Title	Mechanisms of silver diamine fluoride on arresting caries: a literature review
Author(s)	Zhao, IS; Gao, SS; Hiraishi, N; Burrow, MF; Duangthip, D; Mei, L; Lo, ECM; Chu, CH
Citation	International Dental Journal, 2018, v. 68 n. 2, p. 67-76
Issued Date	2018
URL	http://hdl.handle.net/10722/242975
Rights	International Dental Journal. Copyright © John Wiley & Sons Ltd, co-published with FDI World Dental Federation.; This is the peer reviewed version of the following article: International Dental Journal, 2018, v. 68 n. 2, p. 67-76, which has been published in final form at https://doi.org/10.1111/idj.12320. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.; This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

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

Irene Shuping Zhao[1], Sherry Shiqian Gao[1], Noriko Hiraishi[2], Michael Francis Burrow[3], Duangporn Duangthip[1], May Lei Mei[1], Edward Chin-Man Lo[1], Chun-Hung Chu[1]*

[1] Faculty of Dentistry, The University of Hong Kong, Hong Kong, China

[2] Cariology and Operative Dentistry, Tokyo Medical and Dental University, Tokyo, Japan

[3] Melbourne Dental School, University of Melbourne, Melbourne, Australia

Correspondence

*Prof. C. H. Chu

Faculty of Dentistry,

The University of Hong Kong.

34 Hospital Road, Hong Kong SAR, China.

Tel: +852 2859 0287

Fax: +852 2858 7874

E-mail: chchu@hku.hk

Running title: Mechanisms of SDF on arresting caries

Keywords: silver diamine fluoride, caries, mechanism, review

Abstract

Objective: To review the evidence on the mechanisms of silver diamine fluoride (SDF) on arresting caries.

Methods: A literature search was conducted using the keywords silver diamine fluoride and its alternative names in seven databases, which are 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 carious lesion remineralisation and 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 the mineral loss of demineralised enamel and dentine were 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 the 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 restrains the growth of cariogenic bacteria. It inhibits demineralisation and promotes the remineralisation of demineralised enamel and dentine. It also hampers the degradation of the dentine collagen.

1 INTRODUCTION

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

Silver diamine fluoride (SDF) is the most common names and keywords in the dental literature. There have been different nomenclatures for this dental product. It has been called '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 due to 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 appearing to meet the standards of the United

States (U.S.) Institute of Medicine and the Millennium Goals of the World Health Organisation[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 to treat dental caries for many years[14]. In 2014, the U.S. Food and Drug Administration (FDA) cleared the first SDF product for use in the U.S.[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 directly applied on a carious lesion to arrest the caries or on a caries-free surface for prevention. Clinical studies demonstrated that SDF was effective in reducing enamel carious lesions in first permanent molars[20] and dentine caries in the primary anterior teeth[8].

Although studies 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. Since 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 meta-analysis was carried out to evaluate the effectiveness of SDF in arresting dental caries[21]. Up to now, there is no comprehensive review in the literature to evaluate studies investigating the mechanisms of actions of SDF in different languages. This paper is a literature review of SDF on publications published in Chinese, English, Japanese, Portuguese

and Spanish. The aim of this review 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.

2 MATERIALS AND METHODS

2.1 Search strategy

A literature search was conducted to identify papers that could be included in 7 databases containing 5 different languages. English publications were searched in PubMed, Embase and Scopus, using the keywords 'silver diamine fluoride' OR 'silver diammine fluoride' OR 'silver fluoride' OR 'diamine silver fluoride' OR 'diammine silver fluoride'. A Chinese literature search was conducted in the China National Knowledge Infrastructure (CNKI) with the keywords '氟化氨銀' OR '氟化銀'. Spanish publications were searched in Biblioteca Virtual en Salud Espana (BVSE) with the keywords 'fluoruro diaminico del plata' OR 'fluoruro del plata'. A Portuguese literature search was conducted in Biblioteca Virtual em Saude (BVS) with the keywords 'diamino fluoreto de prata' OR 'fluoreto de prata'. Japanese papers were searched in the Ichushi-Web 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).

2.2 Study selection and data extraction

Records identified from database searching from the seven databases were checked for duplication. After eliminating the duplicate publications, the titles and abstracts of initially identified articles from the potentially eligible list were screened. Publications which did not study the mechanisms of SDF on caries or bacteria 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 inclusion criteria: 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 included studies, 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, micro-hardness of teeth surfaces, surface morphology, collagen degradation and bacteria counts) and the main findings.

3 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, 8 articles in BVSE and 116 articles in Ichushi-Web). Two hundred and seventy-three duplicated records were removed (Figure 1). After the screening of 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 included publications 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]. Finally, 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), 9 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 4 studies examined the effect of SDF on the dentine organic matrix (Table 4).

3.1 Actions of SDF on cariogenic bacteria

Dentine surfaces treated with SDF had significantly less *Streptococcus mutans* growth than those without SDF treatment[11]. Colony-forming unit (CFU) counts of mono-species strains of *Streptococcus mutans* and *Actinomyces naeslundii* were reduced after SDF application, with very few bacteria found to be alive[7]. CFU counts of dual-species cariogenic bacteria of *Streptococcus mutans* and *Lactobacillus acidophilus* on demineralised dentine treated with SDF were significantly lower than that treated with water; the dead-to-live ratios of the bacteria were significantly higher after SDF application than that after water application[6]. A further study used multi-species cariogenic biofilms consisting of *Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Actinomyces naeslundii*, with the results showing that CFU were reduced with SDF treatment[5]. The growth of *Streptococcus mutans, Streptococcus oralis* and *Lactobacillus casei* was restrained after SDF treatment[23-26]. SDF also inhibited the adherence of *Streptococcus mutans* to tooth surfaces[27, 28]. The minimum inhibition concentration and minimum bactericidal concentration of SDF for *Streptococcus mutans* were 33.3 μg/ml and 50.0 μg/ml, respectively[29], which was more effective than silver ammonium nitrate and sodium fluoride[23, 27].

3.2 Effects of SDF on mineral content of enamel and dentine

Demineralised tooth surfaces became black after SDF application[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 micro-hardness on the surface, to a depth of approximately 150 µm, compared with the control lesions receiving deionised water[5, 7, 23, 32, 33]. The concentration of calcium in the remineralisation solution was found to be reduced[23, 34], which indicated that SDF promoted calcium absorption. In addition, the concentration of calcium in the demineralisation solution was also decreased[35], which showed 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 SDF application[3]. SDF appeared to produce calcium fluoride and metallic 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 that 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 SDF application[40, 41]. However, the investigators did not mention the detail of these precipitates. Cross-sectional scanning electron micrographs revealed that dense granular structures of spherical grains were found in the inter-tubular area in dentine after SDF treatment[3]. X-ray diffraction found a reduced loss of dentine crystallinity resulting from the dissolution of hydroxyapatite in dentine treated with SDF[6].

3.3 Effects of SDF on organic content of dentine

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

4 DISCUSSION

Rosenblatt et al., performed a literature review of publications on SDF in three languages, namely, English, Portuguese and Spanish[17]. SDF has also been widely used in China and Japan for several decades[9, 23]. Thus, there may be a number of research publications written in Chinese and Japanese on SDF that have been added in the literature search, making the search more comprehensive and providing a wider evidence base. The most common concentration of SDF used for caries management was 38%, but concentrations of SDF at

30% and 12% were also available for caries management. In this review, most laboratory studies used 38% SDF for their experiments, but some old 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 application[21]. The findings of the selected laboratory studies generally concur that SDF concentration at 38% is more effective to inhibit collagenase activity and prevent collagen degradation than low concentrations[42, 43]. Since 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 the contemporary format. The methodology and outcome assessment varied between studies, making quantitative analysis difficult to be undertaken. Last but not the least, this review summarized 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 the consideration to interpret the results and conclusion of this review.

After analysing the results of the studies, this review 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 an antimicrobial action against cariogenic mono-species strains of *Streptococcus mutans* or *Actinomyces naeslundii*[7], dual-

species cariogenic biofilms of *Streptococcus mutans* and *Lactobacillus acidophilus*[6] and multi-species 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 *Lactobacillus rhamnosus* are the most plentiful species easily found in both deep and superficial carious lesions. *Actinomyces naeslundii* is linked to root caries that has a potential to invade dentinal tubules. It is suggested that the concentrations of antibacterial agents for inhibiting biofilms are more than 100 times higher than that for 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 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 ppm can inhibit the growth of *Streptococcus mutans*[12]. A review concluded that silver ions have three antimicrobial effects[16]. Firstly, silver ions can destroy the cell wall structure of bacteria, secondly, it can inhibit enzyme activities and influence metabolic processes, and thirdly, it can inhibit the deoxyribonucleic acid (DNA) replication of bacteria.

SDF at a concentration of 38% contains 44,800 ppm fluoride. Its fluoride concentration is the highest among the available fluoride agents for dental use. Fluoride promotes the remineralisation of hydroxyapatite in enamel and dentine. One proposed chemical reaction between SDF and the mineral of 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 of its similar crystal structure with hydroxyapatite. Calcium fluoride is less acid resistant than fluorapatite. The calcium fluoride formed after SDF application is considered to be a pH-regulated slow-release fluoride reservoir on the tooth surface. Nevertheless, calcium fluoride could be removed easily from a tooth surface by tooth brushing 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 a 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 their removal[39]. The changes of the micro-hardness of a tooth are directly linked to its mineral content[33]. Laboratory studies reported that the micro-hardness of demineralised enamel and dentine increased after SDF treatment[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 micro-hardness of the outermost dentine surface of an arrested carious

lesion was increased after SDF treatment[33]. Another *ex vivo* study reported that a dense and highly remineralised surface layer rich in calcium and phosphate was found after SDF application[39], which could directly reflect the clinical situation. This may explain why the arrested lesion surface 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 due to bacterial acid attacks. 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 due to 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 the 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), type IV collagen, but also other proteins to a lesser degree. Cysteine cathepsins are proteolytic enzymes contributing to dentine collagen degradation 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 break down dentine[42, 43]. Therefore,

the inhibition of MMPs and cysteine cathepsins activities may preserve collagen from degradation and contribute to the arrest of the caries process. It is suggested that silver ions may contribute more than fluoride to the SDF inhibition effect on cysteine cathepsins[43].

Studies found caries removal is not necessary before SDF application. This results suggested dentists do not need to remove caries in their SDF treatment on their patients. SDF is a non-invasive, simple and low-cost approach to arresting dental caries. However, the main disadvantage of its use is the discolouration effect on carious teeth[23], which can cause patient dissatisfaction. Some researchers have proposed using potassium iodide after SDF topical application for reducing 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, whereas it was 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 with no black staining on the carious lesions[46]. This new agent is shown to have low toxicity to living cells and has an antibiotic property similar to that of SDF against *Streptococcus 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.

5 CONCLUSION

This literature review concluded 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.

ACKNOWLEDGEMENTS

The authors thank Dr. Leticia Ito for her assistance in the literature search. This study was supported by the Research Grant Council General research fund (no. 760413M).

COMPETING INTERESTS

The authors declare that they have no competing interests.

References

1. Selwitz RH, Ismail AI, Pitts NB. Dental caries. *Lancet* 2007 **369**: 51-59.
2. Margolis H, Moreno E. Composition and cariogenic potential of dental plaque fluid. *Critical Reviews in Oral Biology and Medicine*. 1994 **5**: 1-25.
3. Mei ML, Ito L, Cao Y *et al*. Inhibitory effect of silver diamine fluoride on dentine demineralisation and collagen degradation. *J Dent* 2013 **41**: 809-817.
4. Horst JA, Ellenikiotis H, Milgrom PL. UCSF protocol for caries arrest using silver diamine fluoride: rationale, indications and consent. *J Calif Dent Assoc* 2016 **44**: 16-28.
5. Mei ML, Li QL, Chu CH *et al*. Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries. *Ann Clin Microbiol Antimicrob* 2013 **12**: 4.
6. Mei ML, Chu CH, Low KH *et al*. Caries arresting effect of silver diamine fluoride on dentine carious lesion with S. mutans and L. acidophilus dual-species cariogenic biofilm. *Med Oral Patol Oral Cir Bucal* 2013 **18**: 824-831.
7. Chu CH, Mei L, Seneviratne CJ *et al*. 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.
8. Chu CH, Lo ECM, Lin H. Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children. *J Dent Res* 2002 **81**: 767-770.
9. Nishino M, Ono S, Kita Y *et al*. Caries prevention in pits and fissures with diammine silver fluoride solution and fissure sealant. Sealing properties of pits and fissures and adhesive characteristics to enamel. *J Osaka Univ Dent Sch* 1974 **14**: 1-7.

10. Klein U, Kanellis M, Drake D. Effects of four anticaries agents on lesion depth progression in an in vitro caries model. *Pediatr Dent* 1999 **21**: 176-180.

11. Knight G, McIntyre J, Craig G *et al*. An in vitro model to measure the effect of a silver fluoride and potassium iodide treatment on the permeability of demineralized dentine to Streptococcus mutans. *Aust Dent J* 2005 **50**: 242-245.

12. Knight GM, McIntyre JM, Craig GG *et al*. Inability to form a biofilm of Streptococcus mutans on silver fluoride-and potassium iodide-treated demineralized dentin. *Quintessence Int* 2009 **40**: 155-161.

13. Rosas SGP, Téllez MÁA, EspinozaII EV. In vitro efficiency of fluoride-containing compounds on remineralization of carious enamel lesions under cyclic pH conditions. *Revista Odontológica Mexicana* 2014 **18**: 96-104.

14. Chu CH, Lo ECM. Promoting caries arrest in children with silver diamine fluoride: a review. *Oral Health Prev Dent* 2008 **6**: 315-321.

15. Mei ML, Lo ECM, Chu CH. Clinical use of silver diamine fluoride in dental treatment. *Compend Contin Educ Dent* 2016 **37**: 93-98.

16. Peng JY, Botelho M, Matinlinna J. Silver compounds used in dentistry for caries management: A review. *J Dent* 2012 **40**: 531-541.

17. Rosenblatt A, Stamford T, Niederman R. Silver diamine fluoride: a caries "silver-fluoride bullet". *J Dent Res* 2009 **88**: 116-125.

18. Yamaga R, Yokomizo I. Arrestment of caries of deciduous teeth with diamine silver fluoride. *Dent Outlook* 1969 **33**: 1007-1013.

19. Tan H, Lo E, Dyson J *et al*. A randomized trial on root caries prevention in elders. *J Dent Res* 2010 **89**: 1086-1090.

20. Llodra J, Rodriguez A, Ferrer B *et al*. Efficacy of silver diamine fluoride for caries reduction in primary teeth and first permanent molars of schoolchildren: 36-month clinical trial. *J Dent Res* 2005 **84**: 721-724.

21. Gao SS, Zhao IS, Hiraishi N *et al*. Clinical trials of silver diamine fluoride in arresting caries among children: a systematic review. *JDR Clinical & Translational Research* 2016 **1**: 201-210.

22. Zhi Q, Lo E, Kwok A. An in vitro study of silver and fluoride ions on remineralization of demineralized enamel and dentine. *Aust Dent J* 2013 **58**: 50-56.

23. Li Y, Qiu X, Li J *et al*. Effect of a silver ammonia fluoride solution on the prevention and inhibition of caries. *Zhonghua Kou Qiang Ke Za Zhi* 1984 **19**: 97-100.

24. Alves S, Maria T, Silva CA *et al*. Atividade Antimicrobiana de Produtos Fluoretados sobre Bactérias Formadoras do biofilme Dentário: Estudo in vitro. *Pesquisa Brasileira em Odontopediatria e Clínica Integrada* 2010 **10**: 209-216.

25. de Almeida LF, Cavalcanti YW, Valenca A. In vitro antibacterial activity of silver diamine fluoride in different concentrations. *Acta Odontol Latinoam* 2011 **24**: 127-131.

26. Igarashi S. Antibacterial Effect of Ag $(NH_3)_2F$ solution on Streptococcus mutans in plaque *Jpn J Paediatr Dent* 1978 **16**: 1-18.

27. Suzuki T, Tsutsum N, Sobue S *et al*. Effect of diammine silver fluoride on plaque formation by Streptococcus mutans 2. Mode of action of plaque inhibition. *Jpn J Oral Biol* 1976 **18**: 268-278.

28. Tsutsumi N. Studies on Topical Application of $Ag(NH_3)_2F$ for the Control of Interproximal Caries in Human Primary Molars 2. Effect of $Ag(NH_3)_2F$ on Streptococcus mutans on interproximal plaque. *Jpn J Paediatr Dent* 1981 **19**: 523-536.

29. Targino AGR, Flores MAP, dos Santos Junior VE *et al*. An innovative approach to treating dental decay in children. A new anti-caries agent. *J Mater Sci Mater Med* 2014 **25**: 2041-2047.

30. Yang D, Li Y, Ge L. Effect of ammonium oxofluoromolybdate solution on the inhibition of artificial caries on root surface in vitro. *J Pract Stomatol* 2004 **20**: 39-42.

31. Yao N, Tang R. Study of the effect of Chinese nutgall water extract on experimental root caries remineralization. *Chin J Conserv Dent* 2006 **16**: 285-287.

32. Li L, Shi S. Comparison of the remineralizations of three preparations on deciduous enamel. *Shanghai Med J* 2001 **24**: 29-31.

33. Chu CH, Lo ECM. Microhardness of dentine in primary teeth after topical fluoride applications. *J Dent* 2008 **36**: 387-391.

34. Wu L, Yang F. The effects of three fluoride-containing agents on the remineralization of deciduous teeth in vitro. *J Pract Stomatol* 2002 **18**: 347-349.

35. Wu L, Yang F. Comparison of the effects of three fluoride-containing agents on the demineralization of deciduous teeth in vitro. *J Modern Stomatol* 2002 **16**: 216-218.

36. Lou Y, Botelho M, Darvell B. Reaction of silver diamine fluoride with hydroxyapatite and protein. *J Dent* 2011 **39**: 612-618.

37. Suzuki T, Nishida M, Sobue S *et al*. Effects of diammine silver fluoride on tooth enamel. *J Osaka Univ Dent Sch* 1974 **14**: 61-72.

38. Li Y, Li J. Study of mechanism of a silver ammonia fluoride solution on inhibition of dentine caries. *Beijng J Stomatol* 1997 **5**: 151-152.

39. Mei ML, Ito L, Cao Y *et al*. An ex vivo study of arrested primary teeth caries with silver diamine fluoride therapy. *J Dent* 2014 **42**: 395-402.

40. Wang Y, Tang R. Scanning electron microscopy observations of surface morphology of initial artificial lesions of enamel treated with Chinese nutgall. *Chin J Conserv Dent* 2005 **15**: 133-135.

41. Guo J, Zhao M, Qi F *et al*. The study of tea polyphenol on the inhibition of artificial caries on root surface in vitro. *J Hebei Med Univ* 2011 **32**: 175-178.

42. Mei ML, Li Q, Chu CH *et al*. The inhibitory effects of silver diamine fluoride at different concentrations on matrix metalloproteinases. *Dent Mater* 2012 **28**: 903-908.

43. Mei ML, Ito L, Cao Y *et al*. The inhibitory effects of silver diamine fluorides on cysteine cathepsins. *J Dent* 2014 **42**: 329-335.

44. Hiraishi N, Sono R, Islam M *et al*. Effect of hesperidin in vitro on root dentine collagen and demineralization. *J Dent* 2011 **39**: 391-396.

45. Chaussain-Miller C, Fioretti F, Goldberg M *et al*. The role of matrix metalloproteinases (MMPs) in human caries. *J Dent Res* 2006 **85**: 22-32.

46. dos Santos VE, Vasconcelos Filho A, Targino AGR *et al*. A new "Silver-Bullet" to treat caries in children–Nano Silver Fluoride: A randomised clinical trial. *J Dent* 2014 **42**: 945-951.

Table 1. Summary of publications studying the action of SDF on cariogenic bacteria

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

CHX–Chlorhexidine, CFU–Colony forming unit, CLSM–Confocal laser scanning microscopy, MBC–Minimum bactericidal concentration, MIC–Minimal inhibitory concentration, MID–Maximum inhibitory dilution, Ag $(NH_3)_2NO_3$–Silver ammonium nitrate, SDF–Silver diamine fluoride, SEM–Scanning electron microscopy, NaF–Sodium fluoride, $AgNO_3$–Sliver nitrate.

Table 2. Summary of publications studying effects of SDF on mineral content of enamel

Authors, Year (Language)	Methods	Main findings
Suzuki *et al*.[37] 1974 (English)	1) 38% SDF-treated human enamel blocks were immersed in artificial saliva for 1 week before EPMA. 2) 38% SDF-treated enamel powder was immersed in artificial saliva containing thiocyanate for 20 weeks before XRD.	1) Fluoride and silver was detected within 20 µm and10 µm from 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 were retained up to 20 weeks.
Li *et al*.[23] 1984 (Chinese)	38% SDF-treated demineralised-enamel blocks with internal control were immersed in lactic acid for 2 days before MCR and MHT.	SDF-treated blocks had less lesion depth and increased in micro-hardness ($p<0.05$) than the negative control. SDF black-stained demineralised but not sound enamel.
Klein *et al*.[10] 1999 (English)	38% SDF-treated demineralised-enamel blocks were subjected to cariogenic biofilm challenge for 2, 4 and 6 weeks before PLM.	SDF-treated enamel blocks had less lesion depth than control up to 4 weeks of biofilm challenge.
Li *et al*.[32] 2001 (Chinese)	Demineralised-enamel blocks were treated with SDF 3 times per week for 4 weeks before MHT.	SDF-treated enamel blocks had increased micro hardness than the control.
Wu *et al*.[34] 2002 (Chinese)	38% SDF-treated and untreated (control) demineralised-enamel blocks were immersed in remineralising solution for 4 days before AAS.	SDF-treated blocks had more calcium uptake than control blocks from the remineralising solution ($p<0.001$).
Wu *et al*.[35] 2002 (Chinese)	38% SDF-treated demineralised-enamel blocks were immersed in demineralising solution for 6 days before AAS.	SDF-treated blocks had less calcium release than control blocks into the demineralising solution ($p<0.01$).
Wang *et al*.[40] 2005 (Chinese)	38% SDF-treated demineralised-enamel blocks were subjected to pH cycling for 10 days before SEM.	Precipitates were formed on SDF-treated surfaces but not water-treated surfaces.
Rosas *et al*.[13] 2014 (Spanish)	38% SDF-treated demineralised-enamel blocks 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*.[36] 2011 (English)	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, 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, SDF–Silver diamine fluoride**,** SEM–Scanning electron microsopy, Ag_3PO_4–Silver phosphate, AgSCN–Silver thiocyanate, TEM–Transmission electron microscopy, XRD–X-ray diffraction.

Table 3. Summary of publications studying effects of SDF on mineral content of dentine

Authors, Year (Language)	Methods	Main findings
Li *et al*.[38] 1997 (Chinese)	Human dentine-powder was immersed in 38% SDF solution. The product after reaction was analyzed by XRD.	CaF_2 and Ag_3PO_4 were formed.
Yang *et al*.[30] 2004 (Chinese)	38% SDF-treated demineralised human root surfaces were subjected to cariogenic biofilm challenge for 2 days before MCR.	SDF-treated root surfaces had less lesion depth ($p<0.05$) and mineral loss than the control.
Yao *et al*.[31] 2006 (Chinese)	38% SDF-treated demineralised human root surfaces were immersed in remineralising solution for 7 days before SEM and MCR.	Precipitates were formed on SDF-treated surfaces but not 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*.[33] 2008 (English)	38% SDF-treated primary teeth with arrested dentine caries 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*.[12] 2009 (English)	29% (1.8 mol/L) SDF-treated demineralised human dentine disks 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*.[41] 2011 (Chinese)	38% SDF-treated demineralised human root surfaces were subjected to cariogenic biofilm challenge for 6 days before SEM. 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*.[7] 2012 (English)	38% SDF-treated demineralised human dentine blocks were subjected to cariogenic biofilm challenge for 7 days before MHT, EDX and FTIR.	SDF-treated dentine blocks had increased micro hardness and calcium/phosphate weight-percentage than the control ($p<0.05$); ratio of amide I to hydrogen phosphate was reduced ($p<0.05$).
Mei *et al*.[6] 2013 (English)	38% SDF-treated demineralised human dentine blocks were subjected to cariogenic biofilm challenge for 7 days before XRD and FTIR.	SDF-treated dentine blocks had reduced mineral loss and ratio of amide I to hydrogen phosphate ($p<0.05$).
Mei *et al*.[5] 2013 (English)	38% SDF-treated demineralised human dentine blocks were incubated in artificial mouth for 21 days before MHT, EDX and FTIR.	SDF-treated dentine blocks had increased micro hardness and calcium/phosphate weight percentage ($p<0.05$); ratio of amide I to hydrogen phosphate was reduced ($p<0.01$).
Mei *et al*.[3] 2013 (English)	38% SDF-treated demineralised human dentine blocks 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*.[39] 2014 (English)	38% SDF-treated primary teeth with arrested dentine caries 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 being exposed with SDF treatment.

AAS–Atomic absorption spectrometry, 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, SDF–Silver diamine fluoride**,** SEM–Scanning electron microsopy, Ag_3PO_4–Silver phosphate, TEM–Transmission electron microscopy, XRD–X-ray diffraction.

Table 4. Summary of publications studying effects of SDF on organic content of dentine

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

MMP–Matrix metalloproteinases, SDF–Silver diamine fluoride**,** NaF–Sodium fluoride, $AgNO_3$–Sliver nitrate.

Figure 1 Flow chart of literature search.