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Ion uptake into demineralized dentine from glass ionomer cement following pretreatment with silver fluoride and potassium iodide

GM Knight,* JM McIntyre,* GG Craig,† Mulyani*

Abstract

Background: Diamine silver fluoride ($\text{Ag}(\text{NH}_3)_2\text{F}$), referred to as AgF, has been shown to provide a pronounced antimicrobial action against caries. The clinical application of this material has been limited by the staining associated with both teeth and tooth coloured restorative materials. The application of potassium iodide (KI) after AgF eliminates stain formation. The purpose of this study was to determine if a prior application of silver fluoride and potassium iodine to demineralized dentine affected the uptake of strontium and fluoride from a glass ionomer cement restoration.

Method: Three cavities were prepared in each of five recently extracted human third molars. The cavities were demineralized and treated as follows. In each tooth, one cavity was left as a control, one cavity was restored with glass ionomer cement and one cavity was treated with 1.8M AgF and a saturated KI solution and then restored with glass ionomer cement. The penetration of the various elements into demineralized dentine was measured by their relative percentage weights using electron probe microanalysis (EPMA).

Results: Fluoride uptake was significantly higher in the AgF and KI treated samples compared to the other two samples and significantly higher in the glass ionomer restored sample compared to the control. The application of AgF and KI did not significantly interfere with the transfer of strontium from glass ionomer cement into dentine. Silver and iodine deposits were present in the demineralized dentine treated with AgF and KI.

Conclusions: The application of AgF and KI onto dentine prior to the placement of glass ionomer cement did not significantly affect the strontium uptake into the subjacent demineralized dentine and the fluoride levels in this zone were significantly increased.

Key words: Dentine, fluoride, glass ionomer cement, silver fluoride, potassium iodide, strontium, silver, iodine, EPMA.

Abbreviations and acronyms: Ag = silver; AgF = diamine silver fluoride; Ca = calcium; EPMA = electron probe microanalysis; F = fluorine; I = iodine; KI = potassium iodide; P = phosphorus; rpm = revolutions per minute; Sr = strontium.

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INTRODUCTION

Glass ionomer cement restorations placed into cavities surrounded by softened dentine have been shown to induce hypermineralization in that dentine after exposure to the oral environment.¹ Studies using an electron probe microanalysis technique (EPMA) have confirmed the transfer of strontium and fluoride ions into the dentine from glass ionomer cement.²

Diamine silver fluoride ($\text{Ag}(\text{NH}_3)_2\text{F}$), referred to as AgF, has been shown to be effective as a caries inhibitor in deciduous teeth³⁻⁵ and has been used under glass ionomer cement restorations in primary teeth as an atraumatic means of treating remaining infected dentine and encouraging reparative dentine formation.⁶ The mechanism of caries protection by the silver moiety in AgF has yet to be determined although it has been linked to the formation of silver phosphate on the tooth surface,⁴ fluoride uptake into the dentine, blocking the dentine tubules with silver precipitates or inactivation of cariogenic bacteria that come into contact with it.⁷

An accepted disadvantage of the use of silver salts has been the propensity to cause staining of surrounding tooth structures and glass ionomer cements. An alternative approach is to use a follow-up treatment that produces tooth coloured, as distinct from black reaction products. One of the salts with the ability to do this is potassium iodide (KI). It produces a white reaction product, silver iodide, after reaction with silver ions. Silver iodide has been previously used in dentistry as a possible dentine desensitizing agent.⁸ Although the application of AgF and KI to dentine surfaces prior to placing glass ionomer cement restorations does not interfere with the bond strength of GIC to dentine,⁹

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there is a lack of information as to how this pretreatment affects the uptake of fluoride and other elements into demineralized dentine.

The purpose of this study was to use an EPMA technique to determine if the prior application of AgF and KI to demineralized dentine affects the uptake of strontium and fluoride from a glass ionomer cement restoration.

MATERIALS AND METHODS

Sample preparation

The teeth used in this study were collected within the guidelines set by the Committee for the Ethics of Human Experimentation at The University of Adelaide. The crowns of five recently extracted human third molar teeth that had been stored in 0.5% chloramine were each prepared with three cavities on the occlusal surface. An end cutting cylindrical diamond bur was used in a high speed handpiece to cut cavities (under a water spray) approximately 2mm in diameter and penetrating approximately 1mm into dentine. With the exception of the cavity preparations, all exposed surfaces were coated with nail varnish and allowed to dry. The samples were immersed in 40mL acetate demineralization solution at 37°C for seven days to create 150µm depth of demineralization in the dentinal walls of the cavity preparations.¹⁰

Pretreatment of samples

The coronal portions of each tooth were separated with a diamond disc into three sections each containing a cavity preparation. Each cavity preparation received one of the following treatments (the treatments were assigned at random): (1) One preparation was etched with 37% phosphoric acid gel (Southern Dental Industries, Melbourne, Australia) for five seconds, washed with water, dried with oil-free air and used as a control; (2) One preparation was etched with 37% phosphoric acid gel (SDI) for five seconds, washed with water, dried with oil-free air and restored with Fuji VII (GC Corp. Tokyo, Japan) auto cure glass ionomer cement; (3) One preparation was etched with 37% phosphoric acid gel (SDI) for five seconds, washed with water and dried with oil-free air. Following this, an application of 1.8M AgF solution was applied with a microbrush, immediately followed by an application with a microbrush of saturated solution of KI. The residual precipitate was immediately washed off the cavity, dried with oil-free air and restored with Fuji VII (GC Corp) auto cure glass ionomer cement. The samples were stored in separate 5ml vials containing double distilled water at 37°C for two weeks.

Experimental method

Following water storage the samples were then placed in a fixing solution containing 1.25% glutaraldehyde, 4% sucrose, 4% paraformaldehyde in PBS at pH 7.2 (Adelaide Microscopy, Adelaide, Australia) for 12 hours, and then placed into a washing

buffer solution, containing PBS, 4% sucrose (Adelaide Microscopy) for 1.5 hours with changes every 30 minutes. Samples were then dehydrated with ascending grades of ethanols (25% ethanol for 20 minutes, 50% ethanol for 20 minutes, 75% ethanol for 20 minutes, 95% ethanol for 30 minutes, 100% ethanol for one hour).

The dehydrated specimens were placed face down on the base of cylindrical mounting blocks into which epoxy resin 100:25 (Epoxy resin LC 191: Epoxy hardener HY 956) was poured under vacuum and left to set for 24 hours at room temperature. After setting, the specimens were polished using an Abramin polishing machine (Struers, Denmark). The upper and lower surfaces of the mounting blocks were made parallel to each other using a leveling device and the samples mounted on a polisher. The surfaces in which the specimens were embedded were polished using a p80 grit silicone carbide disc (Struers, Denmark), at 150 revolutions per minute (rpm), lubricated with water for 30 seconds under a load of 100N. The specimen containing surfaces were then polished using p500 grit silicon carbide discs at 150rpm, lubricated with water under a load of 100N to grind away the surface resin.

Once the specimen containing surfaces were flat, the surfaces were further polished using diamond polishing discs (Struers) with diamond paste (Kemet, UK). First the surface was polished with 15mm diamond paste on a 15mm diamond polishing disc at 150rpm for five minutes at 200N, lubricated with DP-lubricant Green (Struers). After that the surfaces were polished with 3mm (Kemet) and 1mm (Kemet) diamond polishing discs and diamond pastes, respectively. Both cycles were at 150rpm for three minutes at 200N and lubricated with DP-Lubricant Green. The surfaces were cleaned with water, air dried and viewed under a stereo microscope (Zeiss, West Germany) at 25x magnification to determine that the embedded specimens had been polished adequately.

The samples were carbon coated for ion analysis using an EPMA instrument (CAMECA, SX51, France). Line scans were carried out on the specimens to measure the relative percentage weights of the following elements: calcium (Ca); strontium (Sr); silver (Ag); iodine (I); phosphorus (P) and fluorine (F). Scans were conducted from the floor of the cavity over the surface of the specimens every 5µm to a depth of 400µm. Three scans were made at each location and the three readings averaged. Measurements were expressed as a relative percentage weight of the identified element as part of the total weight of the sample where the measurement was taken.

Data analysis

Differences amongst the samples were determined by comparing the areas under the curves for each element. Although scans were carried out to a depth of 400µm, as the study was looking at an interface reaction, data were not analysed beyond a depth of 250µm. Since the

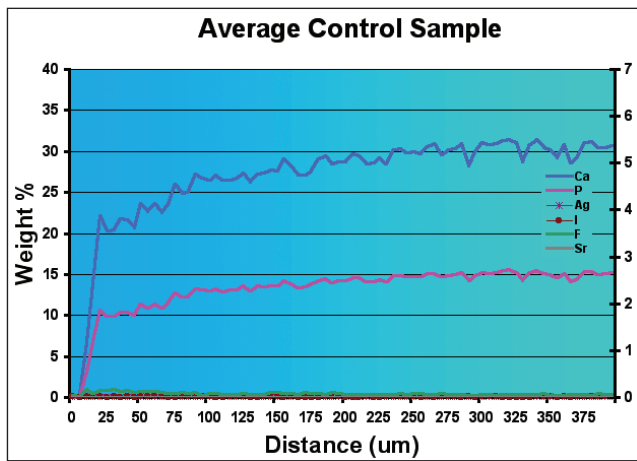


Fig 1. Average relative percentage weight of elements in demineralized dentine control sample. Percentage weights of Ag, I, F and Sr are on right-hand scale.

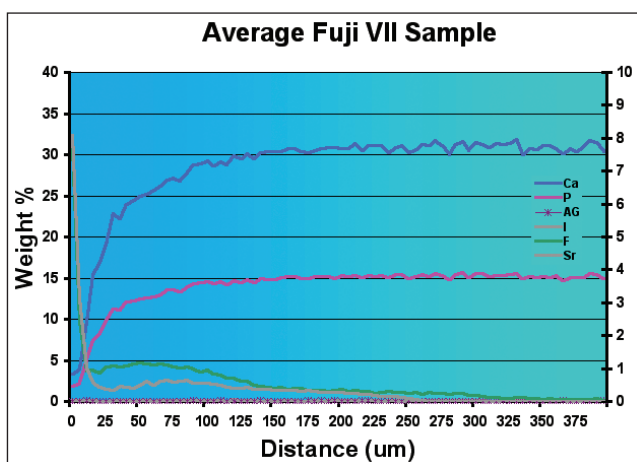


Fig 2. Average relative percentage weights of elements in demineralized sample restored with Fuji VII. Percentage weights of Ag, I, F and Sr are on right-hand scale.

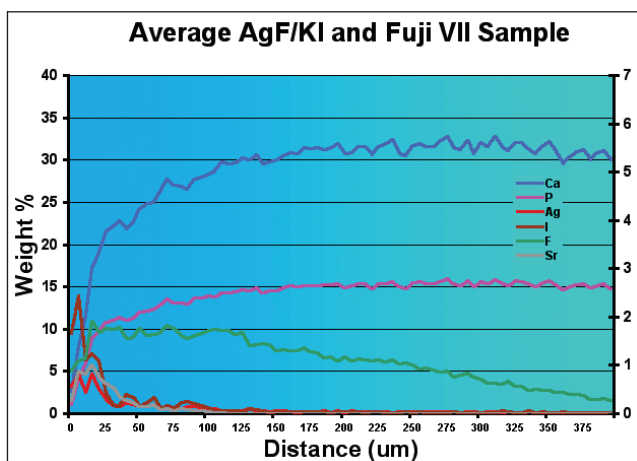


Fig 3. Average relative percentage weights of elements in demineralized sample treated with AgF/KI and restored with Fuji VII. Percentage weights of Ag, I, F and Sr are on right-hand scale.

data were not normally distributed, the Kruskal-Wallis test was used to determine if there was a difference amongst the groups. *Post hoc* testing was used to make pairwise comparisons with no adjustment made for multiple comparisons.

Table 1. Significant levels of difference ($P < 0.05$) amongst the elements in the three groups

Fluoride		
Control/Fuji VII and AgF/KI	<0.000	Significant
Control/Fuji VII	0.001	Significant
Fuji VII/Fuji VII and AgF/KI	0.0063	Significant
Strontium		
Control/Fuji VII and AgF/KI	0.0101	Significant
Control/Fuji VII	0.0012	Significant
Fuji VII/Fuji VII and AgF/KI	0.2703	Not significant
Silver		
Control/Fuji VII AgF/KI	0.0006	Significant
Control/Fuji VII	0.5744	Not significant
Fuji VII/Fuji VII AgF/KI	0.0016	Significant
Iodine		
Control/Fuji VII AgF/KI	<0.0001	Significant
Control/Fuji VII	0.0377	Not significant
Fuji VII/Fuji VII AgF/KI	0.0018	Significant

RESULTS

The graphs in Figs 1–3 show the mean values of each element in dentine in each of the experimental groups up to the analysis depth of 375µm. The left-hand scale represents the percentage weights of Ca and P and the right-hand scale represents the percentage weights of Sr, Ag, F and I. Table 1 shows the analyses of the differences between the groups in the elements analysed. Except for the first 15µm, the calcium and phosphorus levels were comparable in all three treatment groups.

The levels of strontium showed a high peak in the surface zone in the group in which glass ionomer cement was used alone. A similar peak was not seen in the group where AgF/KI was used as the pretreatment. However, the area under the curve analysis (Table 1) showed that there was no significant difference between the two groups in strontium uptake.

Fluorine uptake was less pronounced in the dentine subjacent to glass ionomer cement than in the dentine that had been treated with AgF/KI prior glass ionomer cement placement (Figs 2 and 3). The difference (Table 1) between the two was statistically significant ($P < 0.05$).

Detectable levels of silver and iodine were only seen in the group treated with AgF/KI prior to the application of the glass ionomer cement. Demineralization of each group occurred to a depth of about 150µm although fluoride penetration into the dentine exceeded well beyond the 250µm depth which was the finishing point for statistical analysis.

DISCUSSION

The hypermineralization of softened dentine associated with a glass ionomer cement restoration¹ was attributed by ten Cate and Duinen¹ to the release of fluoride and possibly silica from the restoration into adjacent tooth structure. In the current study glass ionomer cement was placed in cavities where the dentine had been exposed to a demineralizing solution. Two weeks after placement an appreciable uptake of ions had occurred in the outer dentine of the specimens

restored with glass ionomer cement or glass ionomer cement with an AgF and KI dentine pretreatment. It is not known if hypermineralization occurred in these specimens.

The two glasses commonly used in glass ionomer cements are calcium aluminium fluorosilicate and strontium aluminium fluorosilicate.¹¹ Forss¹² showed that depending upon the type of glass used, as well as fluoride and silica, these materials can release sodium, aluminium, strontium and calcium. Strontium aluminium fluorosilicate glass is used for improved radiopacity and is the type of glass used in Fuji VII.¹³ Strontium glasses release little or no calcium^{11,12} although strontium has a similar ionic radius to calcium and has the potential to be substituted for calcium in remineralization type reactions. Recent attempts to increase the calcium release from glass ionomer cements have included the addition of a casein phosphopeptide amorphous calcium phosphate.¹⁴

The findings of this study show that, overall, pretreatment with AgF and KI did not significantly inhibit the uptake of strontium from Fuji VII into the subjacent dentine. However, there are indications that this pretreatment may have caused some inhibition of strontium and fluoride uptake in the first 15mm of the dentine. This is not surprising in that silver can react with phosphate and thus compete with strontium for the available phosphate sites. Furthermore, the possibility exists that the reaction between silver and proteins in this outer area may have produced some form of superficial barrier.

The findings that pretreatment of AgF and KI gave higher fluoride levels in the deeper layers on dentine than those seen with Fuji VII alone is no doubt attributable to the additional fluoride uptake from the topical treatment. The EPMA instrument used in this study was unable to determine whether the elements under study were located in dentinal tubules, inter-tubular dentine or peritubular dentine.

The measurements of fluoride and strontium levels were the result of an additive process and "an area under the curve" analysis was appropriate in these circumstances. Compared to other glass ionomer cements, Fuji VII is a high fluoride releasing glass ionomer cement.¹³ It is specifically designed as an interim restorative to facilitate remineralization of caries-affected dentine and an appropriate material to use in conjunction with AgF application.

The dentine surfaces were etched for five seconds with 37% phosphoric acid prior to applying the AgF and KI. Although the dentine conditioning protocol for Fuji VII is to apply a 10% solution of polyacrylic acid conditioner (GC Corp.) to dentine surfaces prior to bonding,¹³ phosphoric acid removes the surface biofilm as well as the smear layer and smear plugs¹⁵ and Tay *et al.* have shown there is no significant difference in bond strengths between dentine that has been conditioned with 10% polyacrylic acid or etched with 37% phosphoric acid for up to 15 seconds.¹⁶

The findings of this study indicate a number of areas for future investigation including: (i) whether substitution of polyacrylic acid for phosphoric acid in cleaning the dentine surfaces would have influenced the results; and (ii) whether the AgF and KI pretreatment had an effect on the hardness of the dentine subjacent to the glass ionomer cement restorations.

CONCLUSIONS

The study has shown that placing a high fluoride release auto cure glass ionomer cement onto demineralized dentine enables fluoride and strontium within the glass ionomer cement to penetrate into the demineralized dentine. The application of AgF and KI to the surface of the demineralized dentine prior to placing the glass ionomer cement restoration does not interfere with strontium uptake and significantly increases the concentration and penetration depth of fluoride into the dentine compared to the samples restored with auto cure glass ionomer cement alone.

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DISCLOSURE

The corresponding author was associated with the development of Fuji VII and has a financial interest in this product. Authors Craig and Knight are named on a process patent for the application of silver fluoride followed by potassium iodide.

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