Inability to form a biofilm of *Streptococcus mutans* on silver fluoride– and potassium iodide–treated demineralized dentin

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Objective: The presence of a biofilm is necessary for both initiation and progression of dental caries. Silver-based preparations incorporated into, or applied onto, various materials designed for medical use have been shown to be effective in inhibiting biofilm formation. The purpose of this in vitro study was to measure whether a topical application of diamine silver fluoride (AgF) followed by potassium iodide (KI) on partially demineralized dentin affected the formation of a *Streptococcus mutans* biofilm. **Method and Materials:** Forty partially demineralized dentin disks were divided into 4 groups as follows: 10 disks as a control, 10 disks treated with AgF followed by KI, 10 disks treated with KI, and 10 disks treated with AgF. The outer surfaces of the disks were examined with a scanning electron microscope. Cross sections of the disks were subjected to electron probe microanalysis (EPMA) to determine the levels of calcium, phosphorous, silver, and fluoride in the dentin. **Results:** An *S mutans* biofilm covered the entire exposed surfaces of all control and KI-treated disks. No discernible bacterial biofilm was detected on disks treated with AgF or AgF/KI. Detectable amounts of silver and fluoride were found up to 450 µm in the AgF and AgF/KI sections. **Conclusions:** Demineralized dentin disks treated with AgF and AgF/KI prevented the formation of an *S mutans* biofilm and were significantly more resistant to further demineralization than the control and KI-treated disks over the experimental period. The presence of silver and fluoride in the outer layers of the disks treated with AgF and AgF/KI was the likely cause of the prevention of biofilm formation. Additional studies are required before any clinical recommendations can be made. *(Quintessence Int 2009;40:155–161)*

Key words: biofilm, demineralized dentin, potassium iodide, silver fluoride, Streptococcus mutans

The arrestment of early dentin caries using chemotherapeutic procedures is a desirable goal, especially on root surfaces and in areas where access for restorative procedures is limited. To date, the main chemotherapeutic approaches to the problem have involved the use of fluorides[1] and, more recently, ozone.[2] A metal-based topical fluoride preparation, diamine silver fluoride (referred to as AgF in this article), has been used to arrest dentin caries in the primary dentition[3,4] and reduce caries formation in deciduous teeth and permanent first molars.[5] However, its potential in arresting early dentin caries in sites such as root surfaces of the permanent dentition is not known.

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Following the application of silver salts to a treatment site, excess free silver ions have traditionally been reduced by application of either eugenol or stannous fluoride, resulting in the formation of a black precipitate.[6-8] To circumvent this problem, another approach is currently under investigation that involves the application of potassium iodide (KI) immediately following the application of the silver salt.[9] The resulting creamy white precipitate, silver iodide, eliminates staining and has a history of use in dentistry.[10]

An in vitro study on demineralized dentin disks in a diffusion apparatus compared the efficacy of a topical treatment with AgF with that of AgF followed by KI on the penetration and viability of a test organism, *Streptococcus mutans*.[9] Based on optical density readings and scanning electron microscopy (SEM) analysis, both treatments had significant inhibitory effects on organism penetration and/or growth during a 14-day exposure, although the AgF/KI treatment did yield more consistent results.

These observations, plus others in the medical area, indicate the potential for antimicrobial activity of silver when incorporated into a suitable substrate. For example, it has been shown that the incorporation of silver into vascular grafts can diminish the formation of biofilms by *Staphylococcus aureus*.[11]

In the dental field, the possibility exists that silver incorporated into a surface such as demineralized dentin may exert some inhibitory effect on the formation of a biofilm containing cariogenic organisms. To examine this aspect further, this study was undertaken to determine whether silver could be retained in demineralized dentin following topical application of AgF or AgF/KI and whether this influenced biofilm formation by *S mutans*.

METHOD AND MATERIALS

The crowns of 40 recently extracted human third molars that had been stored in 0.5% chloramine were sectioned horizontally to produce enamel-dentin sections approximately 1.5 mm thick. Only sections with flat, sound dentin on either surface were used. Teeth were collected within the guidelines set by the Committee for the Ethics of Human Experimentation, University of Adelaide.

A rim of composite resin (Glacier, SDI) was bonded to the etched outer enamel surface, after which the disks were reduced to 1 mm in thickness using a graded series of wet and dry papers to 4,000-grit silicon carbide paper (Struers).

The enamel surfaces of all disks were painted with a narrow strip of nail varnish to protect the enamel from the effects of demineralization. The samples were then immersed in 40 mL acetate demineralization solution[12] at 37°C for 4 days to create demineralized lesions with a depth of 150 mm.

To facilitate handling and treatment of the sections, the bases of 45-mL vials were removed to provide an open end. The area inside the open end was roughened using airborne-particle abrasion (Rondoflex, KaVo; 50-mm aluminum oxide particles), and disks were attached to the vials using Resin Bond (3M ESPE) and Glacier composite resin.

Once in place, 40 samples received 1 of the following treatments, assigned at random, to the outer surfaces:

1. Ten samples were coated with a solution of 1.8 mol/L AgF, followed by a saturated solution of KI, and rinsed with copious amounts of distilled water.
2. Ten samples were treated with a saturated solution of KI and rinsed with copious amounts of distilled water.
3. Ten samples were coated with a solution of 1.8 mol/L AgF and then rinsed with copious amounts of distilled water.
4. Ten samples were given no treatment and left as a control.

To avoid any effects from desiccation of the underlying surfaces of the samples, the remainder of each vial was filled with nutrient broth. A batch of nutrient solution was made, consisting of 3% tryptone soya broth (Oxoid), yeast extract 0.5% (Oxoid), and 20% sucrose. To ensure sterility, the samples were placed into a sealed plastic vessel and chilled in a refrigerator before being subjected to gamma radiation (Steritech) at a dose of 15 R.

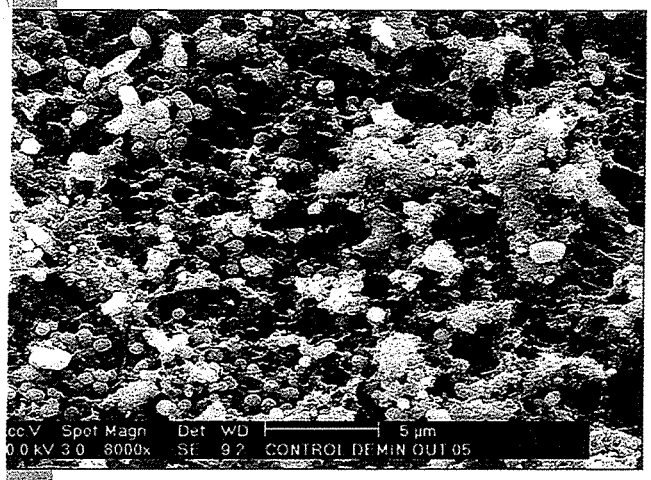

Fig 1 Typical outer surface of a nontreated (control) demineralized dentin disk after 2 weeks of exposure to *S mutans*. Heavy biofilm formation is evident.

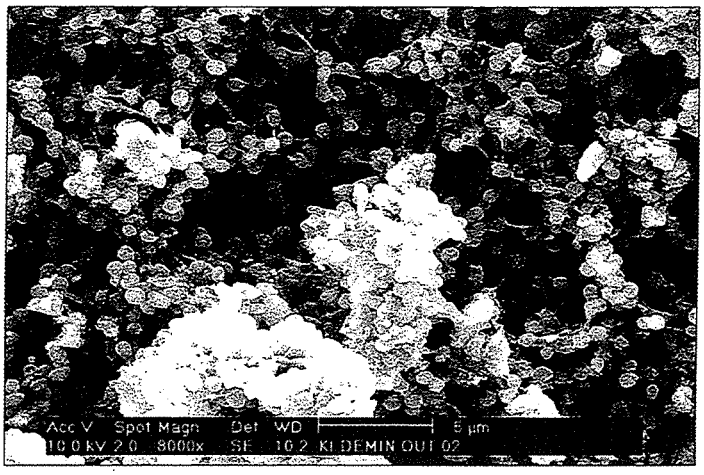

Fig 2 Typical outer surface of a demineralized dentin disk treated with KI after 2 weeks of exposure to *S mutans*. Heavy biofilm formation is evident.

The sterilized vials were then placed in a sterile glass flask connected to the outflow from a chemostat system (New Brunswick Scientific). This provided a constant supply of viable *S mutans* subsp *inbritt* grown by continuous culture. The bacteria were grown in the same medium used to fill the 5-mL vials. Growth was maintained under anaerobic conditions at an imposed dilution rate of $0.1h^{-1}$ (td [doubling time] = 7 h), and the pH was maintained at 7.4 by the automatic addition of KOH (2N) (potassium hydroxide 2 normal). The pH of the flask containing the vials was uncontrolled and remained at about 4.5 throughout the 2-week experiment.

Upon completion of the experiment the disks were removed from the samples and cut in two. One half was prepared for SEM (Philips XL30 field emission scanning electron microscope) and the other half for electron probe microanalysis (EPMA) (Cameca, SX51) following techniques described in detail elsewhere.[13] The elements analyzed by EPMA were calcium, phosphorus, fluoride, and silver using a technique developed by Ngo et al.[14,15]

Because the data were not normally distributed, the Kruskal-Wallis test was used to determine if there was a difference among the groups. Post hoc testing was used to make pairwise comparisons with no adjustment for multiple comparisons. As calcium and phosphorous were removed from the system, data were compared by measuring the areas above the curve, Delta Z. As fluoride and silver were being added to the system, data were compared by measuring the areas below the curve. Although measurements were carried out to a depth of 500 mm, the experimental model was set up to examine the changes occurring at the dentin surface interface, and data was not analyzed beyond a depth of 300 mm, the depth to which secondary demineralization had occurred during the experiment.

RESULTS

The SEMs showed an appreciable *S mutans* biofilm covering the entire exposed surface of all control disks (Fig 1). A similar situation was seen with the disks treated with KI (Fig 2). In contrast, no discernable bacterial biofilm was detected on disks treated with either AgF (Fig 3) or AgF/KI (Fig 4). However, there was evidence of some reaction product precipitates at the orifices of some dentinal tubules in the disks treated with AgF/KI (see Fig 4).

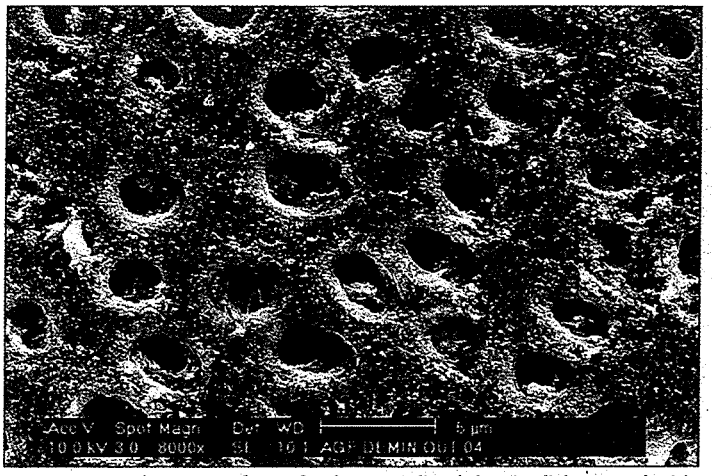

Fig 3 Typical outer surface of a demineralized dentin disk treated with AgF after 2 weeks of exposure to *S mutans*. There is no evidence of biofilm formation.

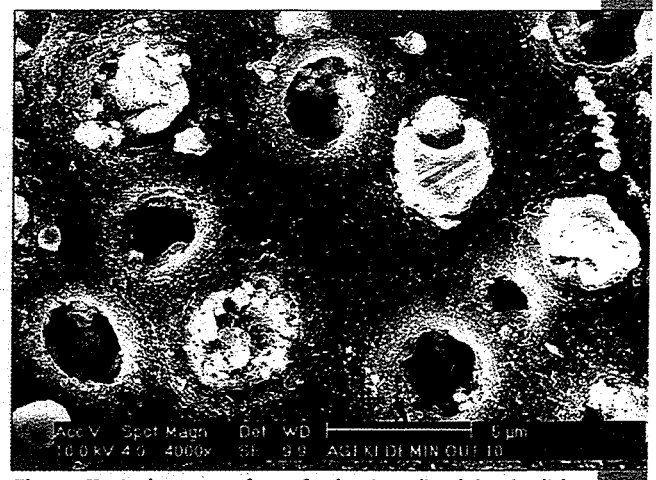

Fig 4 Typical outer surface of a demineralized dentin disk treated with AgF followed by KI after 2 weeks of exposure to *S mutans*. There is no evidence of biofilm formation; however, the openings of many of the dentinal tubules are blocked with inorganic-type deposits.

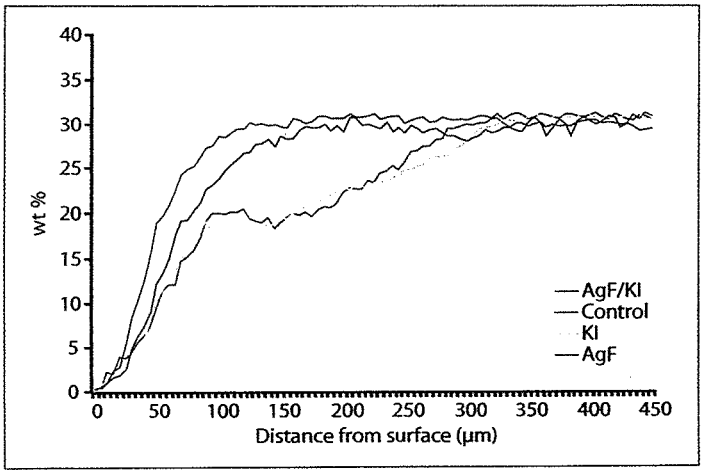

Fig 5 EPMA graph of the percentage weights of calcium in treated and control specimens. Statistical analysis (differences at the .05 level; NS = not significant, S = significant): control versus KI (NS); control versus AgF (S); control versus AgF/KI (S); KI versus AgF (S); KI versus AgF/KI (S); AgF versus AgF/KI (NS).

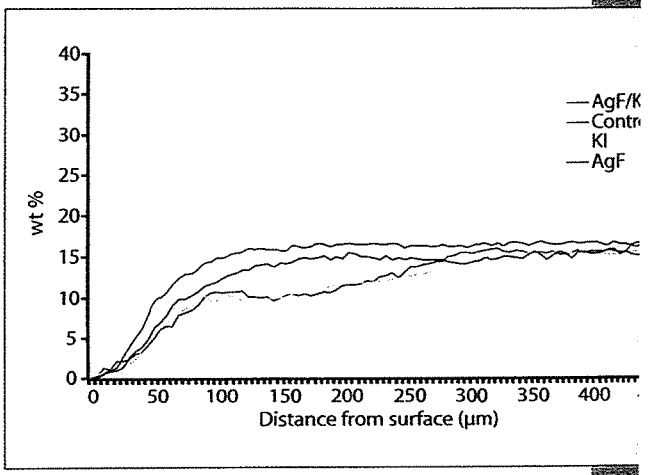

Fig 6 EPMA graph of the percentage weights of phosphorus in treated and control specimens. Statistical analysis (differences at the .05 level; NS = not significant, S = significant): control versus KI (NS); control versus AgF (S); control versus AgF/KI (S); KI versus AgF (S); KI versus AgF/KI (S); AgF versus AgF/KI (NS).

The EPMA analyses showed that the levels of calcium (Fig 5) and phosphorus (Fig 6) increased gradually from the surface to the final measurement depth of 300 mm. The calcium and phosphorus loss from control and KI-treated disks was significantly higher overall than from AgF- and AgF/KI-treated sections ($P < .05$).

Similarly, the fluoride levels in all disks tended to be lower in the surface area than in the deeper zones (Fig 7). They were significantly higher overall in the dentin disks treated with AgF and AgF/KI ($P < .05$). There was a significantly higher uptake of fluoride in the first 300 μm in the AgF/KI-treated disks compared to the AgF-treated disks ($P < .05$). High

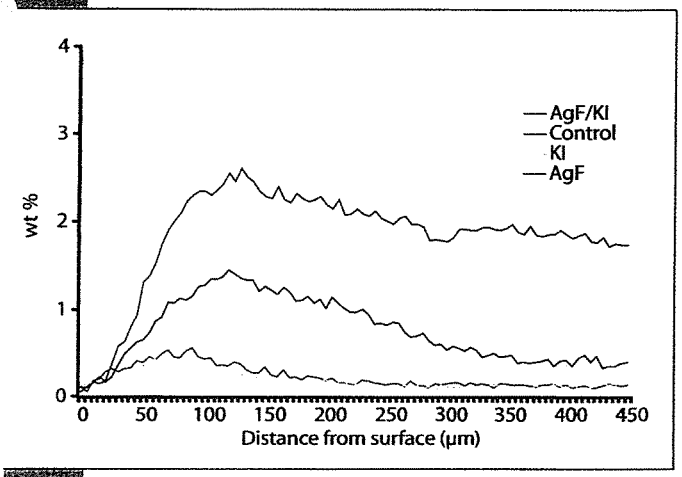

Fig 7 EPMA graph of the percentage weights of fluoride in treated and control specimens. Statistical analysis (differences at the .05 level; NS = not significant, S = significant): control versus KI (NS); control versus AgF (S); control versus AgF/KI (S); KI versus AgF (S); KI versus AgF/KI (S); AgF versus AgF/KI (S).

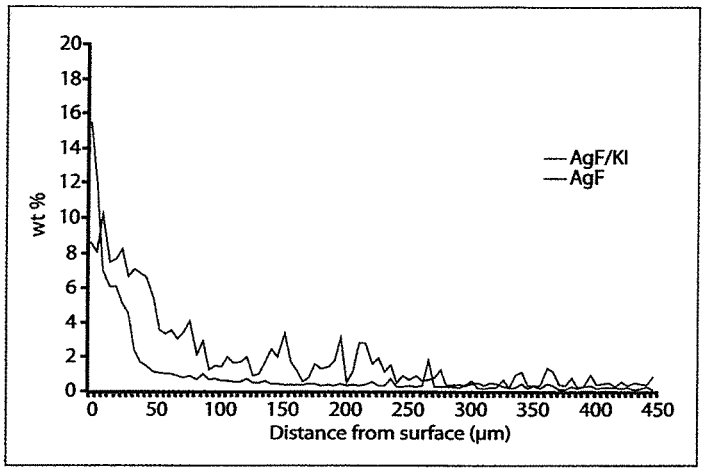

Fig 8 EPMA graph of the percentage weights of silver in the treated and control specimens. Statistical analysis (differences at the .05 level; NS = not significant, S = significant): AgF versus AgF/KI (NS). (The levels of silver in the control and KI-treated samples were below the detectable limit).

levels of fluoride were observed up to a depth of 500 μm in AgF/KI-treated disks.

Extremely high levels of silver were found to a depth of approximately 100 μm in the disks treated with AgF or AgF/KI (Fig 8). There was no significant difference overall between the silver uptake in AgF- and AgF/KI-treated disks, although more silver was precipitated at the surface of disks treated with AgF/KI.

DISCUSSION

When considering the outcome of this investigation, the limitations of such an in vitro study must be taken into account. No attempt was made to mimic the environmental conditions likely to be encountered in the oral cavity. For example, only a single organism was used, and salivary constituents or a salivary substitute were absent. Therefore, the results cannot be extrapolated to the in vivo situation, and caution should be exercised in their interpretation.

The findings of this study confirm those of other investigations showing that the incorporation of silver preparations into surface layers of items such as catheters, prosthetic heart valves, and vascular grafts can inhibit biofilm formation.[11,16] In this study, the effects of the topical treatment with either AgF followed by KI or AgF alone were still evident at the end of the 14-day experimental period. EPMA analyses showed that at 14 days levels of silver were still high in the surface layers of the AgF- and AgF/KI-treated dentin disks.

The complete inhibition of biofilm formation by *S mutans* on disks treated with AgF and AgF/KI points to the major inhibitory effects being due to the silver and the fluoride moieties. Although no attempt was made to quantify the amount of biofilm formed on the control or KI-treated disks, the SEM findings indicate there were marked deposits on both. Consequently, the role of iodine in inhibiting biofilm formation appeared to be either nonexistent or, at best, limited.

Depending on concentration, ionized silver can either kill bacteria or interfere with their metabolic processes.[17,18] While the amount of silver released during the 14-day exposure to *S mutans* from the chemostat is not known, it has been established that silver ion concentrations as low as 20 ppm can inhibit the growth of *S mutans* and *St aureus*.[19]

The levels of silver released in this study, if not sufficient to kill the test organism, could have interfered with the synthesis of cellular polysaccharides produced by *S mutans* by

inactivation glycosyltransferase enzymes. These enzymes are responsible for the synthesis of soluble and insoluble glucans that not only contribute to the bulk of the biofilm but also play essential roles in the sucrose-dependent adhesion of the organism to tooth surfaces.[20] Metal cations have been shown to inhibit the activity of several glycosyltranferases.[21]

The role of fluoride release in the findings is not clear. Numerous studies have shown that fluoride can inhibit growth of *S mutans* and other plaque bacteria.[22] In this study, at the end of the experimental period, EPMA analyses showed that the level of fluoride to a depth beyond 300 μm was significantly higher in the disks treated with AgF or AgF/KI. The role of the elevated fluoride levels in inhibiting biofilm formation in this study is not known. To ascertain the role of fluoride in the results obtained, it would have been necessary to run another group of specimens treated with a nonmetallic fluoride salt with a fluoride concentration equivalent to the AgF preparation used. This was not possible. Nonmetallic fluoride salts are far less soluble than AgF, and no such preparation was found that could equal the fluoride level used in this study, namely 37,500 ppm.

In terms of toxicity, the levels of silver and fluoride employed in this investigation were at least one-third lower than the levels in the AgF preparations used as a topical application to arrest existing caries in primary teeth[4] and for the treatment of carious dentin before placing glass-ionomer cement restorations.[23]

The significantly greater calcium and phosphorus loss from control and KI-treated disks compared to the AgF- and AgF/KI-treated disks suggests that the AgF-based topical treatments had a discernible protective effect on the dentin surfaces. It is estimated that the surfaces of all sections were exposed to a pH of around 4.5 for the entire 2 weeks of the experiment. Once the organisms left the chemostat, the pH dropped because there was no neutralization of acid production as was the case in the chemostat.

SEMs of dentin treated with AgF/KI showed evidence of deposits at the entrance to some dentinal tubules. These may be deposits of silver iodide (AgI) formed as a result of the interaction between free silver ions and free iodine ions, as they were not present on sections treated with AgF alone. Their presence would explain the high silver peak noted at the surface of the AgF/KI-treated specimens (see Fig 8). Residual surface deposits of AgI could be beneficial; as AgI is a sparingly soluble salt, it could act as a slow-release source of antimicrobial silver and iodide ions.

Overall, the additional step of applying KI after the AgF treatment had no adverse effects on the results obtained, and the KI application significantly increased the uptake and depth of fluoride penetration compared to AgF alone.

While the results of the present investigation point to a useful role of AgF/KI in the inhibition of biofilm formation by *S mutans*, no attempt was made to determine the effect of lower concentrations on biofilm formation or the viability of planktonic organisms in groups showing no biofilm growth. Further studies are indicated in this area using confocal laser scanning microscopy to provide further information on the nature of any biofilms formed.

These findings need to be confirmed experimentally in vivo. For example, it would be interesting to know the nature of any biofilm formed on demineralized disks of dentin treated with AgF or AgF/KI, placed in a removable appliance, and exposed to the oral environment.

CONCLUSIONS

Demineralized dentin disks treated with AgF or AgF/KI resisted the formation of an *S mutans* biofilm during a 14-day exposure to the organism. In contrast, control disks and those treated with KI alone showed marked biofilm formation. Electron probe microanalyses showed high levels of silver incorporated into the surface layers. The possibility exists that the release of silver and fluoride from the treated dentin either killed the test organism or interfered with its glycosyltransferase activity, thereby inhibiting the synthesis of the insoluble glucans required for adhesion to a tooth surface.

Additional studies are required before any clinical recommendations can be made.

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REFERENCES

1. Lynch E, Baysan A. Reversal of primary root caries using a dentifrice with a high fluoride content. Caries Res 2001;35(suppl):60–64.
2. Holmes J. Clinical reversal of root caries using ozone, double-blind, randomized controlled 18-month study. Gerodontology 2003;20:106–114.
3. Yamaga R, Nishino M, Yoshida S, Yokomizo I. Diammine silver fluoride and its clinical application. J Osaka Univ Dent Sch 1972;12:1–20.
4. Chu CH, Lo ECM, Lin HC. 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.
5. Llodra JC, Rodriguez A, Ferrer B, Menardia V, Ramos T, Morato M. Efficiency of 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.
6. Craig GG, Powell KR, Cooper MH. Caries progression in primary molars: 24-month results from a minimal treatment programme. Community Dent Oral Epidemiol 1981;9:260–265.
7. Englander HR, James VE, Massler M. Histologic effects of silver nitrate on human dentin and pulp. J Am Dent Assoc 1958;57:621–630.
8. Langeland K, Langeland LK. Indirect capping and the treatment of deep carious lesions. Int Dent J 1968; 18:326–380.
9. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. An in vitro model to measure the effect of silver fluoride and potassium iodide treatment on the permeability of demineralized dentine to *Streptococcus mutans*. Aust Dent J 2005;50:242–245.
10. Obwegeser H, Von Wachter R. The treatment of hyperesthetic dentin with nascent silver iodide [in German]. Zahnarztl Welt 1954;9:429–430.
11. Strathmann M, Wingender J. Use of an oxonol dye in combination with confocal laser scanning microscopy to monitor damage to *Staphylococcus aureus* cells during colonization of silver-coated vascular grafts. Int J Antimicrob Agents 2004;24:234–240.
12. Ten Cate JM, Duijsters PPE. Alternating demineralization and remineralization of artificial caries lesions. Caries Res 1983;16:201–210.
13. Knight GM, McIntyre JM, Craig GG, Mulyani. Ion uptake into demineralized dentine from glass-ionomer cement following pre-treatment with silver fluoride and potassium iodide. Aust Dent J 2006;51: 237–241.
14. Ngo H, Ruben J, Arends J, et al. Electron probe microanalysis and transverse microradiography studies of artificial lesions in enamel and dentin: A comparative study. Adv in Dent Res 1997;11:426–432.
15. Ngo H. Ionic exchanges between glass ionomers and demineralized dentine. [PhD thesis]. Adelaide, South Australia: University of Adelaide, 2005.
16. Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15:167–193.
17. Slawson RM, Lee H, Trevors JT. Bacterial interactions with silver. Biol Met 1990;3:151–154.
18. Russell AD, Hugo WB. Antimicrobial activity and action of silver. Prog Med Chem 1994;31:351–371.
19. Hall RE, Bender G, Marquis RE. Inhibitory antimicrobial actions of electrically generated silver ions. J Oral Maxillofac Surg 1987;45:779–784.
20. Tamesada M, Kawabata S, Fujiwara T, Hamada S. Synergistic effects of streptococcal glucosyltransferases on adhesive biofilm formation. J Dent Res 2004;83:874–879.
21. Wunder D, Bowen WH. Action of agents on glucosyltransferases from *Streptococcus mutans* in solution and adsorbed to experimental pellicle. Arch Oral Biol 1999;44:203–214.
22. Li YH, Bowden GH. The effect of environmental pH and fluoride from the substratum on the development of biofilms of selected oral bacteria. J Dent Res 1994;73:1615–1626.
23. Gotjamanos T. Pulp response in primary teeth with deep residual caries treated with silver fluoride and glass ionomer cement ('atraumatic' technique). Aust Dent J 1996;41:328–334.