Arresting dentine caries with silver diamine fluoride: What's behind it?

Short title: mechanism of silver diamine fluoride

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Abstract

Unlike other fluoride-based caries-preventive agents, silver diamine fluoride (SDF) can simultaneously prevent and arrest coronal and root dentine caries. The profound clinical success of SDF has drawn many clinicians and researchers to study the mechanism of SDF in arresting dentine caries. This critical review discuss how silver and fluoride contribute to caries arrest, in terms of their effects on bacteria, mineral and organic content of dentine. Silver interacts with bacterial cell membrane and bacterial enzymes which can inhibit bacterial growth in both planktonic and biofilm form. Silver can also dope into hydroxyapatite and bring the antibacterial effect to silver-doped-hydroxyapatite. Furthermore, silver is also a strong inhibitor of cathepsins and inhibits dentine collagen degradation. Early studies proposed that silver hardened caries lesion by forming silver phosphate. However, recent studies found little silver phosphate remained on the arrested dentine lesion. The principle silver precipitate was silver chloride which could not contribute to the significant hardening of the arrested lesions. On the other hand, fluoride enhances mineral formation by forming fluorohydroxyapatite with reduced solubility. There is a significant increase in microhardness with elevated level of calcium and phosphorus but not silver in the surface layer of the arrested dentine caries lesion after SDF treatment. Fluoride also inhibits matrix metalloproteinases activities and therefore inhibits dentine collagen degradation. The combination of silver and fluoride in an alkaline solution has a synergistic effect in arresting dentine caries. The alkaline property of SDF provides an unfavourable environment for collagen enzyme activation. Understanding the mechanisms of SDF in arresting dentine caries helps clinicians to develop appropriate protocol for the use of SDF in clinical care.

1. Introduction

Dentine caries refers to the situation in which caries have progressed into dentine and caused significant lesion depth (Ten Cate et al. 2008). Many clinicians believed that in this situation caries would become irreversible and could spread rapidly. Hence the traditional management of dentine caries has focused primarily on treatment via the excision of diseased tissues and subsequent restoration of the defect. It should be note that mechanical tooth preparation is a destructive and irreversible procedure in which natural dental tissues are removed (Tsang et al. 2006). Contemporary caries management philosophy has changed from the traditional surgical approach to a medical model, which often includes the use of fluoride therapy (Chu et al. 2010). Among the fluoride agents, silver diamine fluoride (SDF) is drawing much attention from both researchers and dental clinicians recently. The ability of SDF to halt the caries process and simultaneously prevent the formation of new caries makes SDF different from other caries-preventive agents like sodium fluoride (5%) and stannous fluoride (2% to 8%) (Rosenblatt et al. 2009). Clinical trials reported success of using SDF to arrest coronal caries (Chu et al. 2002; Duangthip et al. 2016; Fung et al. 2016; Yee et al. 2009; Zhi et al. 2012) and root caries (Tan et al. 2010; Zhang et al. 2013). A meta-analysis has found that the overall caries arrest rate for SDF was 81% (Gao et al. 2016).

SDF solution composed of diammine-silver ion and fluoride ion. Diammine-silver ion is a complex produced by attaching two ammonia molecules to a silver ion. Ammonia is a stronger field ligand than water in the spectrochemical series. Therefore, metal ammine complexes are more stable compare to the corresponding aquo complexes and are less strongly oxidized than the corresponding aquo complexes (Nilsson et al. 2006). This diammine-silver complex is also more stable and less oxidizing than silver fluoride, and the position of equilibrium lies within the diammine-silver ion (Chu and Lo 2008b). The stability of the reagent is crucial in arresting the progress of caries. In a study that measured concentrations of fluoride and silver ion in several commercially available SDF products, no significant change in the fluoride and silver ion concentrations or the acidity was detected over 28 days after the products were opened (Mei et al. 2013a).

Though 12% and 30% SDF solutions are available in commercial market, most SDF products are prepared at a concentration at 38% (Mei et al. 2016). Studies have shown that 12% SDF is not as effective as 38% SDF in arresting caries among children (Fung et al. 2016; Yee

et al. 2009). Manufacturers do not disclose all the ingredients of the SDF products, and the ingredients of different brands of SDF products may differ. According to available information, a SDF product (Cariestop 30%, Biodinamica, Brazil) contains fluoridic acid, silver nitrate, and ammonia hydroxide. Fluoridic acid is a double fluoride, consisting essentially of a solution of boron fluoride in hydrofluoric acid. Fluoridic acid has one free fluoride ion. The remaining three fluoride moieties bind with boron covalently and may not be freely detectable by an ion-selective electrode. If the fluoride moieties cannot form an SDF complex through chemical reaction, they will most probably remain bound to the boron covalently and will not be detectable (Mei et al. 2013a). A study reported the concentrations of free fluoride ions were not always consistent with the ones manufactures claimed (Mei et al. 2013a). The fluoride concentration of Cariestop 30% (30% SDF) was 13,200 ppm, which was only 37% of the expected 35,400 ppm. In contrast, the mean free fluoride concentration of Saforide (38% SDF; Toyo Seiyaku Kasei, Japan) was 55,800 ppm as measured by means of an ion-selective electrode, which was 25% higher than the expected 44,800 ppm (Mei et al. 2013a).

In a case report three applications of SDF within six weeks lead to arrest of rampant caries in a young teenager (Figure 1) (2014). The arrested caries lesions were coal-black in appearance and hard to probing after the treatment (Chu and Lo 2008a; Mei et al. 2014b). This non-invasive and efficient caries-arresting capability of SDF has drawn much attention from dental clinicians and researchers. Understanding the mechanism of SDF that causes these changes will endow scientific evidence of its clinical success and will inspire ideas for making improvements. This paper reviews studies on the mechanisms of SDF in arresting dentine caries. Since dentine caries is a biofilm-mediated oral disease that involves bacteria and dentine, and SDF is composed of silver and fluoride, this paper also discusses how silver and fluoride contribute to caries arrest, in terms of their effects on bacteria, mineral and organic content of dentine.

2. Silver

2.1. Anti-bacteria activity

Silver compounds ionize in the presence of water and biologic fluids to release silver ion (Marx and Barillo 2014). Therefore, SDF solution releases silver ions which can exhibit three oxidation states, silver ion (I), divalent silver ion (silver (II)), and trivalent silver ion (silver (III)) (pure metallic silver is silver (0)). Of these, only the silver ion state is sufficiently

stable for use as an antibiotic, as the other silver-cations are highly reactive and short-lived (Lansdown 2006). Silver ion expresses its antimicrobial effect in several possible ways. Firstly, silver ion can interact with life-sustaining enzymes and block the electron transport system in bacteria. Silver ion can also interact with thiol group of the enzymes and deactivate the enzyme, resulting in bacterial cell death (Russell and Hugo 1994). Secondly, silver ion can bind to bacterial cells through interacting with their cell membrane or cell wall. The surface of bacterial cell membrane contains both cationic and anionic charges. Silver ion can also electrostatically bind to the anionic portions of the membrane. This can inhibit the movement of the organism or cause the membrane to leak or rupture (Slawson et al. 1990). Thirdly, silver ion can interact with the deoxyribonucleic acid (DNA) of bacterial cell. Unless DNA is contained within the nucleus such as in eukaryotic cells, the interaction between silver ion and bacterial DNA will result in mutation of the DNA and ultimately in the death of a bacterial cell (Russell and Hugo 1994). Fourthly, silver ion can destroy bacterial cells by binding to the amino acids in the cell. When silver ion binds to the amino acids, an organometallic complex is formed. Silver ion can be generated inside the bacterial cell when this organometallic complex breaks down. The accumulation of silver ions in the cell can impair the electron transport chain, inactivate bacterial DNA and ribonucleic acid (RNA), damage and rupture the cell membrane, and bind and precipitate proteins with cysteine and thiol groups, causing cell death (Lansdown 2002). Therefore, bacterial resistance against silver is difficult to achieve due to its multiple antibacterial mechanisms. A study reported the minimal inhibitory concentration of SDF on *Streptococcus mutans* was 0.06 μmol/ml, which was slightly lower than that of diammine silver nitrate (0.12 μmol/mL) (Lansdown 2002). Another study reported similar results (Li 1984). A study demonstrated silver ion of SDF inhibited both the dextran-induced and sucrose-induced agglutination of *S. mutans* (Suzuki et al. 1976). The study also found that silver ion inhibited both glucosyl- and fructosyl-transferase activities, which are related to the synthesis of polysaccharide (Suzuki et al. 1976).

Although silver ion has an antimicrobial effect on planktonic bacterial cells, it is less effective on microorganisms in biofilm because the extracellular matrix of the biofilm probably acts as a physical barrier against the antimicrobial action of silver ion (Harrison et al. 2004). Despite this, it has been shown that 38% SDF, which has a high concentration of silver ion, can have a highly effective antibacterial action against cariogenic biofilms. A laboratory study showed 38% SDF treated tooth surface inhibited the growth of *S. mutans* mono-species biofilm for 48 hours (Savas et al. 2015). Other studies have shown similar results in terms of the strong

inhibitory effect of 38% SDF on cariogenic biofilm including mono-species of *S. mutans* or *Actinomyces naeslundii* (Chu et al. 2012), and dual-species of *S. mutans* and *Lactobacillus acidophilus* (Mei et al. 2013b). The adjunctive use of silver fluoride with potassium iodide, a compound intended to reduce the silver staining on tooth, also showed an inhibition effect on *S. mutans* growth (Hamama et al. 2015; Knight et al. 2005; 2007). A study showed SDF inhibited development of a multi-species biofilm composed of *S. mutans, Streptococcus sobrinus, L. acidophilus, Lactobacillus rhamnosus* and *Actinomyces naeslundii* on SDF treated dentine surface for 14 days (Figure 2) (Mei et al. 2013f). It should be noted that these studies were conducted in *in vitro* biofilm models in which particular species biofilms were formed. In contract, the biofilm in human mouth involves 30 genera representing at least 500 different species that interact (Davey and Costerton 2006). It remains to be determined in an *in situ* situation whether the caries-arresting effect of SDF is caused by suppression of a single or specific consortium of bacteria or whether dental plaque as a whole undergoes a more complicated shift in multiple groups of bacteria.

2.2 Effect on dentine minerals

SDF can react with hydroxyapatite to form calcium fluoride, silver phosphate and silver oxide (Zhao et al. 2017). Suzuki and co-workers (1974) demonstrated the formation of silver phosphate by mixing enamel powder with an SDF solution, and suggested the relatively insoluble silver phosphate might contribute to the hardening of the arrested surface. However, silver phosphate disappeared after being immersed in artificial saliva and was replaced by silver chloride and silver thiocyanate. When chloride presents in the oral environment such as in saliva, silver phosphate and silver oxide also react with chloride ions to form silver chloride. Studies found silver chloride to be the principal precipitate (Mei et al. 2013c; Mei et al. 2017; Zhao et al. 2017). This is because the solubility product of silver chloride (8.9×10^{-5} g/100 ml) is lower than that of silver phosphate (6.5×10^{-4} g/100 ml) and silver oxide (1.3×10^{-3} g/100 ml). Some metallic silver in very limited amount was also found in some studies (Lou et al. 2011; Mei et al. 2014b). The above mentioned silver compounds are the reason for the black staining of the SDF treated caries lesion. The clinical observation of a coal-black colour is an indication of arrested caries (Lou et al. 2011; Mei et al. 2014c; Mei et al. 2017). Studies have tried different approaches to solve this problem. Zinc fluoride and ammonium hexafluorosilicate have been used but they are inferior to SDF in the inhibition of dentine demineralisation and collagen degradation (Kawasaki et al. 2005; Suge et al. 2008; 2010; Thanatvarakorn et al. 2016). Knight and co-workers (2006) applied saturated potassium iodide

solution immediately after the application of silver fluoride. Iodide ions will react with the excess silver ion to form a yellowish precipitate of silver iodide to minimise the staining. However, the result of a clinical trial found that the application of potassium iodide did not have a no long-term effect on improving the aesthetic problem caused by the black stains on arrested root surface caries (Li et al. 2016). Silver nanoparticles have also been used to address this staining problem (Besinis et al. 2014). One study used nano silver fluoride and found that the treated caries lesion had no black staining (Santos et al. 2014). More laboratory and clinical studies should be carried out before it is recommended for clinical use.

Yamaga and co-workers (1972) suggested that the formation of silver phosphate could be one of the reasons for the hardening of a caries lesion after application. Seto and co-workers (2017) also claimed that the hardening of SDF arrested caries is due to reaction with silver, rather than classic fluoride-mediated remineralization. However, whether silver compounds contribute to the hardening of caries lesion is questionable. First, whether the depth of penetration of silver into dentine is consistent with the depth of microhardness increase is unknown. *Ex vivo* studies demonstrated that the outmost 150 μm of the dentine caries lesion is hardened (Figure 3) (Chu and Lo 2008a) while increases in calcium and phosphate were observed in the corresponding region (Figure 4) (Mei et al. 2014c). However, there was no increase of silver in the region (Figure 4). Second, if silver compounds can increase the hardness of caries lesion, then silver nitrate which contains silver shall be able to harden the caries lesion as well. However, a study found that silver nitrate treated caries lesion showed clearly exposed collagen fibres in both inter-tubular and intra-tubular dentine when compare to SDF treated caries lesion; and there is no evidence to support an increased hardness of silver nitrate treated caries lesion (Mei et al. 2013d). Third, the density of the silver compounds in dentine caries lesions is unknown. This is important because density of the material should be high enough to cause a change in microhardness (Buchalla et al. 2008).

Although whether silver compounds contribute to the increase in hardness of treated caries lesion is uncertain, it is plausible that silver can be incorporated into the crystal during hydroxyapatite formation and produce silver-containing hydroxyapatite (Chen et al. 2006; dos Santos et al. 2015). The formula of silver-containing hydroxyapatite is $Ca_{10-x}Ag_x(PO_4)_6(OH)_2$ with $0.0 \leqslant x \leqslant 0.5$, with a very small amount of calcium ions substituted by silver ions (Singh et al. 2011). This silver-containing hydroxyapatite was shown to reduce bacterial adhesion and

minimise tissue cytotoxicity (Chen et al. 2006). Feng and co-workers (1998) prepared silver-containing hydroxyapatite coating through an ion exchange reaction by treating hydroxyapatite with silver nitrate at 20 ppm for 48 hours, and they proved that some of the calcium ions in hydroxyapatite were replaced by silver ions. Therefore, there is a possibility that the silver ions in SDF can diffuse into the hydroxyapatite crystal and substitute calcium ion. The fact that this result was not found in earlier studies, it may be due to the minute amount of silver incorporation and the limitation of their detection methods.

2.3. Effect on dentine collagen

Silver has an indirect protection effect on dentine collagen by inhibiting dentine collagenase. The common caries-related collagenases are matrix metalloproteinases (MMPs) and cathepsins (Tjaderhane et al. 2013). Collagens are partially exposed to the environment when minerals are lost due to caries attack (Souza et al. 2001). MMPs mediate the degradation of practically all extracellular matrix molecules, including native and denatured collagen (Chaussain-Miller et al. 2006). The activities of cathepsins were reported to be associated with MMPs activities in dentine (Tersariol et al. 2010). SDF at 38% solution showed an inhibitory effect on activities of both MMPs and cathepsins (Mei et al. 2014a; Mei et al. 2012a). By comparing SDF with silver nitrate and sodium fluoride solutions, it was suggested that silver is a stronger inhibitor of cathepsin B and cathepsin K and moderate inhibitor of MMP-8 and MMP-9. The large ionic radius and low oxidation state of silver have great affinity with protein and this may contribute to its inhibitory effect on the elastase and cathepsin proteinases. Silver probably also interacts with a reactive side chain of the enzymes to inactivate their catalytic functions (Mei et al. 2013d).

3. Fluoride

3.1. Anti-bacterial effect

Fluoride has been shown to inhibit acid production in dental plaque. It inhibits plaque metabolism by a direct inhibition of cellular enzymes or enhancing proton permeability of cell membranes in the form of hydrogen fluoride (Koo 2008). However this inhibition is only observed for a short time, and the effect could be negligible in caries reduction by topical fluorides applied professionally every several months (Van Loveren 1990). In fact very few *in vivo* studies are available to quantify the antimicrobial effect in relation to the overall effect of fluoride on dental caries. The dominated effect of fluoride on caries arrest is the direct

interactions of fluoride with the dental hard tissue during caries lesion development and progression.

3.2. Effect of firmly bound fluoride on dentine minerals

Most of a tooth's hard tissues structure is composed of minerals. Dentine contains 70% mineral by weight (Goldberg et al. 2011). When caries occurs, the tooth surface will be chemically dissolved and therefore mineral will be lost. Remineralisation of a caries lesion requires the presence of partially demineralised apatite crystal that grows to their original size as a result of exposure to solutions supersaturated with respect to apatite. Fluoride may react with apatite in several different ways: ion exchange of fluoride ion for hydroxyl ion, crystal growth of fluorapatite from supersaturated solutions, or apatite dissolution with calcium fluoride formation (Ogard et al. 1994). Fluoride-substituted hydroxyapatite is chemically more stable than hydroxyapatite in acid environments. A higher concentration of fluoride-substituted hydroxyapatite in tooth enamel decreases tooth dissolution and therefore prevents the tooth from developing caries (Okazaki et al. 1999). Fluoride-substituted hydroxyapatite are always referring to firmly bound fluoride because fluorine is incorporated into the apatite crystal structure (Ogard et al. 1994).

Some studies of SDF on mineral have failed to find fluorapatite, primarily due to the similarity of its crystal structure with hydroxyapatite, and sometimes the residual fluoride in the samples is below the detection limit, such as with energy-dispersive X-ray spectroscopy (Lou et al. 2011; Mei et al. 2013d; Mei et al. 2014b). A recent study adopted a chemical system to simulate the salivary environment using calcium (calcium chloride) and phosphate ions (potassium phosphate dibasic) provided in Tris-buffered saline solution (Mei et al. 2017). The study found SDF reacted with the system and formed fluorohydroxyapatite after a 24-hour incubation (Figure 5). In addition, a noticeable contraction was detected in the *a*-axis dimensions of the lattice, which indicated ion exchange of fluoride ion for hydroxyl ion because fluoride ion is smaller than hydroxyl ion. This isotropic distribution of the charge on fluoride anions allows a better fit in the lattice compared with the larger asymmetric hydroxyl ion and produces a fairly well-ordered apatite structure, which is characterised by increased thermal and chemical stability compared with hydroxyapatite. The percentage of substitution of fluoride ion determines the different chemical formula of the apatite. If the fluoride ion fully substitutes hydroxyl ion, the product is fluorapatite ($Ca_{10}(PO_4)_6F_2$). If fluoride ion partially substitutes hydroxyl ion, then fluorohydroxyapatite ($Ca_{10}(PO_4)_6(OH)_{2-2x}F_{2x}$, $0<x<1$) is formed

(Figure 6) (Chen and Miao 2005). Fluorapatite alone is not a desirable biomaterial because it is stable (or bio-inert) and thus lacks good biological properties (Chen et al. 2015). Besides, it is difficult to attain pure fluoroapatite in a clinical situation, as the full substitution of hydroxyl ion is hard to achieve. Previous study found that fluoride content in the apatite increased when SDF concentration increased (Mei et al. 2017). Therefore, SDF may well reacts with calcium and phosphate and produces a mixture of fluorohydroxyapatite. The mixture contains a different percentage of fluoride. These crystals are firmly bounded to the caries lesion and therefore induce remineralisation.

3.3. Effect of loosely bound fluoride on dentine minerals

Yamaga and co-workers (1972) suggested that in the case of SDF treatment, formation of calcium fluoride and silver phosphate could be responsible for the prevention of dental caries and the hardening of a caries lesion. However, Suzuki and co-workers (1974) reported the formation of calcium fluoride by mixing enamel powder with an SDF solution, but the amount dropped significantly when the materials were immersed in artificial saliva. In addition, Lou and co-workers (2011) also found a calcium fluoride-like material was formed by mixing 38% SDF with hydroxyapatite powder and gelatine (as a chemically-representative protein), but the calcium fluoride-like material dissolved and disappeared after washing with water (Figure 7). The globular structure of calcium fluoride is thought to be due to incorporation of phosphate during its formation on the tooth surface, which is different from the cubical structure of pure calcium fluoride. Therefore, this material is described as calcium fluoride-like (Christoffersen et al. 1988). Mei and co-workers (2017) did not observe the formation of calcium fluoride when they reacted SDF with a buffer solution containing calcium and phosphate. Nevertheless, formation of calcium fluoride is considered to be the major reaction product when topical fluoride is applied onto the tooth (Ogard et al. 1994) and is believed to serve as a source of fluoride for the formation of fluoridated apatite. Calcium fluoride is adsorbed onto rather than incorporated into the tooth surface. Thus, it is always referred to as loosely bound fluoride. The succinct chemical reaction between hydroxyapatite and fluoride is as follows:

$$Ca_{10}(PO_4)_6(OH)_2 + 20\ F^- + 8\ H^+ \rightarrow\ 10\ CaF_2 + 6\ HPO_4^{2-} + 2\ H_2O$$

However, the formation and retention of calcium fluoride has received a different appraisal over the years. First, the intensity of calcium fluoride is found to be greatly affected by the acidity of the fluoride solution (Petzold 2001). This can be attributed to the accelerated

dissolution of apatite at low acidity that provides more free calcium ions and consequently favours the precipitation of calcium fluoride-like material after fluoride treatment. This assumption is not applicable to SDF because SDF is alkaline (Mei et al. 2013a), and this alkaline property is unlikely to dissolve the tooth structure and promote the release of free calcium ions unless there was a caries attack. The amount of calcium fluoride produced in this circumstance is expected to be lower than other fluoride treatments such as acidulated phosphate fluoride or neutral sodium fluoride varnish. Another source of calcium ions in the mouth is saliva. Although 38% SDF solution has a high concentration of silver (255,000 ppm) and fluoride (448,000 ppm), clinical treatment involves a one-time application of a minute amount of the solution (0.22 ± 0.07 mg total amount / 8.8 ± 2.8 μg fluoride) onto a caries lesion (Chu et al. 2012). In the clinical setting, the SDF is readily diluted by saliva in the mouth. Study which mimiced this situation and found that fluorohydroxyapatite was the major product (Mei et al. 2017). Another question is how the calcium fluoride is retained on the tooth surface. Previous *in vitro* studies found that the amount of calcium fluoride after SDF treatment significantly dropped after being immersed in artificial saliva (Suzuki et al. 1974) or disappeared after washing with water (Lou et al. 2011). Some clinical studies found that 80% of the calcium fluoride was lost in 5 days after fluoride varnish application (Attin et al. 1995), or even within 24 hours when it was subsequently exposed to the oral environment (Brudevold et al. 1967). Others, however, believe that calcium fluoride can persist on the tooth due to the presence of a phosphate or protein rich "pellicle" covering the calcium fluoride globular deposits (Chander et al. 1982). Nevertheless, it is generally accepted that calcium fluoride in oral cavity could act as a depot and release fluorides at acidic condition such as caries attack to form fluorohydroxyapatite gradually (ten Cate 2013). Future studies seem to be necessary to take into account the influence of different locations of the oral cavity and of the real oral conditions on these phenomena.

3.4. Effect on dentine collagen

Fluoride protects dentine collagen mainly through two possible ways. First, fluoride promotes remineralisation and the apatite crystallites in turn cover and protect the collagen. In an *ex vivo* study which collected the exfoliated primary teeth treated by SDF (Mei et al. 2014b), the surface morphology of the arrested caries lesion showed a relatively smooth surface with few dentine collagen fibres exposed (Figure 8a) while that of the active dentine lesion was porous and rough with collagens exposed, disorganised, and sparsely distributed (Figure 8b). By using immunolabeling technology, researchers also found more sound collagen I in SDF

treated caries lesion than that in water treated caries lesion (Mei et al. 2013b). Second, fluoride inhibits the activities of collagenases (Selwitz et al. 2007). By comparing SDF with silver nitrate and sodium fluoride solutions, it was suggested fluoride is a strong inhibitor to MMP-2, MMP-8 and MMP-9 (Mei et al. 2012b). Kato and co-workers (2014) reported that 200 ppm F completely inhibited both inactive and active forms of MMP-2 and MMP-9, Hannas and co-workers (2016) also demonstrated that when MMP-9 was incubated with 150 ppm fluoride, the activity of the enzyme was inhibited 79% within a few minutes. The mechanism proposed by previous researchers on fluoride inhibitory on MMPs is limited. Some study indicated the inhibitory effect was attributed to the high electronegativity of fluoride ions that could bind to zinc ion and calcium ion, which are required cations for the catalytic function of MMPs (Kato et al. 2014). Fluoride was also found to inhibit cathepsins B and K activities (Altinci et al. 2016; Mei et al. 2012b), but the mechanisms are not clear.

4. Silver-fluoride complex

The concept of fluoride-metal complexes has been adopted for decades (Peng et al. 2012). When silver combine with fluoride in ammonia solution, it releases silver ions and fluoride ions. Silver was proven to be antibacterial (Mei et al. 2013e), it also interacts with a reactive side chain of the enzymes to inactivate their catalytic functions. Fluoride is well-known as a remineralisation agent (Mei et al. 2017), it also inhibits collagenase activities. Silver and fluoride are proven to have synergic effect, rather than pure addition effect on arresting dentine caries (Mei et al. 2013d). In addition, SDF is an alkaline solution, the acidity (pH values) of SDF solutions (12%, 30% and 38%) are around 9 to 10 (Mei et al. 2013a). Most enzymes are activated in an acidic environment, and the alkaline property of SDF may also contribute to neutralisation of the acidity and therefore inactivate the enzymes.

5. Summary

The understanding of the mechanisms of silver diamine fluoride in arresting dentine caries has increased in recent years. Unlike other fluoride products that mainly have an effect on preventing formation of new caries, 38% SDF is capable of efficiently arresting the caries process. The reason for this phenomenon could be the synergic effect of silver ion and fluoride ion. Silver ion inhibits biofilm growth while fluoride enhances mineral formation. The formation of fluorohydroxyapatite with reduced solubility could be one of the main structures produced after SDF application. Silver ion and fluoride ion also inhibit the activity of collagen

389 enzyme and therefore protect the collagen from degradation. In addition, the alkaline property
390 of this reagent could also alter the microenvironment around the caries lesion in which the
391 enzymes could be inactivated.
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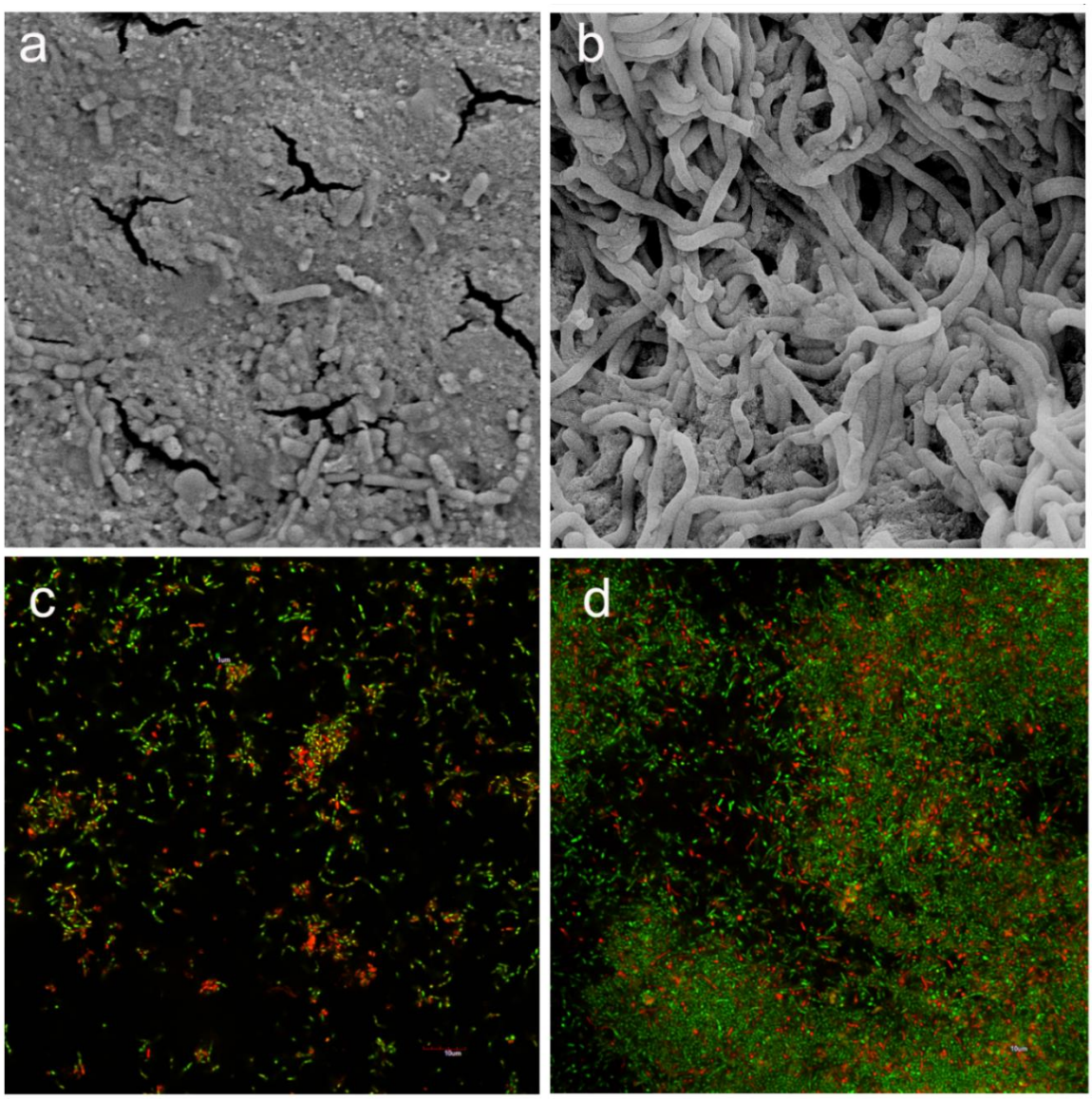
Figure 1 Rampant caries before and after silver diamine fluoride application

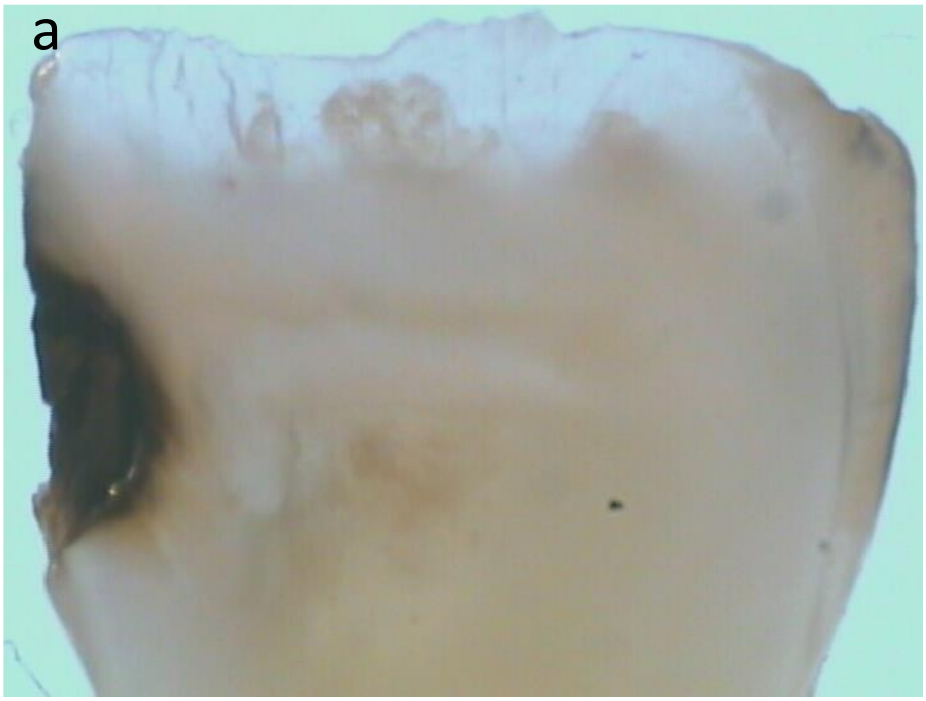
Images from Chu et al. 2014 [reprinted with approval]

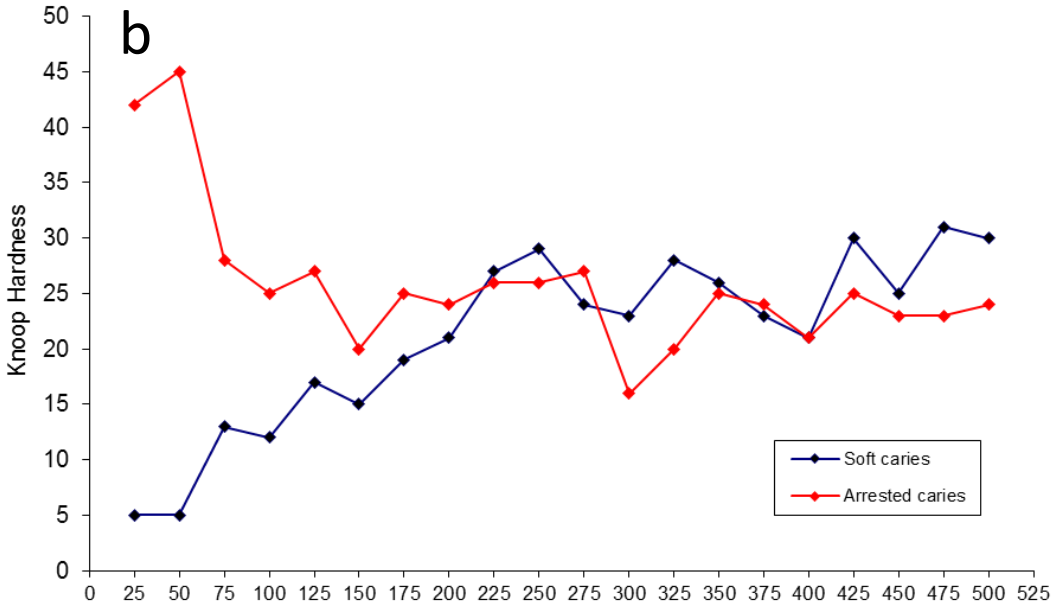
Figure 2 Growth of multi-species cariogenic biofilm (*Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Actinomyces naeslundii)* on dentine treated with silver diamine fluoride (SDF) and water (control) after 14 days



a: Scanning electron micrograph of SDF treated dentine.
b: Scanning electron micrograph of water treated dentine. SDF aggregates of bacteria were observed on the dentin surface, while multi-species cariogenic biofilm in the control group was confluent.
c: Confocal laser scanning micrograph of SDF treated dentine.
d: Confocal laser scanning micrograph of water treated dentine. The red-to-green ratio was calculated to denote the ratio of dead-to-live bacteria, and it showed significantly higher ratios in the SDF group than in the water groups.
Images from Mei et al. 2013e [reprinted with approval]

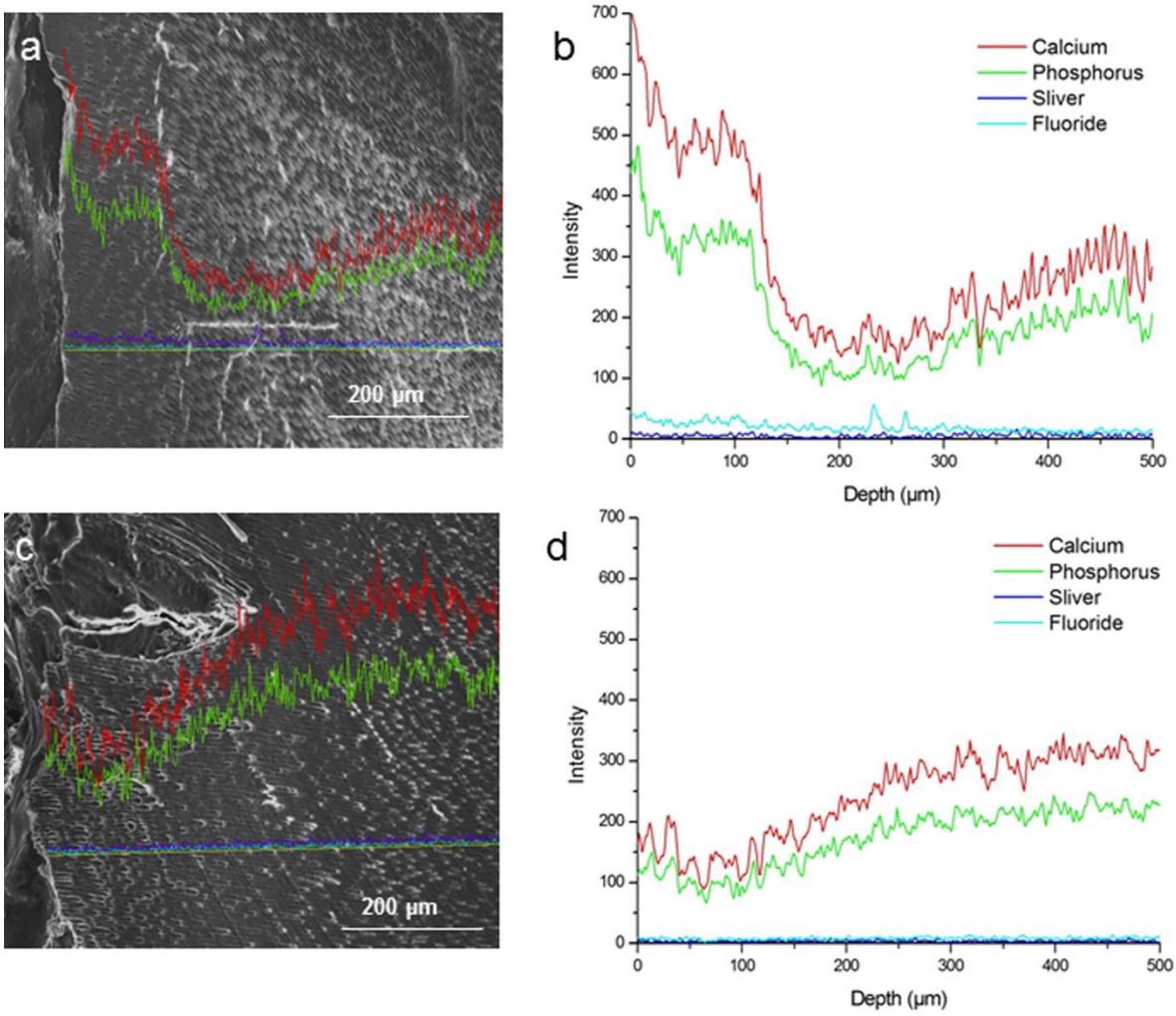
Figure 3 Ground section (a) and microhardness (b) of arrested caries lesion after silver diamine fluoride (SDF) treatment





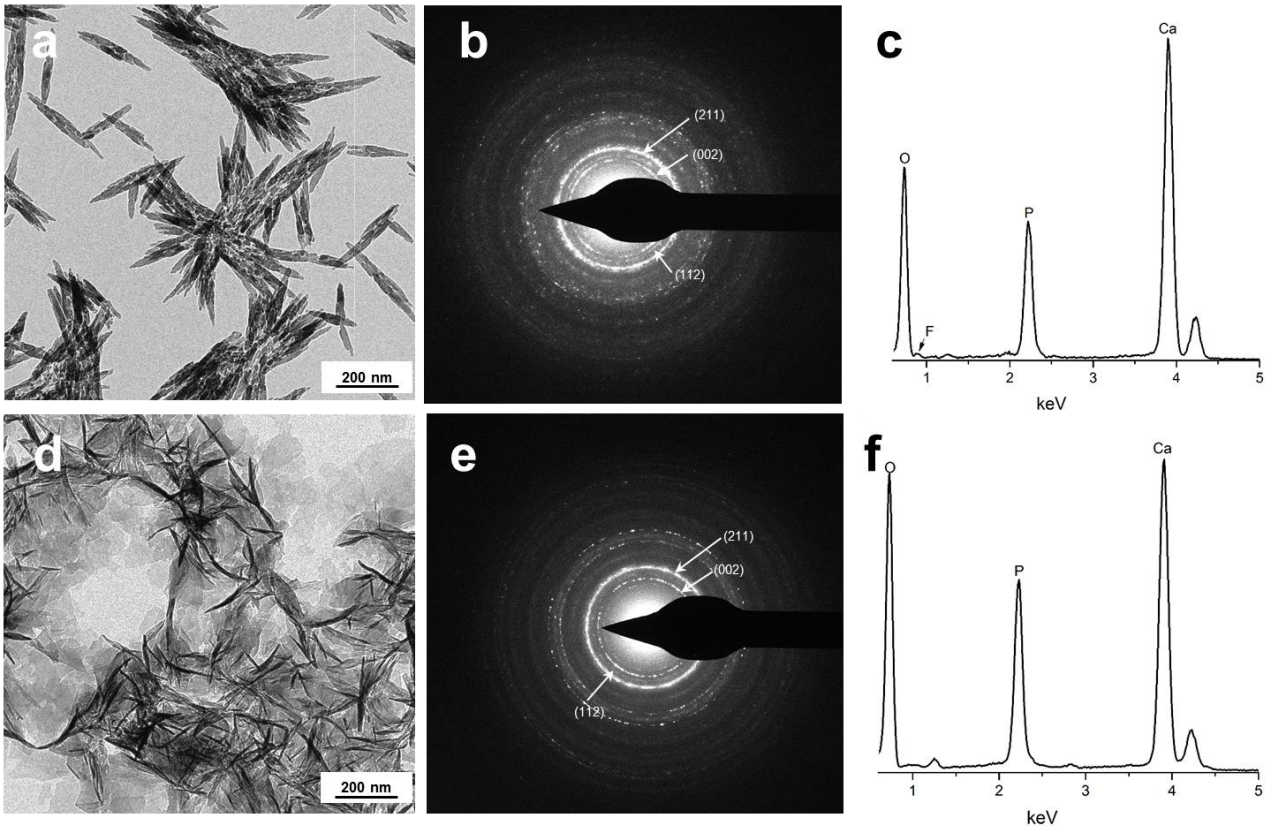
Images from Chu and Lo, 2008a [reprinted with approval].

Figure 4 Elemental distribution of calcium, phosphorus, silver and fluoride along the depth in the arrested caries lesion and active caries lesion



a: Cross-sectional image of SDF-arrested caries lesion
b: Cross-sectional image of active caries lesion
c: Corresponding line-scan elemental profile of a) along the depth of arrested caries lesion
d: Corresponding line-scan elemental profile of b) along the depth of active caries lesion
Images from Mei et al. 2014b [reprinted with approval]

Figure 5 Apatite crystal formed in silver diamine fluoride solution and calcium phosphate solution (control) under transmission electron microscopy



a: Transmission electron micrograph of apatite crystal formed in silver diamine fluoride (0.38 mg/mL) solution

b: Image of a) under selected-area electron diffraction

c: Energy dispersive X-ray spectroscopy spectrum of a). Fluoride was detected suggesting the formation of fluorohydroxyapatite.

d: Transmission electron micrograph of apatite crystal formed in calcium phosphate solution (control).

e: Image of d) under selected-area electron diffraction

f: Energy dispersive X-ray spectroscopy spectrum of d). No significant amount of fluoride was detected.

Images from Mei et al. 2017 [reprinted with approval]

Figure 6 Crystal structure of hydroxyapatite, fluorohydroxyapatite and fluorapatite

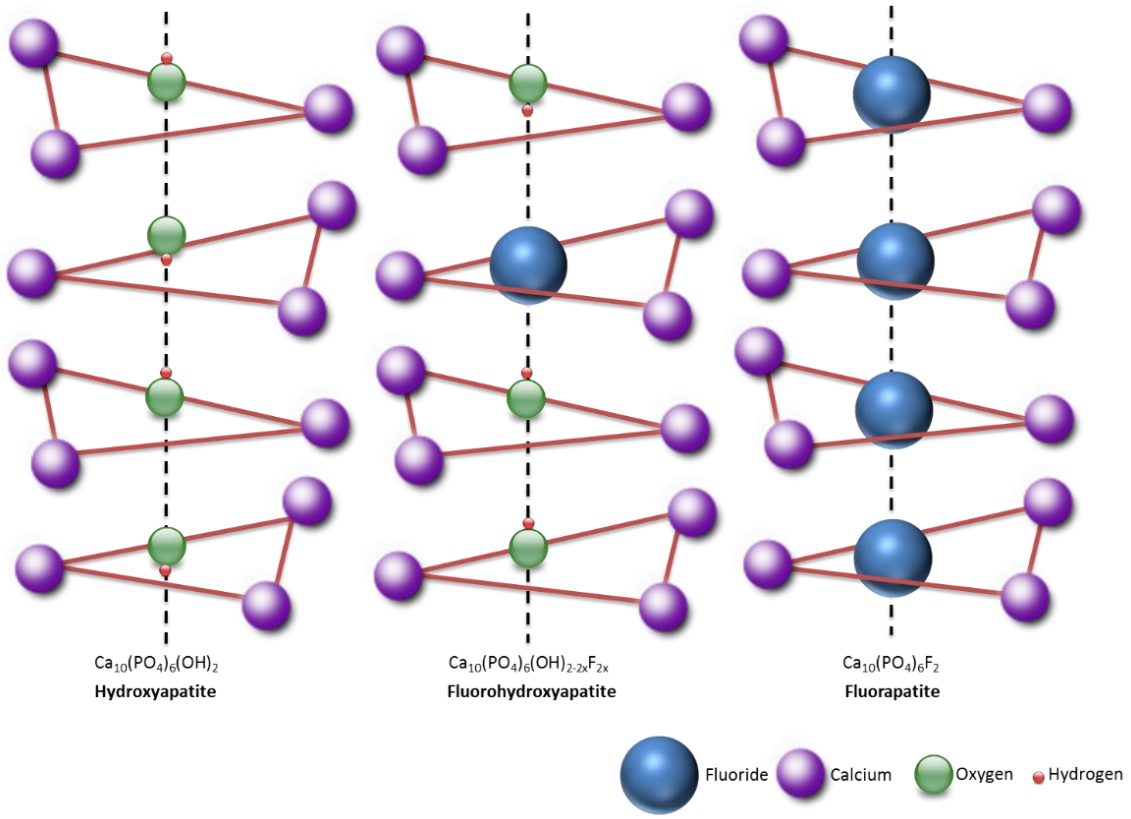
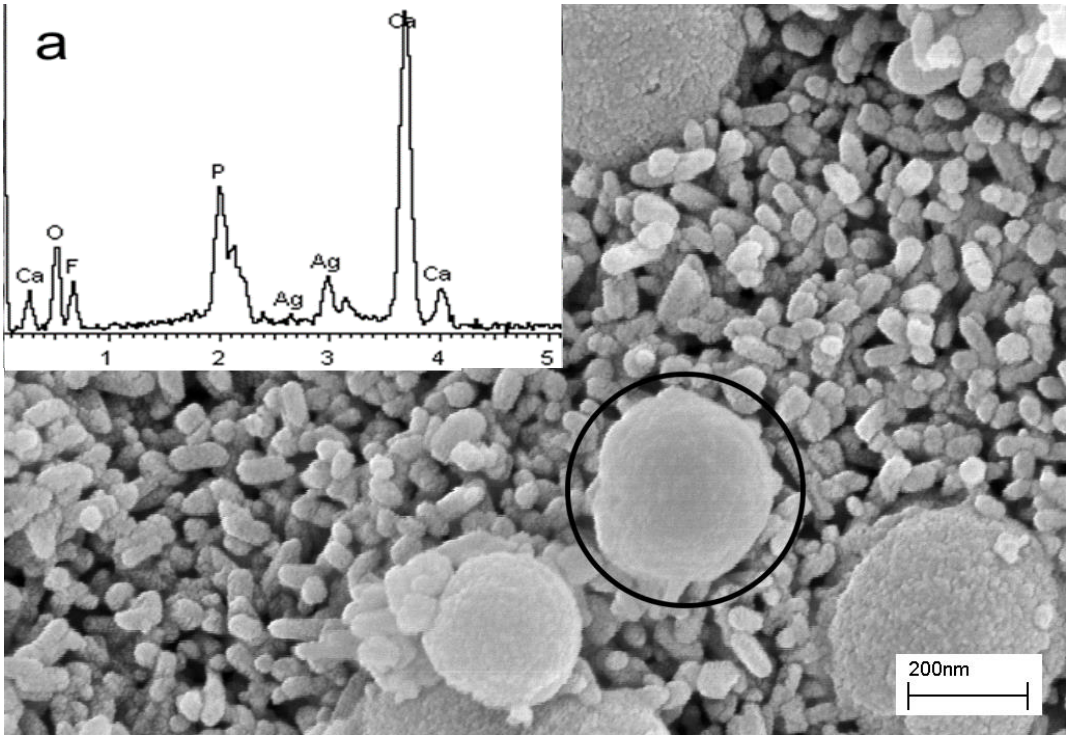
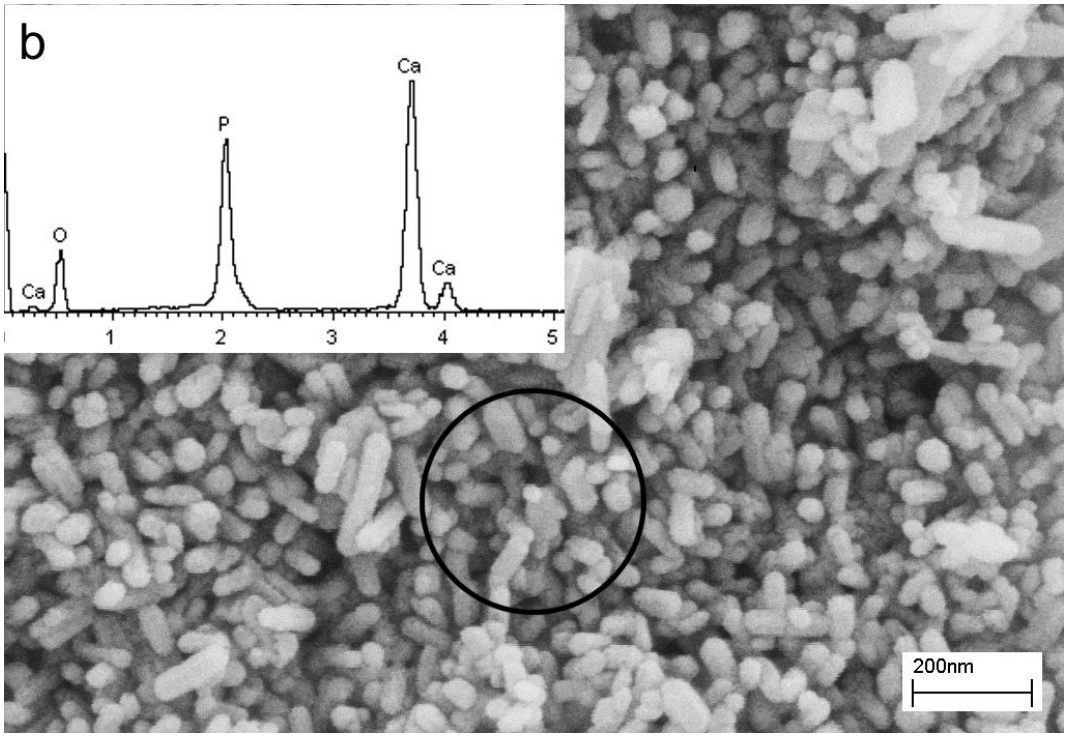


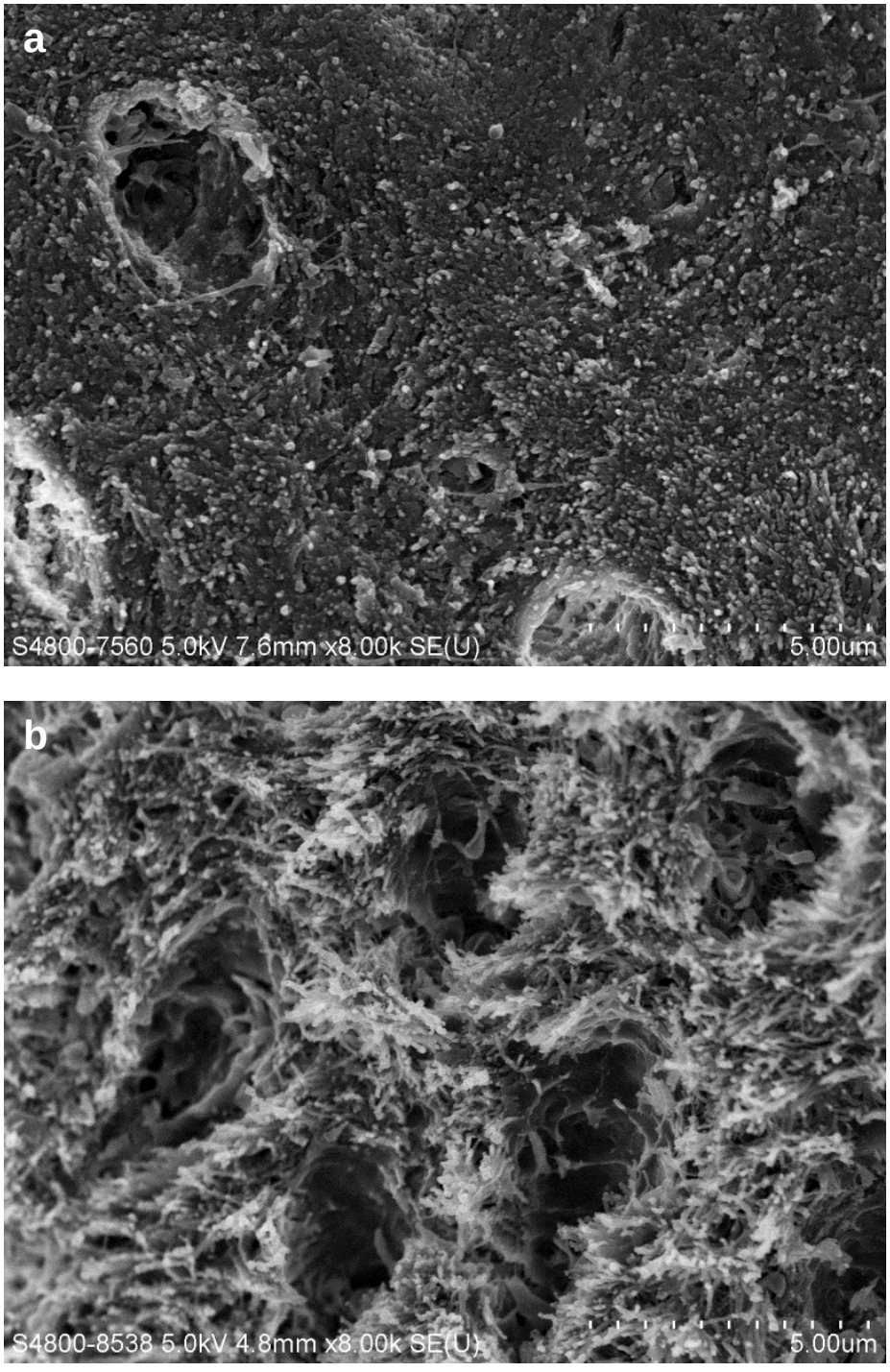
Figure 7 Silver diamine fluoride treated hydroxyapatite power exposed to light before (a) and after (b) washing





Scanning electron micrographs and energy dispersive X-ray spectroscopy spectrum of circled areas of hydroxyapatite power treated with 38% silver diamine fluoride exposed to light, before (a) and after (b) washing. Calcium fluoride-like material (circled areas) was found and disappeared after washing. *Images from Lou et al. 2011 [reprinted with approval]*

Figure 8 Surface morphology of arrested dentine caries lesion after silver diamine fluoride treatment (a) and active dentine caries lesion (b) under scanning electron microscopy



Images from Mei et al. 2014 [reprinted with approval]