studies suggested that virtually insoluble or less soluble silver chloride and silver phosphate were detected on the dentine surface when treated with SDF[3,38]. An insoluble protective layer was formed, according to these precipitates, to decrease calcium and phosphorous loss from demineralised enamel and dentine. These laboratory studies simulated some factors of clinical conditions by using different *in vitro* models, while *ex vivo* studies are more appropriate for representing the complicated oral environment. An *ex vivo* study found that the microhardness of the outermost dentine surface of an arrested carious lesion was increased after treatment with SDF[33]. Another *ex vivo* study reported that a dense and highly remineralised surface layer, rich in calcium and phosphate, was found after application of SDF[39], which could directly reflect the clinical situation. This may explain why the surface of the arrested lesion could be hardened with SDF treatment.

The pathogenesis of caries in dentine and enamel are different. By weight, dentine contains approximately 10% fluid, 20% organic matrix and 70% mineral[44]. Thus, the process of dentine caries cannot be merely explained by mineral loss as a result of attack by bacterial acid. Dentine type I collagen accounts for approximately 90% of the organic component in dentine, and the residual part is composed of noncollagenous proteins. Type I collagen can act as a scaffold for the deposition of mineral crystals, and the organic dentine matrix might inhibit the diffusion of calcium and phosphate for further demineralisation[44]. In this review, a study showed that SDF can preserve collagen from degradation in demineralised dentine[3]. In the past, the dentine organic matrix was considered to be destroyed mainly as a result of the action of bacterial collagenases, while recent studies have suggested that collagen can be degraded by MMPs. MMPs are present in saliva and the dentine matrix[45]. They can be activated by the low pH in carious dentine[42]. MMP-8 (neutrophil collagenases) cleaves interstitial collagen types I, II and III. It is capable of digesting other extracellular matrix and non-extracellular matrix molecules. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) not only degrade the denatured collagen molecules (gelatin) and type IV collagen, but also other proteins to a lesser degree. Cysteine cathepsins are proteolytic enzymes that contribute to degradation of dentine collagen by breaking down type I collagen and proteoglycans[43]. Cathepsins can be identified from the degradation of extracellular matrix components and are considered to be associated with MMP activities in teeth. Cathepsin-B cleaves in the non-helical telopeptide extensions of collagens, and Cathepsin-K can catabolise collagen and degrade dentine[42,43]. Therefore, the inhibition of MMP and cysteine cathepsin activities may prevent collagen degradation and contribute to the arrest of the caries process. It is suggested that silver ions may contribute more than fluoride to the inhibitory effect of SDF on cysteine cathepsins[43].

Studies have found that removal of caries is not necessary before application of SDF. This suggests that dentists do not need to remove caries from patients' teeth during treatment with SDF. SDF is a non-invasive, simple and low-cost approach to arresting dental caries. However, the main disadvantage of its use is discoloration of carious teeth[23], which can cause patient dissatisfaction. Some researchers have proposed using potassium iodide after topical application of SDF to reduce the staining effect by generating silver iodide[11]. However, this white product, silver iodide, is considered to be photosensitive and can turn dark with exposure to light. Ammonium hexafluorosilicate has been suggested to exclude silver and its staining effect, but it is less effective than SDF in arresting caries[16]. A recent study has used nano-silver fluoride, which was found to be effective in arresting dentine caries and did not result in black staining of the carious lesions[46]. This new agent is shown to have low toxicity to living cells and has antibiotic efficacy similar to that of SDF against *S. mutans*[29]. Further research is necessary to find an approach to solve the staining problem of SDF without reducing its effectiveness in arresting dental caries.

CONCLUSION

This literature review concludes that SDF reduces the growth of cariogenic bacteria. The silver ion is bactericidal. SDF can also remineralise both enamel and dentine caries. The possible mode of action of SDF for arresting caries may be attributed to its inhibition of mineral demineralisation, promotion of mineral remineralisation and protection of the collagen matrix from degradation.

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Competing Interests

The authors declare that they have no competing interests.

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