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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*

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Abstract

Background: Diamine silver fluoride ($Ag(NH_3)_2F$), referred to as AgF, has been used to reduce the incidence of caries in primary dentitions but has been limited by the associated staining of both teeth and restorative materials. The application of potassium iodide (KI), following AgF prevents staining but its effects on the ability of AgF to reduce caries are not known. The aim of this study was to develop an *in vitro* model that would provide an indication of the permeability of demineralized dentine to *Streptococcus mutans* after treatment of the dentine with AgF followed by KI.

Methods: Forty dentine discs were bonded to the base of forty 5mL polycarbonate screw top vials (that had had their bases removed), filled with nutrient medium, sterilized and placed into a continuous culture of *S. mutans*. Samples were divided into four groups as follows: 10 samples of demineralized dentine as a control, 10 samples of demineralized dentine treated with AgF/KI, 10 samples of demineralized dentine treated with KI and 10 samples of demineralized dentine treated with AgF. After two weeks the optical density of the growth medium chambers was measured to determine bacterial penetration and growth. Cultures were plated out to determine migration through the discs by *S. mutans*.

Results: S. mutans migrated through all dentine discs. However, the samples treated with AgF and AgF/KI had significantly lower optical densities than the corresponding controls. The range of optical densities was least amongst demineralized samples treated with AgF/KI.

Conclusions: Under the conditions of this study, treatment of demineralized dentine discs with AgF followed by KI allowed the penetration of *S. mutans*. Based on optical density measurements, the treatment resulted in significantly fewer micro-organisms being present subjacent to the discs treated with AgF and KI than the control discs at the end of the experimental period.

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Key words: dentine, *Streptococcus mutans*, silver fluoride, potassium iodide, bacterial migration, growth.

Abbreviations and acronyms: AgF = diamine silver fluoride; KI = potassium iodide.

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INTRODUCTION

The clinical success of a dental restoration relies in part upon the formation of a biological seal at the tooth restoration interface even if some of carious dentine is left within the preparation.[1] Mertz-Fairhurst *et al.*[2] demonstrated this in a 10-year study with lesions bounded by enamel. When viable bacteria in the remaining carious dentine at the base of a cavity are completely sealed in by a restoration they gradually lose their viability.[1] Nonetheless, as an additional safety measure it would be desirable if all remaining organisms were rendered non-viable at the time of restoration placement.

Silver salts can provide a pronounced antimicrobial action and have a long history of use in medicine and dentistry.[3-5] Several silver preparations including silver nitrate ($AgNO_3$), diamine silver fluoride ($Ag(NH_3)_2F$, referred to as AgF), followed by stannous fluoride, have been used in an attempt to prevent or arrest open carious lesions, particularly in the primary dentition.[6-8]

Another clinical application has been to use a silver salt to treat any residual infected dentine at the base of a cavity preparation prior to restoration placement and, in so doing, promote sclerotic and reparative dentine formation. $AgNO_3$ and, more recently, AgF have been used for this purpose.[4,9-11] The application of AgF under glass ionomer cement restorations in primary teeth has been shown to produce a favourable pulpal response and to be effective in promoting reparative dentine formation.[11] However, for silver salts such as AgF to be acceptable on a wider scale as a dentine treatment, any untoward side effects, such as staining of tooth structure and adjacent tooth-coloured restorations, should be eliminated.[6]

After the application of silver salts to a treatment site there is invariably an excess of free silver ions that have not been involved in the reaction. It has been customary to reduce these with either eugenol or stannous fluoride.[7-10] Both treatments result in the formation of black precipitates.

A novel approach to this problem is to use a follow-up treatment that produces white, as distinct from black, reaction products. One of the salts to do this is KI, as it produces a creamy white reaction product, silver iodide, which has been used previously in dentistry.[12]

The aim of this study was to develop an *in vitro* model that would provide an indication of the effects of the application of silver fluoride and potassium iodide singly and in combination on the permeability of demineralized dentine to *Streptococcus mutans*.

MATERIALS AND METHODS

Preparation of demineralized dentine slabs

The crowns of 40 recently extracted human third molar teeth that had been stored in 0.5 per cent chloramine were sectioned horizontally, using an Isomet Low Speed Saw (Buehler, Illinois, USA) to produce enamel dentine sections approximately 1.5mm thick. Only sections with flat, sound dentine on either surface were used. Teeth were collected within the guidelines set by the Committee for the Ethics of Human Experimentation, The University of Adelaide.

A rim of composite resin (Glacier SDI, Melbourne, Australia) was bonded to the etched outer enamel surface after which the sections were reduced to 1mm in thickness using a graded series of wet and dry papers to 4000 grit Silicone Carbide paper (Struers, Denmark).

The enamel surfaces of all sections were painted with a narrow strip of nail varnish to protect the enamel from the effects of demineralization. The samples were then immersed in 40mL acetate demineralization solution[13] at 37°C for four days to create 150µm depth of demineralized lesions.

Construction of the bacterial migration model apparatus

Forty 5mL polycarbonate vials were prepared by removing the base of each vial, leaving the lip for added retention. The cut bases were then roughened using air abrasion (Rondoflex, Kavo, Germany) (50 micron Al_2O_3 particles) around the base and over the inner lip of the container. Specimens were attached onto the base by applying resin bond (3M ESPE, Minnesota, USA) and then securing with composite resin (Glacier, SDI, Australia) to ensure there was no leakage through the composite seal (Fig 1).

Pre-treatment of dentine prior to experimentation

Samples were treated as follows: (1) 10 samples were coated with a solution of 1.8M AgF, followed by a saturated solution of KI and then washed off with copious amounts of distilled water; (2) 10 samples used as a demineralized control; (3) 10 samples treated with a saturated solution of KI and washed off with copious amounts of distilled water; (4) 10 samples were coated with a solution of 1.8M AgF and then washed off with copious amounts of distilled water.

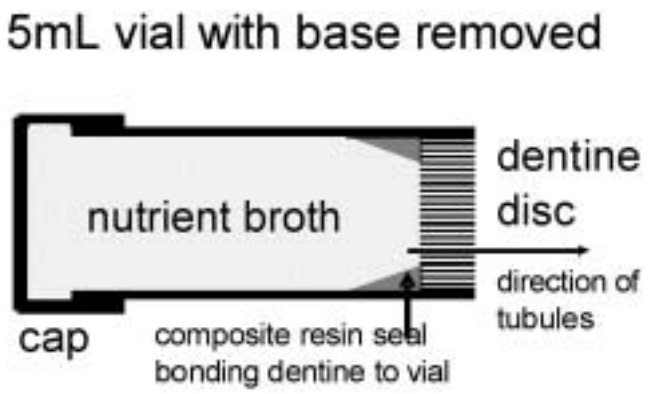

Fig 1. 5mL vial showing the various components.

Preparation of the culture medium

A batch of nutrient solution was made up consisting of 3%w/v Tryptone Soya Bean broth (Oxoid, Basingstoke, UK), Yeast Extract 0.5%w/v (Oxoid Basingstoke, UK) and 20 per cent sucrose in distilled water.

The resultant solution was poured into each of the 5mL vials so as to minimize the amount of entrapped air. After the caps were screwed on and the containers dried each container was sealed around the cap with nail varnish to prevent leakage at the seal.

Three pairs of containers filled with nutrient were subjected to gamma irradiation of 5R, 10R and 15R by Steritech Pty Ltd (Dandenong, Victoria). The samples were then incubated at 37°C for eight days. Contents of the 5R sample became turbid whilst the 10R and 15R samples did not. As there was no evidence of radiation damage to the 15R samples this was the dose chosen to sterilize the samples in the study.

The containers were placed into a sealed plastic vessel and chilled in a refrigerator prior to being dispatched for gamma irradiation (Steritech Pty Ltd, Dandenong, Victoria) at a dose of 15R to sterilize the plastic vessel and its contents.

Experimental method

The sterilized vials were then placed into a sterile glass container that was connected to the outflow from a Chemostat system. This provided a constant supply of viable *S. mutans* (sub species Inbritt) grown by continuous culture. The bacteria were grown in the same medium used to fill the 5mL vials. Growth was maintained under anaerobic conditions at an imposed dilution rate of $0.1h^{-1}$ (generation time = 7 hours) and the pH was maintained at approximately 7.4 by the automatic addition of KOH (2N). The pH of the flask containing the vials was uncontrolled and remained at

Table 1. Mean and standard deviation of optical density of nutrient broth of demineralized dentine samples

Sample	Control	AgF/KI	KI	AgF
1	1.752	0.196	0.202	0.163
2	0.176	0.166	1.217	0.199
3	1.490	0.203	0.176	0.126
4	1.865	0.201	0.188	0.170
5	0.163	0.206	0.201	0.145
6	0.171	0.182	1.346	0.127
7	1.070	0.177	1.902	0.125
8	2.060	0.155	0.227	0.161
9	0.158	0.170	0.210	0.844
10	0.993	0.189	0.246	1.813
mean	0.990	0.185	0.590	0.387
SD	0.778	0.017	0.641	0.546

around pH 4.5 throughout the two week duration of the experiment.

After two weeks the vials were removed from the Chemostat system and the optical density of the nutrient solution in the vials was measured using a Lambda 5 dual beam spectrophotometer (Perkins Elmer, Uberlingen, Germany) at a wave length of 560nm to determine the level of bacterial growth within each vial. There is no data available to give a correlation between the optical density readings and concentrations of *S. mutans* in the solution.

Further to this, cultures were plated from each container onto *Mitis salavarius* agar (Difco-Becton Dickinson, Maryland, USA) to confirm the presence of *S. mutans* in the nutrient broth.

Data analysis

Since the data was not normally distributed, the Kruskall-Wallis Test was used to determine if there was a difference amongst the groups. *Post hoc* testing was used to look at pair wise comparisons with no adjustment made for multiple comparisons.

RESULTS

S. mutans were present on every plated culture, confirming the bacteria's ability to migrate through all the samples irrespective of the surface treatment applied prior to the experiment.

The mean and standard deviation of optical density readings and the levels of variability amongst the samples are presented in Table 1. The mean values with their standard deviations are shown in Fig 2.

The median and range of the optical density readings of the control samples and those treated with either, AgF followed by KI, AgF alone and KI alone are shown in Table 2. The lowest value (0.125) was obtained for a sample treated with AgF and the highest (2.060) was obtained with a control specimen. Table 3 shows the level of significance of the differences in optical density readings between the samples.

The median optical density of the control samples was significantly ($P<0.5$) higher than that of the AgF/KI and AgF samples. However, there were no significant differences between the control samples and KI samples, between the AgF/KI and AgF samples or between the AgF/KI, AgF samples and the KI samples.

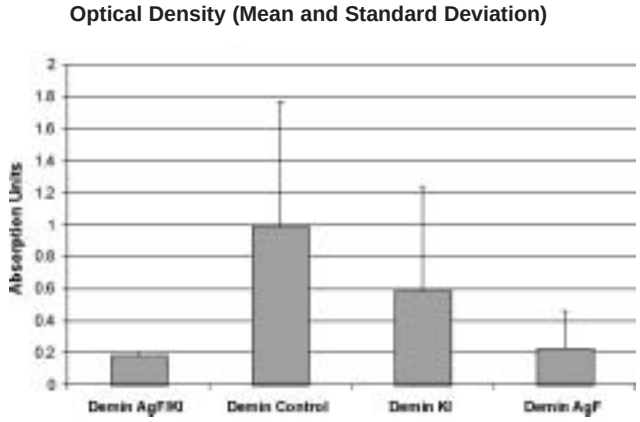

Fig 2. Graph depicting the means and standard deviations of the optical density readings in the nutrient broth samples.

DISCUSSION

The pharmacological management of dental caries has become increasingly important in restorative dentistry. An *in vitro* model to measure bacterial migration through dentine and determine the extent to which a biological seal can be achieved to prevent the passage of micro-organisms through the dentinal tubules has applications in cariology. It is also useful to have a mechanism that would measure the level of growth of micro-organisms once they had migrated through the treated samples of dentine.

Although evidence suggests that some infected dentine can be left under biologically sealed restorations with enamel margins[1] without adverse effects[2] it is unquestionably a desirable goal to try to achieve a total elimination of any potentially harmful residual organisms at the time of restoration placement.

The present study shows that *S. mutans* were able to migrate through all the samples. The unencumbered migration of bacteria into a contained nutrient solution should result in a rapid propagation of bacteria over a relatively short period.

Hall *et al.*[14] have shown that inclusion of 20ppm of $Ag(NO)_3$ in a nutrient broth inhibited growth of *S. mutans* and *Staphylococcus aureas* and inclusion of 20ppm AgF into a nutrient broth was able to inhibit growth of *S. aureas*, suggesting that silver salts at this concentration act specifically as a growth inhibitor

Table 2. The median and range of optical density of samples of nutrient broth following dentine treatment with AgF and/or KI (n: 10)

Sample	Range	Median
AgF/KI	0.155-0.206	0.186
AgF	0.125-1.813	0.162
KI	0.176-1.902	0.233
Control	0.158-2.060	1.031

Table 3. Statistical significance (P < 0.05) amongst the optical density of the samples applying Kruskall-Wallis regression analysis

Control and AgF/KI	0.0034 – S
Control and AgF	0.0245 – S
Control and KI	0.1303 – NS
AgF/KI and AgF	0.4346 – NS
AgF/KI and KI	0.1207 – NS
KI and AgF	0.4295 – NS

rather than a bactericide, although unspecified higher concentrations were found to be bactericidal.

The lower optical density readings associated with some of the treated samples indicate that some of the applied agents had the ability to interfere with the growth of the migrated bacteria. Yamaga *et al*.[5] found a wide range of bacterial inhibition amongst untreated control samples compared to a relatively narrow range of pronounced inhibition amongst teeth treated with AgF.

The wide range results obtained with the control and KI treated samples shows that the protective ability can vary within different specimens. However, the very narrow range of the results obtained with discs that were treated with AgF/KI suggests that the application of these solutions may be able to inhibit the growth of micro-organisms once they have come into close proximity with them. Further studies are required to ascertain whether this could be a possible mechanism.

There were no significant differences between the AgF/KI and the AgF samples, suggesting that the application of KI over AgF does not interfere with the effect of AgF.

The difference in the range of the readings between the AgF/KI and AgF samples suggests that AgF/KI treated teeth are more capable of exerting a reproducible effect on optical density values than samples treated with AgF alone.

CONCLUSIONS

This study has demonstrated an *in vitro* model that can be used to determine the permeation of micro-organisms through samples of demineralized samples of dentine.

The model is also able to evaluate the concentration of micro-organisms surviving in a nutrient broth once they have passed through the dentine samples. The study has shown that the materials applied to dentine samples were unable to prevent the permeation of *S. mutans* through them although there is a significant reduction in the optical density in samples treated with AgF and AgF/KI compared to control samples. The application of KI onto AgF did not appear to affect the ability of AgF to lower the optical density readings.

Finally, the low optical density readings and the narrow range amongst the observed readings suggests that application of AgF/KI to demineralized dentine exerts a reproducible effect on the ability of *S. mutans* organisms to survive in a nutrient broth.

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