The pharmacological management of dentine to protect against plaque microorganism degradation

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Abstract

Background

There is a transition towards minimally invasive restorative techniques in restorative dentistry based upon reducing bacterial viability and encouraging remineralization of caries infected tissue. To improve the predictability of the antibacterial and remineralization potential of carious dentine by either the application of medicaments or placement of restorative materials that encourage remineralization would be a significant benefit in disease management.

Materials and Methods

An experimental model was developed using a chemostat for *in vitro* analysis of the effects of silver fluoride followed by potassium iodide (AgF/KI) and ozone treatment on non demineralized and demineralized dentine.

Electron Probe Micro Analysis (EPMA) and Scanning Electron Microscopy (SEM) on the treated dentine were conducted to investigate ion transfer, and biofilm formation. Bacteria growth was measured by optical density.

IV

An *in vitro* caries model using a chemostat was developed to determine the ability of glass ionomer cement and composite resin to inhibit dentinal degradation in adjacent dentine and to measure ion exchange at the restorative interface.

Tests were made to determine the bond strength between dentine and glass ionomer cement after application of silver fluoride to the surface of the dentine.

Results

S. mutans migrated through all dentine samples. Samples treated with AgF/KI had significantly lower optical densities than the corresponding controls. Optical density readings were significantly lower in demineralized dentine treated with AgF/KI than non demineralized dentine.
There were lower but not significant differences in the optical density readings between ozonated and non ozonated dentine.

An *S. mutans* biofilm covered all control discs. No biofilm was detected on discs treated with AgF/KI and these discs were significantly more resistant to further demineralization than the control discs. Detectable amounts of silver and fluoride were found up to 450 μm in the AgF and AgF/KI sections.

Ozone infusion prevented *S. Mutans* and *L. acidophilus* biofilm formation on all the treated dentine samples, biofilm was present on all control specimens.

There was calcium and phosphorus present in all auto cure glass ionomer cements to a depth beyond 50 microns. Aluminium and strontium ions were also present in dentine except strontium subjacent to Ketac Molar restorations.

Fluoride uptake was significantly higher under glass ionomer cement restorations where the dentine was pretreated with AgF/KI compared to non treated specimens. Silver and iodine deposits were present in demineralized dentine treated with AgF/KI.

Calcium and phosphorus levels up to 130 microns from the restorative interface were similar to non demineralized dentine adjacent to auto cure glass ionomer cements and half that adjacent to composite resin. There was significant surface degradation in auto cure glass ionomer cements compared to composite resin.

Washing away the AgF/KI precipitate produced higher bond strengths to dentine than samples where the precipitate remained.

VI

Conclusions

Under the conditions of these *in vitro* studies, the application of AgF/KI and ozone pharmacologically reduces the initiation and rate of dentine caries.

Glass ionomer cements were shown to protect dentine from experimental carious degradation and assist with remineralization. AgF/KI application enhances remineralization beneath glass ionomers and does not interfere with bond strengths.

Statements of Originality and Authorship

Statement of Originality

This work contains no material that has been accepted for the award of any other degree or diploma in any university or tertiary institution, and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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VIII

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Disclosure

The candidate was associated with the development of Fuji VII restorative material (GC Corporation, Tokyo, Japan) and has a financial interest in this product. Authors Knight and Craig are jointly named on a process patent associated with the use of silver fluoride and potassium iodide.

Geoffrey M Knight date

Statements of Authorship

1. 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

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Gully NJ

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2. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. Inability to form a biofilm of *Streptococcus mutans* on silver fluoride and potassium iodide treated demineralized dentin Quintessence Int Accepted for publication July 2007

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3. The inability of *Streptococcus mutans* and *Lactobacillus acidophilus* to form a biofilm *in vitro* on dentine pretreated with ozone. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS Aust Dent J. Accepted for publication May 2008

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4. Knight GM, McIntyre JM, Craig GG, Mulyani , Zilm PS , Gully NJ

Differences between normal and demineralized dentine pretreated with silver fluoride and potassium iodide after an *in vitro* challenge by *Streptococcus mutans* Aust Dent J 2007;52:16-21

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5. Knight GM, McIntyre JM, Craig GG, Mulyani. Electron Probe Microanalysis of ion exchange of selected elements between dentine and adhesive restorative materials Aust Dent J 2007; 52:128-132

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6. 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

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7. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. An *in vitro* investigation of marginal dentine caries abutting composite resin and glass ionomer cement restorations. Aust Dent J 2007; 52: 187-192

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Zilm PS

Assisted with specimen preparation, data collection and interpretation

Signed PS Zilm date

Gully NJ

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Signed NJ Gully date

8. Knight GM, McIntyre JM, Mulyani The effect of silver fluoride and potassium iodide on the bond strength of auto cure glass ionomer cement to dentine Aust Dent J 2006;42-45

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Introduction

Dental caries is prevalent in both developing and industrialized economies. In 1982 for the first time child dental caries in developing countries exceed those in developed countries (Sheim 1984). WHO data shows dental caries remains a major public health problem in industrialized economies and in developing economies particularly where dietary changes have had an impact on increasing prevalence (Petersen 2004). In industrialized economies an increasing incidence of root and recurrent caries has already been identified in the aging dentate population (Carpendale 1999). It is predicted that the edentulous population in Australia (and other industrialized societies) will be about one per cent by the middle of this century with an associated increase in demand for dental services (Data Watch Aus Dent J 2007).

There is currently a transition in the concepts of the restoration of carious teeth (Silva *et al.* 2007). While it is acknowledged that remaining caries affected dentine containing undenatured collagen fibres (ten Cate 2001) will remineralize if left under a restoration (McComb 2001; Kidd 2004), there are still doubts about leaving beneath a restoration caries infected dentine that has been degraded by bacterial invasion into the dentinal tubules (Yoshiyama *et al.* 2002).

Glass ionomer cements are known to assist with the remineralization of demineralized tooth structure (Ngo 2005), however its ability to protect teeth from recurrent caries is equivocal (Randall *et al.* 1999; Mjör *et al.* 2000; Van Nieuwenhuysen *et al.* 2003; Burke *et al.* 1999; Tyas 2005). There is however counter subjective evidence to this (Mount 1986; Forsten *et al.* 1994).

The focus of this study was to investigate the ability of various pharmacological substances to protect dentine from bacterial degradation.

The benefits of this investigation could lead to insights into the protection of teeth against the onset of caries and provide a protocol for the restoration and ongoing protection of caries affected teeth.

The aims of this study were as follows:

- To develop an *in vitro* model to investigate the ability of various pharmacological agents that may inhibit oral bacterial plaque, biofilm formation and inhibit the bacterial degradation of dentine;

- To develop an *in vitro* technique to investigate the ability of various restorative materials to inhibit the bacterial degradation of dentine;

- To determine if the pharmacological management of demineralized dentine could improve remineralization below glass ionomer cement restorations;
- To establish if the pharmacological pretreatment of dentine affects the bond strength of glass ionomer cement.

Literature Review

1. Composition of healthy dentine in the dentine pulp complex
2. The effects of caries upon dentine and the tissue response
3. The chemical nature of the remineralization demineralization reaction
4. Current concepts of caries prevention
5. Current management concepts of carious dentine
6. The use of silver fluoride in the prevention and treatment of caries
7. The use of ozone in the prevention and treatment of caries
8. Glass ionomer cement
9. Evidence of ion exchange between tooth and glass ionomer cements
10. Marginal dentine caries and glass ionomer cement
11. Chemostat
12. Scanning Electron Microscopy SEM
13. Electron Probe Microanalysis EPMA

1. Composition of healthy dentine in the dentine pulp complex

Dentine and pulp tissue are embryologically, histologically and functionally the same tissue and should be treated as a single entity. Dentine is the hard portion of the complex and is by weight 70% inorganic, 20% organic and 10% water and by volume, 45% inorganic, 33% organic and 22% water. The inorganic phase of dentine consists predominantly of a carbonated form of hydroxyapatite. The inorganic component is type I collagen with fractional inclusions of glycoproteins, proteoglycans and phosphoproteins (Ten Cate 1998).

Tooth mineral is commonly called hydroxyapatite with the following formula. $Ca_{10}(PO4)_6(OH)_2$ As the apatite crystals form embryologically in a biological soup they are contaminated with carbonate and other ions. This makes the crystals more prone to demineralization than hydroxyapatite. The formula for carbonated apatite may be expressed as follows $Ca_{10-x}(Na)_x(PO4)_{6-y}(CO_3)_y(OH_{2-u})(F)_u$ where “x”,“y”and “u” levels vary slightly throughout the tissue (Featherstone 1999). Typical levels of impurities are carbonate (4%), sodium (0.6%), magnesium (0.2%), and chloride (0.2), and small amounts of fluoride (0.1%) (Driessens *et al.* 1981).

Dentine is characterized by the presence of multiple closely packed dentinal tubules that extend from the cell bodies of the odontoblasts along the entire

thickness of the dentine. Ion concentrations of calcium, phosphorus and sodium in dentinal tubules were found to be at higher than serum levels and these concentrations showed an increase when caries were present (Larmas 1986).

Dentinal tubular fluid also appears to play a role in reducing the rate of demineralization of dentine (Ozok *et al.* 2004).

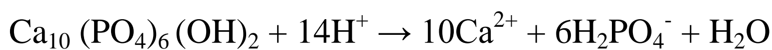
2. The effects of caries upon dentine and the tissue response

Caries effects on dentine occur prior to cavitation of the enamel. Increased permeability of the enamel to acids and various other chemicals stimulate the odontoblasts with possible retraction of the processes and collagen deposition within the periodontoblastic space. This may lead to the deposition of reactionary dentine by the vital odontoblasts. Once the enamel cavitates, bacteria encroach upon the dentine surface and destruction of the dentine begins. Acid diffuses ahead of the invading bacteria and demineralizes the dentine causing mineral precipitation further into the tubules forming sclerotic dentine. Bacteria within the tubules eventually move into the dentine and destroy the collagen matrix (Ten Cate 1998).

One of the effects on dentine of an acidic pH results in the release of matrix metalloproteinases that have been shown to cause destruction of the collagen matrix (Chaussian *et al.* 2006). While it is difficult to comprehend why a tooth would facilitate a bacteria invasion these substances may be initiating an antibacterial reaction at the lesion interface.

3. The chemical nature of the remineralization demineralization reaction

Demineralization is a reaction of protons derived from the dissociation of plaque acids (primarily lactic acid) produced by bacterial carbohydrate metabolism. Cariogenic attack occurs under mildly acidic conditions (pH 5-6). The reaction leads to the release of mineral ions into the solution.



$$Ca_{10}(PO_4)_6(OH)_2 + 14H^+ \rightarrow 10Ca^{2+} + 6H_2PO_4^- + H_2O$$

Mineral solution

The extent to which tooth mineral can dissolve is determined by the thermodynamic ion activity product (IAP). When the IAP equals a constant called the solubility product constant (Ksp) the solution is in equilibrium with the solid.

$$\text{IAP (in a saturated solution)} = \text{Ksp}$$

When the hydroxyl ions are replaced with fluoride ions converting the hydroxyapatite OHAp to fluorapatite FAp ($Ca_{10} (PO_4)_6 F_2$ the Ksp is substantially reduced requiring a lowered pH for dissolution. Tooth mineral low in F content is more susceptible to cariogenic attack than tooth mineral with a higher F content (Chow *et al.* 2001).

Small amounts of fluoride in solution play an important role in remineralization by acting as a catalyst for OHAp precipitation. The low solubility of fluorapatites and the ability of solution F to accelerate remineralization mean that low concentrations of fluorides in oral fluids are essential for significant remineralization to occur (Featherstone *et al.* 1990; ten Cate 1990).

4. Current concepts of caries prevention

Mechanisms that prevent onset of caries are based upon the suppression of plaque organisms, improving the resistance of tooth structure to caries and the physical separation between biofilm and tooth surfaces (Kidd 2004).

Reducing the frequency of ingesting refined carbohydrates (Caufield *et al.* 2005) and mechanical plaque removal using a fluoridated tooth paste (Davies *et al.* 2003) are currently the most widely used means of preventing caries.

Chlorhexidine has been identified as a possible means of suppressing plaque organisms however the effectiveness of this procedure in preventing caries remains inconclusive (Twetman 2004). An array of substances has been professionally applied to teeth to improve caries resistance. To date the most successful of these have been fluorides (in various forms and concentrations). (Twetman 2007) The application of ozone to tooth surfaces has also been cited as a means of suppressing biofilm formation (Baysan 2004).

Since the early seventies, fissure sealing with resin composites has formed the basis of physically separating tooth surface from biofilm (Raadal 1978). Glass ionomer cements have also been advocated although the literature seems to favour resin fissure sealing (Simonsen 1996). Recent *in vivo* studies now suggest that high viscosity glass ionomer cements (Atraumatic Restorative Technique /ART) are four times more effective in preventing caries than resin composite over a five year period (Frencken 2006).

5. Current management concepts of carious dentine

Traditional Approach

The traditional approach to caries management involves the removal of all softened and stained dentine either by rotary instruments or hand excavators to establish a firm base for placement of a restoration. There is an increased

probability of mechanically exposing the pulp using this procedure that has largely been superseded by more conservative techniques leaving some caries in deep asymptomatic lesions (Ricketts *et al.* 2006).

Stepwise

The stepwise technique is based upon removing part of the carious dentine, placing a zinc oxide eugenol dressing and re-entering the preparation for further caries removal prior to placing a permanent restoration. This technique minimized the risk of a mechanical pulp exposure by stimulating the formation of secondary dentine. A practice based study showed a high clinical success rate in reducing exposed pulps than traditional techniques (Bjørndal *et al.* 1998).

ART

The "Atraumatic Restorative Technique" was developed as a technique to treat caries in developing economies (Frencken *et al.* 1996). Carious tooth structure is removed with hand instruments and the cavity is restored using glass ionomer cement. The technique is highly successful in treating single surface lesions but has limitations for multi surfaced cavities.

Indirect Pulp Capping

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 carious dentine is left within the preparation. This was shown 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 (Mertz-Fairhurst *et al.* 1998). This work has recently been confirmed over a 40 month study that showed complete caries removal was not essential for caries control (Maltz *et al.* 2007).

6. The use of silver fluoride in the prevention and treatment of caries

Silver salts can provide a pronounced antimicrobial action and have a long history of use in medicine and dentistry (Stebbins 1942; Crannell 1967; Yamaga *et al.* 1972). 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 (Crannell 1967; Yamaga *et al.* 1972; Craig *et al.* 1981; McDonald *et al.* 1994; Chu *et al.* 2002).

Another clinical application has been to use silver salts 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 (Stebbins 1942; Englander *et al.* 1958; Langeland *et al.* 1968; Gotjamanos 1996). The application of AgF under glass ionomer cement restorations in primary teeth has been shown to produce a favorable pulpal response and to be effective in promoting reparative dentine formation (Gotjamanos 1996). 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 (Chu 2002).

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 (Englander *et al.* 1958; Langeland *et al.* 1968; Craig *et al.* 1981; McDonald *et al.* 1994). Both treatments result in the formation of black precipitates.

A solution 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 potassium

iodide (KI), as it produces a creamy white reaction product, silver iodide, which has been used previously in dentistry (Obwegeser *et al.* 1954).

7. The use of ozone in the prevention and treatment of caries

The use of ozone therapy in the treatment of dental caries is equivocal and literature reviews suggest there is insufficient evidence to regard ozone treatment as a viable alternative to the current management and treatment of dental caries (Rickard *et al.* 2005; McComb *et al.* 2005). Opposing this view are studies showing both *in vitro* (Baysan *et al.* 2000) and *in vivo* (Baysan *et al.* 2004) reduction of viable bacteria in primary root carious lesions and there is *in vitro* evidence showing ozonated water decreased levels of *S. mutans* in an experimental dental plaque (Nagayoshi *et al.* 2004) .

The rationale for using ozone therapy is largely based upon the toxic effect of the gas on bacteria. A recent in *vitro* study however observed that the application of ozone gas onto infected dentine (Baysan *et al.* 2007) was unable to show a reduction in the number of viable bacteria just below the surface of the infected dentine. The authors concluded from this observation that the mechanism of ozonated dentine inhibiting bacteria may occur in a manner other than the direct toxicity of the gas.

8. Glass ionomer cement

Glass ionomer cements were developed by Wilson and Kent (Wilson *et al.* 1972). It is the product of an acid base reaction between a fluoroaluminosilicate glass powder and an aqueous polycarboxylic acid.

Glass ionomer cements are biocompatible, have a good chemical adhesion to tooth structure, are easily clinically manipulated and studies have shown that since 1989 the material releases fluoride ions that can be absorbed into tooth structures (Komatsu *et al.* 1989).

Importantly, 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 (ten Cate *et al.* 1995). Studies using an Electron Probe Microanalysis technique (EPMA) have confirmed the transfer of strontium and fluoride ions into the dentine from glass ionomer cement (Ngo *et al.* 1997).

9. Evidence of ion exchange between tooth and glass ionomer cements

Since Wilson postulated the bonding mechanism of auto cure glass ionomer cement to tooth structure (Wilson *et al.* 1983) there have been a number of attempts using different mechanisms to verify this bond. Watson used fluorescent

dyes in glass ionomer cements to demonstrate the possibility of ion exchange (Watson *et al.* 1991; Watson *et al.* 1998). Lin *et al.* (1982) looked at the bond using secondary ion mass spectrometry and Ngo used a cryo scanning electron microscopy to demonstrate the existence of an interaction zone at the dentine restorative interface that resisted acid etching, suggesting the existence of an ion enriched layer (Ngo *et al.* 1997). Yoshida confirmed ionic bonding between the carboxyl ions from the cement and calcium and phosphorus ions within the tooth using X-ray photon spectrometry (Yoshida *et al.* 2000). Using an Electron Probe Microanalysis (EPMA) (Ngo *et al.* 1997) technique Ngo was able to demonstrate the transfer of strontium and fluoride ions from auto cure glass ionomer cements into demineralized dentine (Ngo 2005).

10. Marginal dentine caries and glass ionomer cement

A number of papers observing the clinical performance of auto cure glass ionomer cement (GIC) have cited the primary reason for replacing these restorations have been marginal caries (Mjör *et al.* 2000; Van Nieuwenhuysen *et al.* 2003; Burke *et al.* 1999; Burke *et al.* 1999). This is in contrast of Mount, who found no marginal caries associated with GIC restorations up to five years (Mount 1986).

In a recent paper, Tyas analyzed the clinical criteria of 28 general practitioners used to place approximately 100 consecutive restorations (Tyas 2005). The failure of GIC restorations due to secondary caries ranged from 17 to 36 per cent. The design of this study did not involve any standardization of clinical assessment criteria amongst the practitioners. Whilst the anecdotal experience of many Australian dental practitioners suggests that GIC's resist caries formation in adjacent tooth structure (Forsten *et al.* 1994) a systematic review of the literature does not support this (Randall *et al.* 1999). Nicholson and co-workers who have shown GIC's capable of buffering plaque acids (Nicholson *et al.* 2000; Czarnecka *et al.* 2002) and although the cement breaks down as a consequence of this process, this may have the effect of maintaining the pH above the levels required to demineralize the surrounding dentine.

11. Chemostat

A Chemostat (from chemical environment is static) is a continuous culture device used in microbiology for growing and harvesting microbes at a known growth rate (Wikipedia). Developed by Novick and Szilard (Novick *et al.* 1950) the defining

feature for chemostats is the ability to keep all fermentation parameters (growth chamber volume, dissolved oxygen, nutrient concentrations, pH, cell density, etc.) constant. Chemostats consist of two primary parts: a sterile nutrient reservoir and a growth chamber. Sterile medium is fed into the growth chamber from the nutrient reservoir at a controlled rate. By controlling the levels of the limiting nutrient, bacterial culture in the growth chamber can be kept at a reduced growth rate over an indefinite period of time. The limiting nutrient is an essential growth factor necessary for bacterial growth but present in the media at a concentration such that balanced growth consumes it prior to exhausting any other of the essential nutrients in the media. As medium flows out from the growth chamber, bacteria and byproducts can be harvested.

The Chemostat can be usefully applied to dental research either using bacterial monocultures or multiple cultures of oral bacteria (Marsh 1995).

12. Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high energy beam of electrons in a scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample. Electronic devices

are used to detect and amplify the signals and display them as an image on a cathode ray tube in which the raster scanning is synchronized with that of the microscope. The image may be photographed from a high resolution cathode ray tube, but in modern machines is digitally captured and displayed on a computer monitor. Back-scattered electrons (BSE) that come from the sample may also be used to form an image. BSE images are often used in analytical SEM along with the spectra made from the characteristic X-rays as clues to the elemental composition of the sample (Wikipedia).

The SEM has wide applications as a research tool looking at various aspects of carious dentine (Kielbassa *et al.* 1986).

13. Electron Probe Microanalysis (EPMA)

EPMA can be used to measure the concentrations and distributions of elements in a given sample. Emitted electrons bombard a sample to produce an emission of X-rays whose energies and relative abundance depends upon the composition of the sample. This enables both qualitative identification and quantitative composition of the sample being analyzed. It is a useful tool for measuring remineralization and demineralization in tooth samples by tracking the percentage concentrations of calcium and phosphorus. The method has further application in measuring the

percentage concentration and distance of penetration of various ions such as fluoride, strontium and aluminium. The preparations of samples for analysis are relatively straight forward and there is minimal surface destruction during analysis of the specimen (Ngo *et al.* 1997; Ngo 2005).

Literature Review Summary

This review has examined the parameters of this proposed study.

An explanation is given of healthy dentine and what changes can occur once dentine becomes affected by caries. Also addressed are some of the basic chemical reactions that occur during the demineralization and remineralization of tooth apatite.

Current concepts of caries prevention and caries management have been reviewed along with the short comings that exist in this area. A broad outline of techniques used to restore teeth is presented with the benefits and shortcomings of each methodology. Particular reference has been given to silver fluoride and ozone as these materials are specifically investigated in this study.

The nature of glass ionomer cement is discussed with particular reference to biocompatibility and ion transfer between glass ionomer cement and tooth structure. The current understanding of ion exchange has been reviewed in some depth as well as the controversy about the relationship of glass ionomer cement and recurrent caries.

Finally, a brief description of specific scientific instruments used in this study and explanations of their applications to caries research.

Parameters of Research

To achieve the overall objectives of this research, eight individual studies were carried out, and their description and results have been either published or accepted for publication in approved refereed journals. The objectives and titles of these publications are listed below and copies of each paper have been included to form the bulk of the requirements being submitted for this PhD Thesis.

1. To determine the effects on *S. mutans* viability by pretreating demineralized dentine with silver fluoride and potassium iodide

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

2. To determine if the pretreatment of demineralized dentine with silver fluoride and potassium iodide would inhibit *S. mutans* biofilm formation

Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. Inability to form a biofilm of *Streptococcus mutans* on silver fluoride and potassium iodide treated demineralized dentin. Quintessence Int Accepted for publication July 2007

3 To determine if the pretreatment of dentine surfaces with ozone inhibited *S. mutans* and *L. acidophilus* biofilm formation

The inability of *Streptococcus mutans* and *Lactobacillus acidophilus* to form a biofilm *in vitro* on dentine pretreated with ozone. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS Aust Dent J. Accepted for publication May 2008

4. To determine if the pretreatment of silver fluoride and potassium iodide was more effective in biofilm inhibition on normal or demineralized dentine

Knight GM, McIntyre JM, Craig GG, Mulyani , Zilm PS , Gully NJ Differences between normal and demineralized dentine pretreated with silver fluoride and potassium iodide after an *in vitro* challenge by *Streptococcus mutans.* Aust Dent J 2007;52:16-21

5. To determine the ion exchange of various ions between various adhesive restorative materials.

Knight GM, McIntyre JM, Craig GG, Mulyani. Electron Probe Microanalysis of ion exchange of selected elements between dentine and adhesive restorative materials. Aust Dent J 2007; 52:128-132

6. To determine if the pretreatment of demineralized dentine with silver fluoride and potassium iodide enhanced remineralization levels

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

7. To determine the ongoing protection from caries of various restorative materials

Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. An *in vitro* investigation of marginal dentine caries abutting composite resin and glass ionomer cement restorations. Aust Dent J 2007; 52: 187-192

8. To determine if the pretreatment of silver fluoride and potassium iodide effected the bond strength of glass ionomer cement to dentine

Knight GM, McIntyre JM, Mulyani. The effect of silver fluoride and potassium iodide on the bond strength of auto cure glass ionomer cement to dentine. Aust Dent J 2006; 42-45

Restorative Dentistry

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

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Abstract

Objectives: The presence of a biofilm is necessary for both the initiation and progression of dental caries. The purpose of this study was to measure the *in vitro* effect of fourteen days in a Chemostat on the formation of a *Streptococcus mutans* biofilm on the surface of partially demineralized dentine discs which had been pretreated with diamine silver fluoride followed by potassium iodide. **Method and Materials:** Forty partially demineralized dentin discs were divided into 4 groups as follows**:** 10 discs as a control, 10 discs were treated with AgF followed by KI, 10 discs were treated with KI and 10 discs were treated with AgF. The outer surfaces of the discs were examined using a scanning electron microscope (SEM). Cross sections of the discs were subjected to Electron Probe Micro Analysis (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 discs. No discernible bacterial biofilm was detected on discs treated with either AgF or AgF/KI and these discs were significantly more resistant to further demineralization than the control and KI treated discs. Detectable amounts of silver and fluoride were found up to 450 µm in the AgF and AgF/KI sections. **Conclusions:** Demineralized dentin discs 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 discs over the experimental period. The presence of silver and fluoride in the outer

layers of the AgF and AgF/KI treated were the likely causes of the prevention of biofilm formation.

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

Clinical Relevance: **Measures that inhibit the formation of a *Streptococcus mutans* biofilm on demineralized dentin may be of particular benefit in helping arrest caries.**

INTRODUCTION

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 (AgF), has been used to arrest dentin caries in the primary dentition, [3,4] and reduce caries formation in primary teeth and first permanent molars. [5] However, its potential in arresting early dentin caries in sites such as root surfaces in the permanent dentition is not known.

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 discs 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 SEM analysis both treatments had significant inhibitory effects on organism penetration and/or growth during a fourteen 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 aureas.*[11]

In the dental area 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 forty recently extracted human third molar teeth 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, Australia) was bonded to the etched outer enamel surface after which the discs were reduced to 1 mm in thickness using a graded series of wet and dry papers to 4000 grit Silicone Carbide paper (Struers, Denmark).

The enamel surfaces of all discs 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 37^0C for 4 days to create demineralized lesions with a depth of 150µm.

To facilitate handling and treatment of the sections, the bases of forty 5ml vials were removed to provide an open end. The area inside the open end was roughened using air abrasion, (Rondoflex, Kavo, Biberach, Germany) (50µm Al_2O_3 particles) and discs were attached to the vials using Resin Bond (3M ESPE, Minnesota, USA) and Glacier composite resin (SDI).

Once in place forty samples were given one of the following treatments, assigned at random, to the outer surfaces:

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 were treated with a saturated solution of KI and washed off with copious amounts of distilled water
3. 10 samples were coated with a solution of 1.8M AgF and then washed off with copious amounts of distilled water.
4. 10 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 up consisting of 3% Tryptone Soya broth (Oxoid, Basingstoke, UK), 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 prior to being subjected to gamma radiation (Steritech Pty Ltd, Dandenong, Vic., Australia) at a dose of 15R.

The sterilized vials were then placed into a sterile glass flask that was connected to the outflow from a Chemostat system (New Brunswick Scientific, Edison, USA). 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 $0.1h^{-1}$ (td=7hrs) and the pH was maintained at pH 7.4 by the automatic addition of KOH (2N). The pH of the flask containing the vials was uncontrolled and remained at ca. pH 4.5 throughout the 2 week duration of the experiment.

At completion of the experiment the discs were removed from the samples and cut in two. One half was prepared for scanning electron microscopy (Philips XL30 Field Emission Scanning Electron Microscope, Netherlands) and the other half for EPMA (CAMECA, SX51, France) 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*. (1997) [14,15]

Since the data were 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 make pairwise comparisons with no adjustment made for multiple comparisons. As calcium and phosphorous were being removed from the system, this data was compared by measuring the areas above the curve, Delta Z**.** As fluorine and silver 30were being added to the system, this data was compared by measuring the areas below the curve. Although measurements were carried out to a depth of 500µm, 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 300µm, the depth to which secondary demineralization had occurred during the experiment.

RESULTS

The scanning electron micrographs showed an appreciable *S. mutans* biofilm covering the entire exposed surface of all control discs (Fig 1). A similar situation was seen with the discs treated with KI (Fig 2).

In contrast no discernable bacterial biofilm was detected on discs 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 discs treated with AgF /KI (Fig 4).

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 µm. The calcium and phosphorus loss from control and KI treated discs was significantly higher overall than from AgF and AgF/KI treated sections ($p<0.05$).

Similarly the fluoride levels in all discs tended to be lower in the surface area than in the deeper zones. (Fig 7) They were significantly higher overall in the dentin discs treated with AgF and AgF /KI ($p<0.05$). There was a significantly higher uptake of fluoride in the first 300 µm in the AgF/KI treated discs compared to the AgF treated discs ($p<0.05$). High levels of fluoride were observed up to a depth of 500 µm in AgF/KI treated discs.

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

DISCUSSION

The complete inhibition of biofilm formation by *S. mutans* on discs treated with AgF and AgF /KI points to the major inhibitory effects being due to the silver

and/or fluoride moieties. Although no attempt was made to quantify the amount of biofilm formed on the control or KI treated discs the SEM findings indicate there were marked deposits on both. Consequently the role of iodine in inhibiting biofilm formation appeared to be either non-existent or, at best, limited.

EPMA analyses showed that at the end of the experiment there were still high levels of silver in the surface layers of the AgF and AgF/KI treated dentin discs. Whilst 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 *Staphylococcus aureas.*[19]

Cellular polysaccharides are synthesized by glycosyltransferase enzymes produced by *S. mutans*. 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.[17] Metal cations have been shown to inhibit the activity of several glycosyltranferases. [18] Consequently at sub-inhibitory concentrations silver ion release from the tooth surface may interfere with this enzyme activity.

The role of fluoride release in the findings is not clear. It has been shown in numerous studies that fluoride can inhibit growth of *S. mutans* and other plaque bacteria.[19] In this study, at the end of the experimental period EPMA analyses showed the level of fluoride to a depth beyond 300µm was significantly higher in the discs treated with AgF or AgF /KI. The role of the elevated fluoride levels in inhibiting biofilm formation in this study is not known.

The significantly higher calcium and phosphorus loss from control and KI treated discs compared to the AgF and AgF/KI treated discs suggest 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.

Scanning electron micrographs 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 (Fig 8). Residual surface deposits of AgI could be beneficial. AgI is a sparingly soluble salt that may 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.

Whilst 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 either biofilm formation or the viability of planktonic organisms in groups showing no biofilm growth.

Silver fluoride has been used successfully in deciduous teeth as a treatment of carious dentine prior to placing glass ionomer cement restorations[20] and as a topical application to arrest existing caries[4]. In both studies there were no reported systemic side effects.

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 discs of dentin that had been treated with AgF or AgF /KI, placed in a removable appliance and exposed to the oral environment.

CONCLUSIONS

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

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Adelaide Microscopy for assistance with the EPMA analysis, statistical support from Ms Emmae Ramsay, Statistician, Dept. of Public Health University of Adelaide and from Steritech Pty Ltd, Dandenong, Victoria, for gamma irradiation of the samples.

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Figure 1. Typical outer surface of a non-treated (control) demineralized dentine disc after 2 weeks exposure to *Streptococcus mutans*. Heavy biofilm formation is evident.

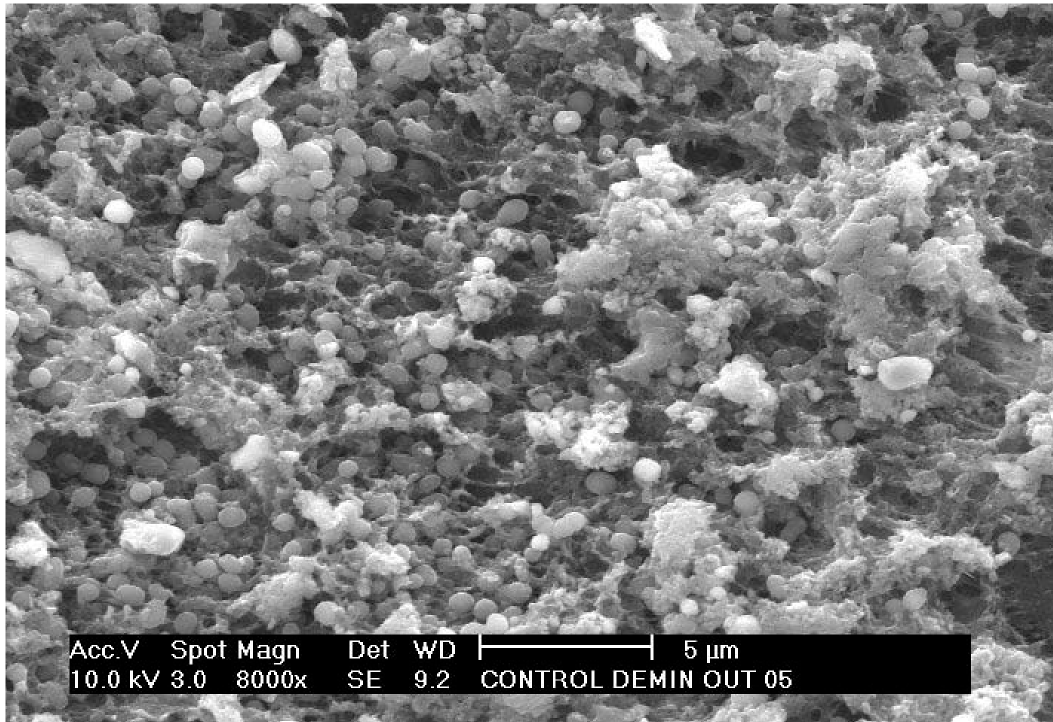


Figure 2. Typical outer surface of a demineralized dentine disc that had been treated with KI after 2 weeks exposure to *Streptococcus mutans*. Heavy biofilm formation is evident.

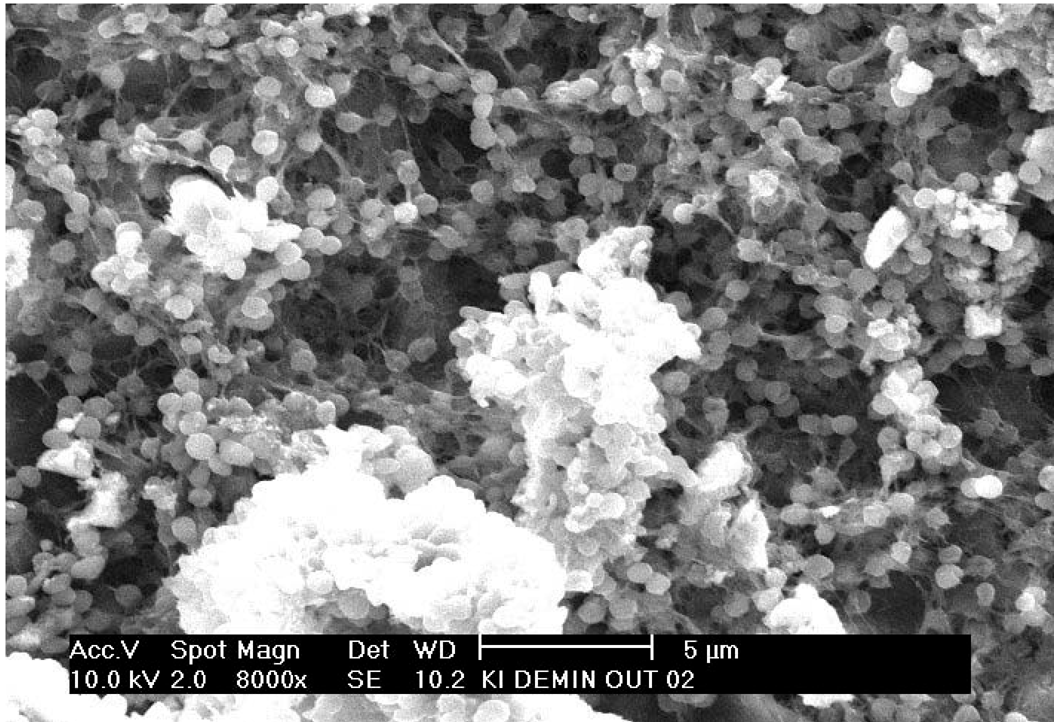
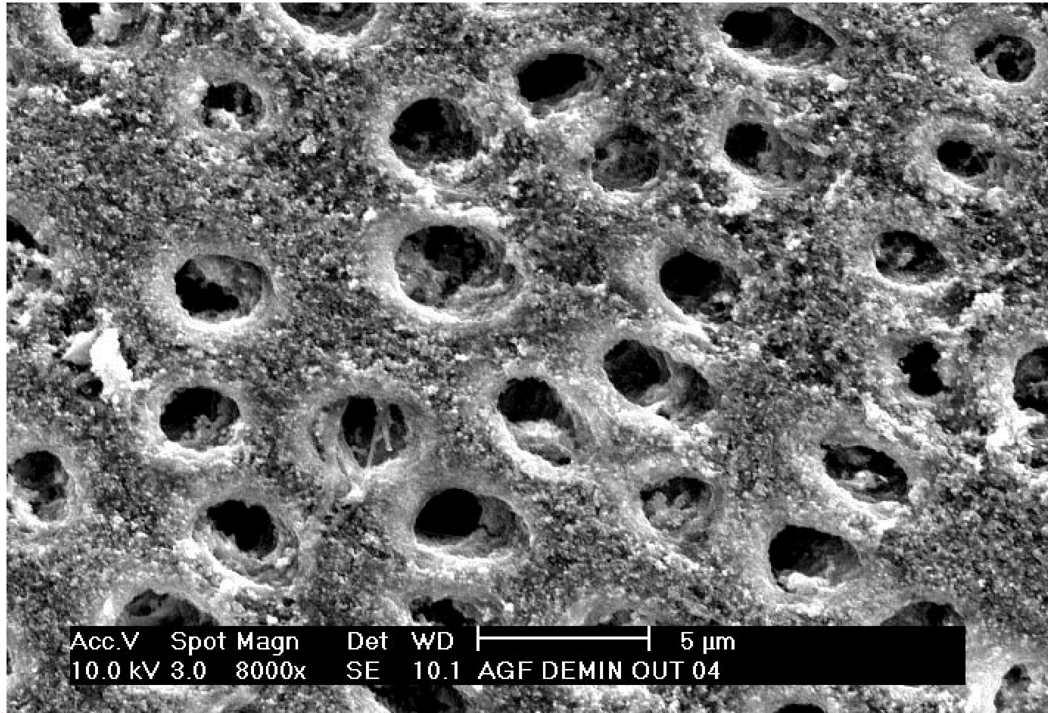


Figure 3. Typical outer surface of a demineralized dentine disc treated with AgF after 2 weeks exposure to *Streptococcus mutans*. There is no evidence of biofilm formation.



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

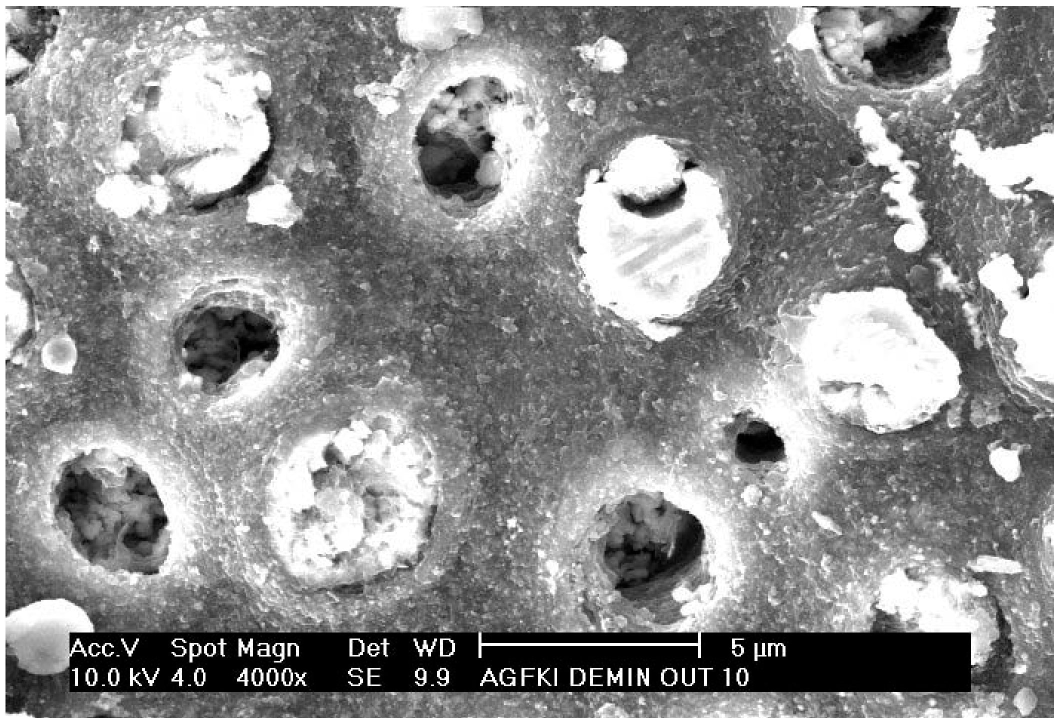
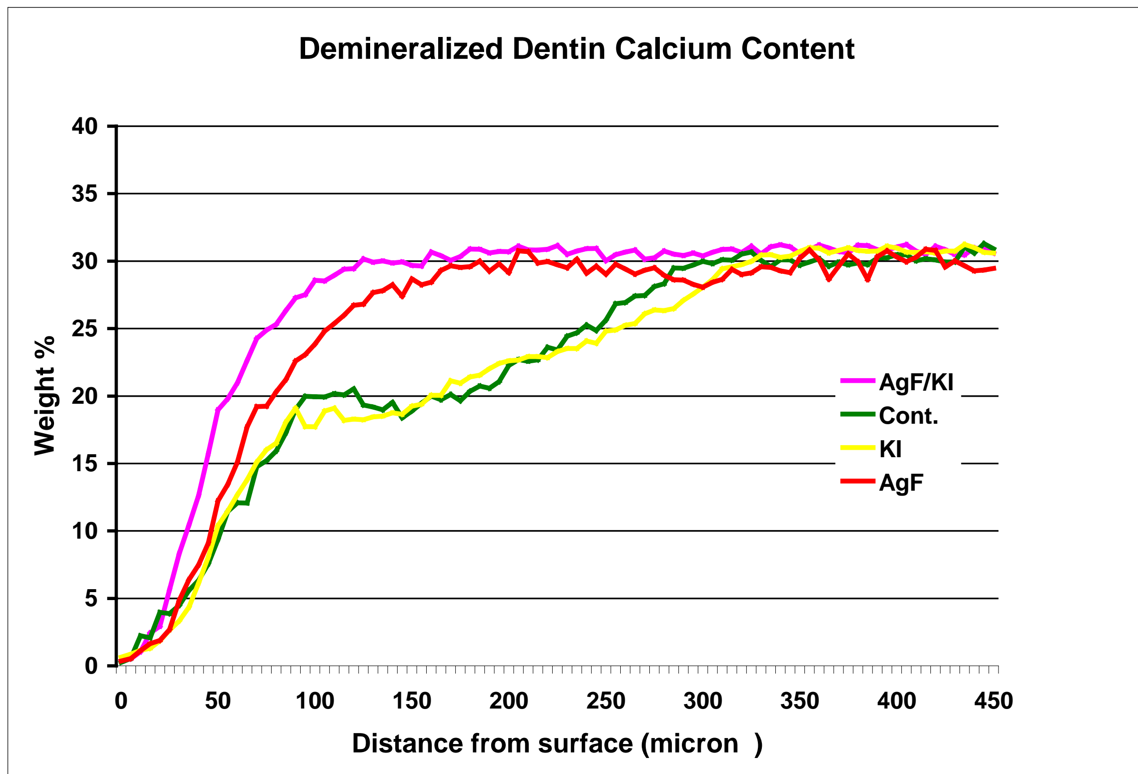


Figure 5. EPMA graph of the percentage weights of calcium in treated and control specimens.



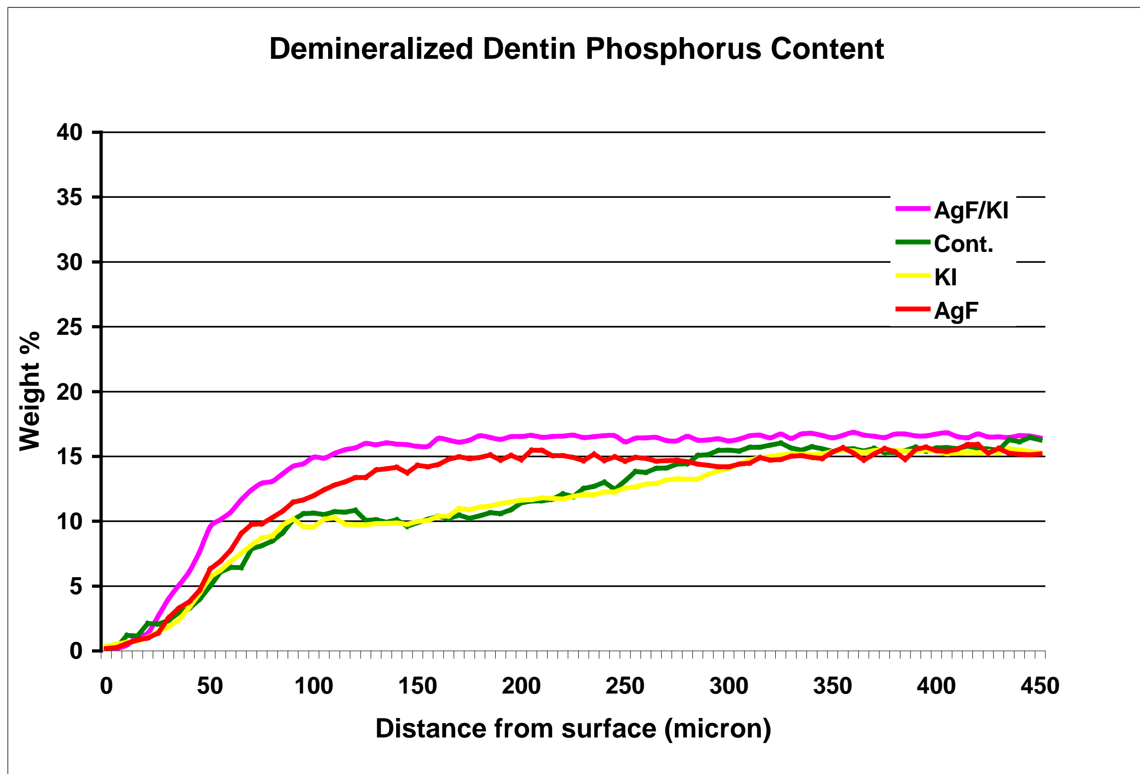
Statistical analysis: Calcium

Differences at the 0.05 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**).

Figure 6. EPMA graph of the percentage weights of phosphorus in treated and control specimens.



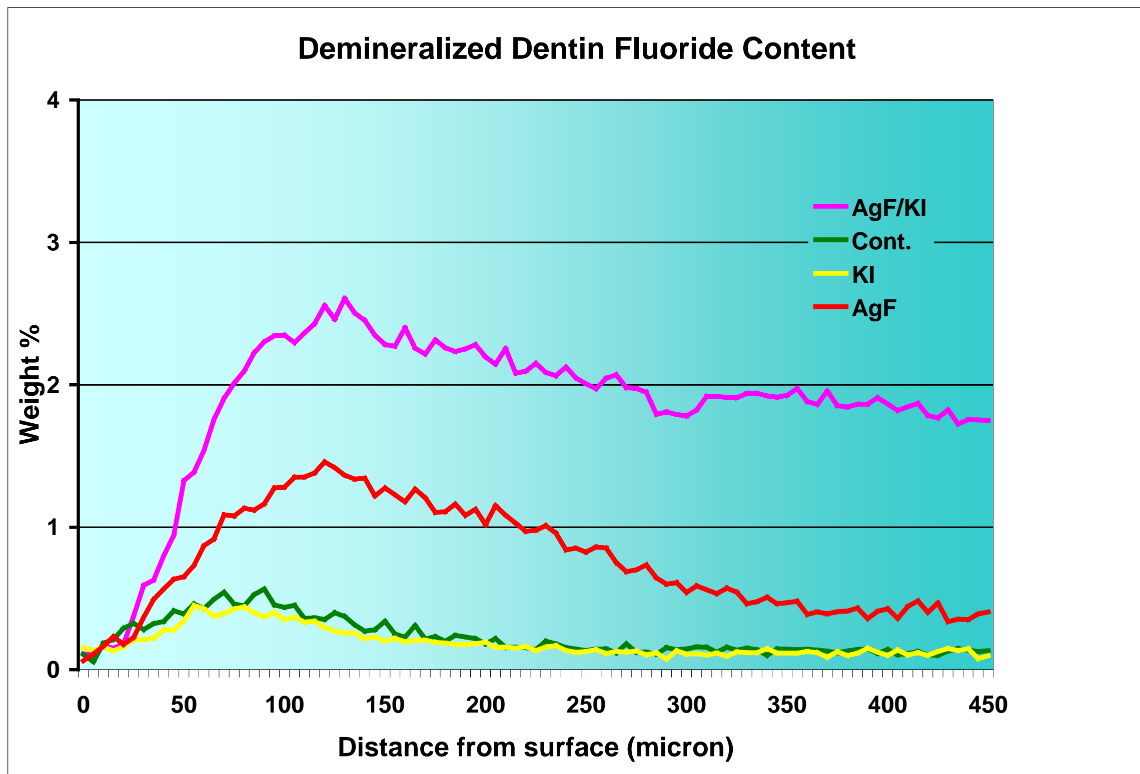
Statistical analysis: Phosphorus

Differences at the 0.05 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**).

Figure 7. EPMA graph of the percentage weights of fluoride in treated and control specimens.



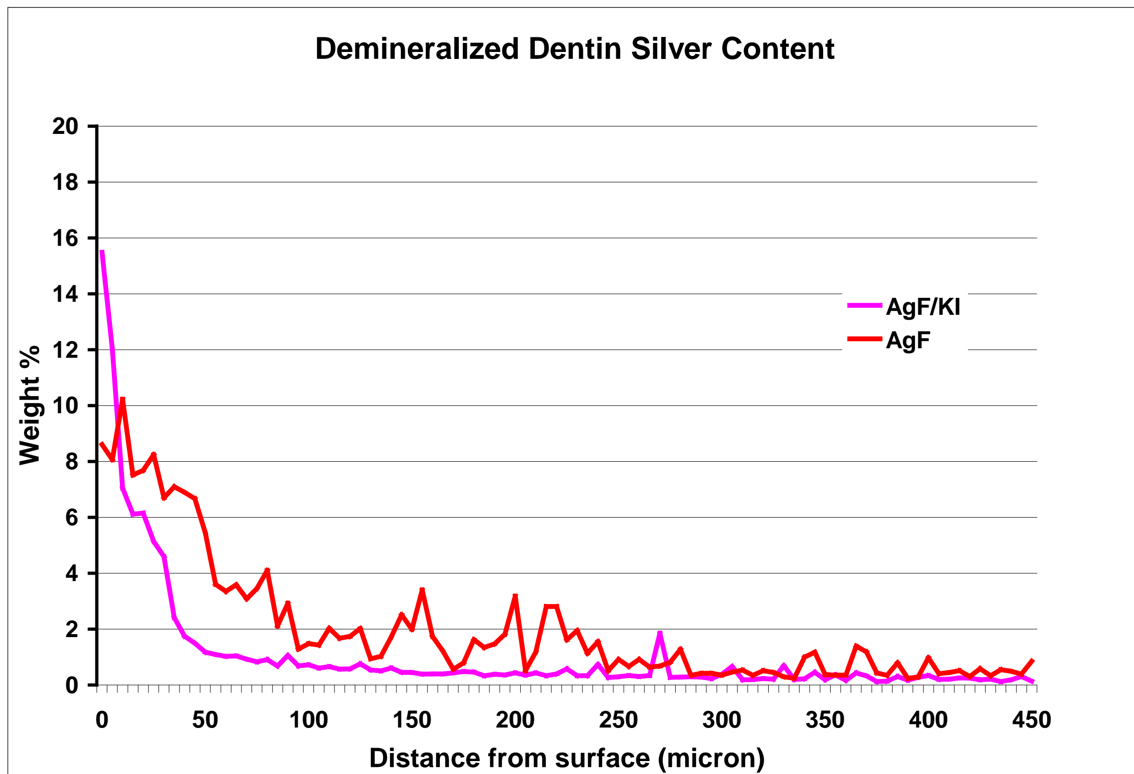
Statistical analysis: Fluoride.

Differences at the 0.05 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**)

Figure 8. EPMA graph of the percentage weights of silver in the treated and control specimens



Statistical analysis: Silver.

Differences at the 0.05 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).

The inability of *Streptococcus mutans* and *Lactobacillus acidophilus* to form a biofilm *in vitro* on dentine pretreated with ozone.

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Key words: dentine, *Streptococcus mutans*, *Lactobacillus acidophilus*, ozone, biofilm

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Abstract

Background

The use of ozone therapy in the treatment of dental caries is equivocal. The aim of this study was to use an *in vitro* model to determine the effects of prior ozone application to dentine on biofilm formation and to measure any associated reduction in bacteria viability.

Method

Twenty dentine discs were bonded to the bases of 5mL polycarbonate screw top vials. Ten dentine discs were infused with ozone for 40 seconds, 10 samples remained untreated as a control. The vials were filled with nutrient medium, sterilized and placed into the outflow from a continuous chemostat culture of *S. mutans* and *L. acidophilus* for four weeks. At the conclusion of the experiment bacterial growth was monitored by taking optical density readings of the growth medium in each vial, and the outer surface of the dentine specimens were examined by scanning electron microscopy as shown by SEM analysis.

Results

Ozone infusion prevented biofilm formation on all the treated samples while there was substantial biofilm present on the control specimens.

While the average optical density of the control specimens was almost twice that of the ozone infused dentine (0.710 for the control with a SD of 0.288 and 0.446

for the ozonated samples with a SD of 0.371, the results were not significant ($P>0.05$)

Conclusions

This preliminary study has shown that the infusion of ozone into non carious dentine prevented biofilm formation *in vitro* from *S. mutans* and *L. acidophilus* over a four week period. The possibility exists that ozone treatment may alter the surface wettability of dentine through reaction with organic constituents.

INTRODUCTION

The formation of a biofilm overlying tooth structure is essential for the initiation and progression of caries.[1, 2] *Streptococcus mutans* and *Lactobacillus acidophilus* are present in cariogenic biofilms and play a significant role in the carious process.[3] The treatment of dentine surfaces to prevent biofilm formation and reduce bacteria growth may assist in the prevention of caries initiation and progression.[4]

The use of ozone therapy in the treatment of dental caries is equivocal and literature reviews suggest there is insufficient evidence to regard ozone treatment as a viable alternative to the current management and treatment of dental caries[5,6] Opposing this view are studies showing both *in vitro*[7]and *in vivo*[8] reduction of viable bacteria in primary root carious lesions and there is *in vitro* evidence showing ozonated water decreased levels of *S. mutans* in an experimental dental plaque[9].

The aim of this study was to use an *in vitro* model to determine the effects of biofilm formation on dentine and any reduction in bacteria viability by the application of ozone prior to placing dentine specimens into the outflows from two Chemostats inoculated respectively with *Streptococcus mutans* and *Lactobacillus acidophilus*.

MATERIALS AND METHODS

Preparation of dentine slabs

The crowns of twenty recently extracted human third molar teeth that had been stored in 0.5% chloramine. Horizontal mid coronal sections were made to produce enamel and dentine sections approximately 1.5 mm 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, 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 1 mm in thickness using a graded series of wet and dry papers to 4000 grit Silicone Carbide paper (Struers, Denmark).

Construction of the bacterial migration model apparatus

Twenty 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 first etching with 37 per cent phosphoric acid (SDI), washing and drying with oil free air prior to applying resin bond (3M ESPE, Minnesota, USA) and then securing with composite resin (Glacier) to ensure there was no leakage through the composite seal[10] (Figure 2).

Pre-treatment of dentine prior to experimentation

Samples were treated as follows:

1. 10 samples were infused with ozone on the outer surface for forty seconds using a HealOzone 2130C device (KaVo Dental, Biberach, Germany)
2. 10 samples had no treatment and were used as a control

Microorganisms

S. mutans Ingbritt was obtained from B Krasse, University of Gotëborg, Gotëborg Sweden. It was maintained on Tryptone Soy agar plates (Oxoid, Basingstoke, UK) grown at 37°C in an atmosphere of $N_2/CO_2/H_2$ (90:5:5). Starter cultures (200ml) were grown in 3% Tryptone Soya broth (Oxoid, Basingstoke, UK), Yeast Extract 0.5% (Oxoid, Basingstoke, UK) and 20% sucrose and, after overnight incubation, was used for chemostat inoculation. Following inoculation cultures were allowed to reach mid-log phase before switching on the medium pump to allow continuous culture. The pH of the culture was not controlled

Lactobacillus acidophilus (a human isolate) was donated by the Division of Microbiology at the Institute of Medical and Veterinary Science, Adelaide. It was maintained on Rogossa agar (Oxoid, Basingstoke, UK) under the same conditions used for *S mutans*. The starter culture was prepared using the following ingredients (in grams) added to a litre of distilled water: Tryptone 10.0 (oxoid), Yeast extract 5.0 (oxoid), glucose 20, Sorbitan mono-oleate 1.0, Potassium dihydrogen phosphate 6.0, Ammonium citrate 2.0, Sodium acetate (anhydrous) 17.0, Magnesium sulphate 0.575, Manganous sulphate 0.12, Ferrous sulphate 0.034.

One litre of solution was boiled to dissolve the ingredients and after cooling 1.32mls glacial acetic acid was added to the solution. Chemostat inoculation followed the same protocol as used for *S. mutans.*

Preparation of the vial culture medium

A batch of nutrient solution was made up consisting of 3% Tryptone Soya Bean broth (Oxoid, Basingstoke, UK), Yeast Extract 0.5 % (Oxoid Basingstoke, UK) and 20% sucrose.

After dissolution in distilled water, the solution was poured into each of the 5 ml 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 minimize the chance of leakage at the seal.

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, Vic., Australia) at a dose of 15 R to sterilize the plastic vessel and its contents.

Experimental method

S. mutans and *L. acidophilus* were grown anaerobically using two separate model C30 Bio Flo Chemostats (New Brunswick Scientific) each with a culture volume of 365 ml as described previously (Rogers et al., 1991[11]) The sterilized vials were placed into a sterile glass container that was connected by a "T" junction to the outflow of each Chemostat system. This provides a constant supply of viable *S. mutans* and *L. acidophilus*. In each Chemostat growth was maintained under anaerobic conditions at an imposed dilution rate of 0.1h -1 (td=7hrs) and the pH was maintained at pH 7.4 for the *S mutans* and pH 5.0 for *L acidophilus* by the automatic addition of KOH (2N). The pH of the flask containing the vials was

uncontrolled and remained at ca. pH 4.5 throughout the 4 week duration of the experiment.

Optical density measurement

After 4 weeks the specimens were removed from the Chemostat system and the optical density of the nutrient solution in the vials was measured using a λ 5 dual beam spectrophotometer (Perkins Elmer, Uberlingen, Germany) at a wavelength of 560 nm to determine bacterial growth within each vial. There is no data available to give a correlation between the optical density readings and concentrations of *S. mutans* and *L. acidophilus* in the solution.

Preparation of sections for SEM analysis

Following optical density readings the discs were removed from the vials, placed in a fixing solution and then dehydrated as described by Knight *et al* (2006).[12] The surface of each specimen that was exposed to the Chemostat was attached to a mounting stub and carbon coated for SEM analysis (Philips XL30 Field Emission Scanning Electron Microscope, Netherlands)

Data Analysis

Calculations were performed using SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

Differences in optical density readings between the control and HealOzone groups were compared using non-parametric Wilcoxon two-sample tests. Non-parametric tests were chosen as there was some evidence of non-normality in the distribution of the outcomes.

RESULTS

SEM

Two of the 20 samples were lost during vial recovery.

The SEM's showed biofilms present on all the control samples. (Figure 3) Higher magnification clearly differentiated *S. mutans* and *L. acidophilus* within the biofilms. (Figure 4) No biofilms were observed on any of the ozonated treated surfaces. (Figure 5) At higher magnification small numbers of planktonic *S. mutans*, and *L. acidophilus* were observed on the ozonated samples. (Figure 6) The pH of the chemostat remained at 4.2 throughout the period of the experiment.

Optical Density

The mean and standard deviation of optical density readings of the two samples were 0.710 for the control with a SD of 0.288 and 0.446 for the ozonated samples with a SD of 0.371. Although the mean value of the control specimens were almost twice that of the ozonated specimens the results were not significant ($P > 0.05$) The results are presented in Table 1 and presented graphically in Figure 2.

DISCUSSION

The existence of an overlying bacterial biofilm is essential for the initiation and progression of dental caries.[14] and a recent paper has show the application of ozone had a minimal effect in reducing bacterial counts within the biofilm.[15]

The ozone treatment of dentine failed to have a consistent effect on the optical density readings in the chamber of the diffusion apparatus. This is possibly not surprising in that the half-life of ozone is relatively short, especially so when dissolved in an aqueous medium. In the latter situation the half-life may be in the order of 20 minutes at 20^0C.[16] Consequently any ozone effect on bacterial viability would probably have dissipated early in the 4-week exposure of the dentine discs to the test organisms.

In regard to the anti-microbial properties of ozone, Baysan *et al* [17] found no significant differences in numbers of viable bacteria between ozonated and non-ozonated infected dentine beneath demineralized enamel.

It is noteworthy there was no evidence of bacterial colonization on the dentine surfaces. In determining the possible mechanisms that may have operated to create this situation, the short half-life of ozone makes it highly improbable that it could have interfered with the synthesis of cellular polysaccharides produced by *S. mutans* by an inactivation of glycosyltransferase enzyme. 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. [18]

A more likely mechanism may have been that the ozone treatment changed the wettability of the dentine surfaces making it more difficult for organisms to colonize. Glantz [19] has shown that reducing the wettability of dentine surfaces can inhibit plaque formation. Ozone is a strong oxidizing agent that can react with almost every organic material. In the case of a dentine surface, the possibility exists that the treatment altered organic surface constituents, such as collagen, in some way. There is evidence for the ability of ozone to induce the release of selected low molecular mass salivary biomolecules from macromolecular binding-sites. This may have the effect of "smoothing" the protein molecules thus affecting

the wettability of the dentine surface[20]. Additional studies are warranted to examine this aspect further.

In this study both the smear layer any biofilm was removed by etching the samples with 37 per cent phosphoric acid during preparation. The ability of ozone treatment to prevent a biofilm formation by *S. mutans* and *L. acidophilus* on dentine suggests there may be therapeutic benefits of ozone application to root surfaces.

The dentine surfaces used in this study were free of a bacterial biofilm prior to exposure to the test organisms. An examination of treatment protocols for root surface caries using ozone that suggests the removal of any bacterial biofilm prior to commencing ozone therapy is necessary for a successful clinical outcome of these procedures. Holmes[21] was able to show a significant remineralization of root caries after cleaning the surfaces and applying ozone. It is suggested that as there was no biofilm forming over the surface of the lesions, the remineralization pH remained above the critical level for remineralization of the lesions to occur

Whilst the results of this preliminary investigation point to a useful role for ozone in the inhibition of bacterial biofilm formation, no attempt was made to determine the effect of varying ozone infusion times on either biofilm formation or the

effects on organism growth. Confocal laser scanning microscopy may be a useful tool to help examine some of these aspects further.

ACKNOWLEDGEMENTS

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LEGEND

Figure1. 5 mL vial showing the various components

Table 1. Mean and standard deviation of optical density of nutritional broth of control and ozone pretreated dentine discs

Figure 2. Typical outer surface of non ozonated sample after four weeks exposure to *Streptococcus mutans*, and *Lactobacillus acidophilus.* Heavy biofilm formation was evident.

Figure 3. Higher magnification view of typical outer surface of non ozonated sample after four weeks chemostat exposure. *Streptococcus mutans* and *Lactobacillus acidophilus* were clearly identified within the biofilm.

Figure 4. Typical outer surface of ozonated sample after four weeks exposure to *Streptococcus mutans*, and *Lactobacillus acidophilus.* A few planktonic bacteria were present but no biofilm formation was evident.

Figure 5. Higher magnification view of typical outer surface of ozonated sample after four weeks chemostat exposure. Only planktonic *Streptococcus mutans* and *Lactobacillus acidophilus* were present on the dentine surface.

Figure1. 5 mL vial showing the various components

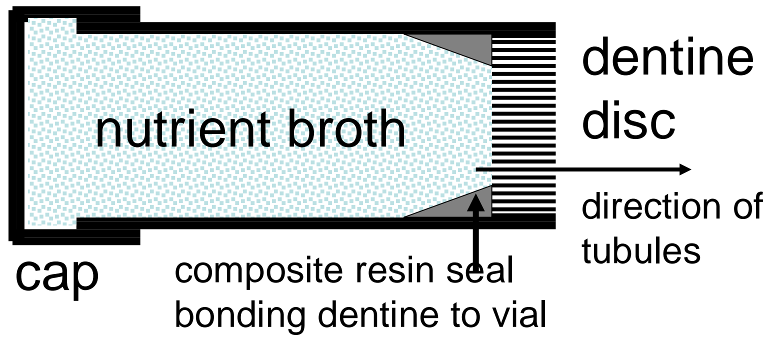


Table 1. Mean and standard deviation of optical density of nutritional broth of control and ozone pretreated dentine discs. The differences in the two samples were not significant ($P > 0.05$)

	Control		HealOzone
1	1.008	1	0.118
2	0.661	2	1
3	0.81	3	0.136
4	0.76	4	0.776
5	0.954	5	0.121
6	0.673	6	0.845
7	0.976	7	0.191
8	0.427	8	0.69
9	0.119	9	0.141
Mean	0.710		0.446
SD	0.288		0.371

Figure 2. Typical outer surface of non ozonated sample after four weeks exposure to *Streptococcus mutans*, and *Lactobacillus acidophilus.* Heavy biofilm formation was evident.

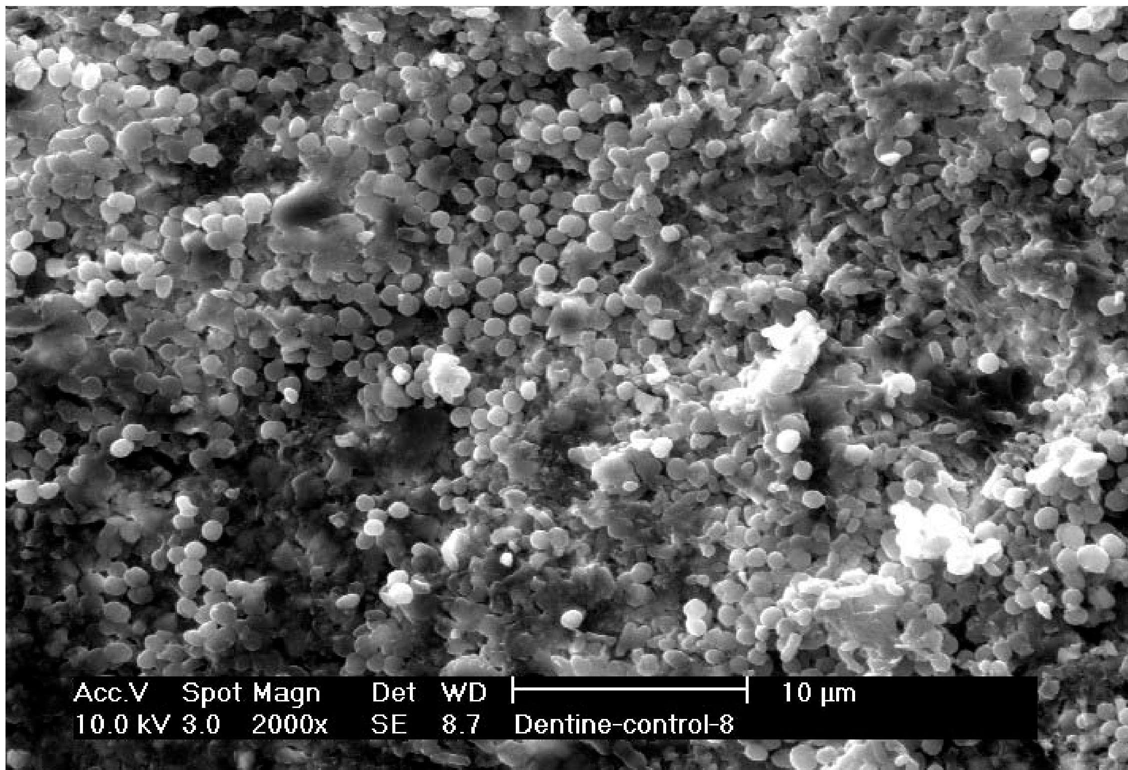


Figure 3. Higher magnification view of typical outer surface of non ozonated sample after four weeks chemostat exposure. *Streptococcus mutans* (Sm) and *Lactobacillus acidophilus* (La) were clearly identified within the biofilm.

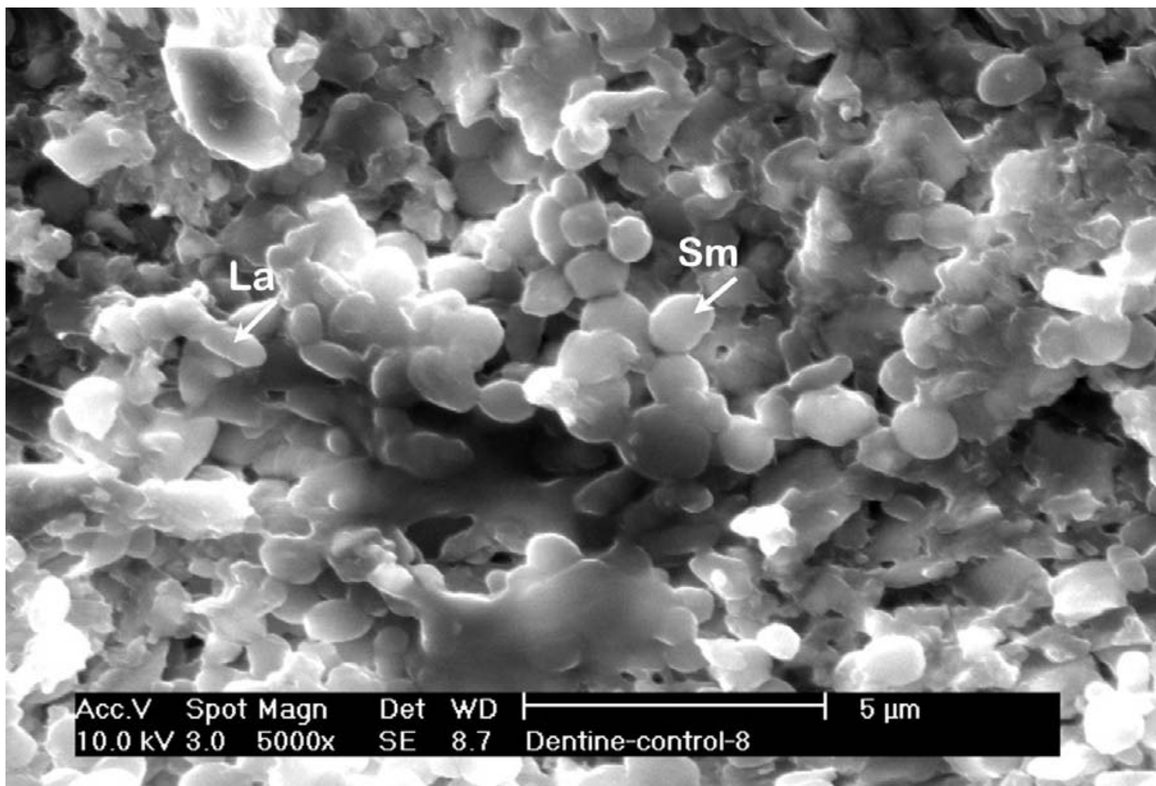


Figure 4. Typical outer surface of ozonated sample after four weeks exposure to *Streptococcus mutans*, and *Lactobacillus acidophilus.* A few planktonic bacteria were present but no biofilm formation was evident.

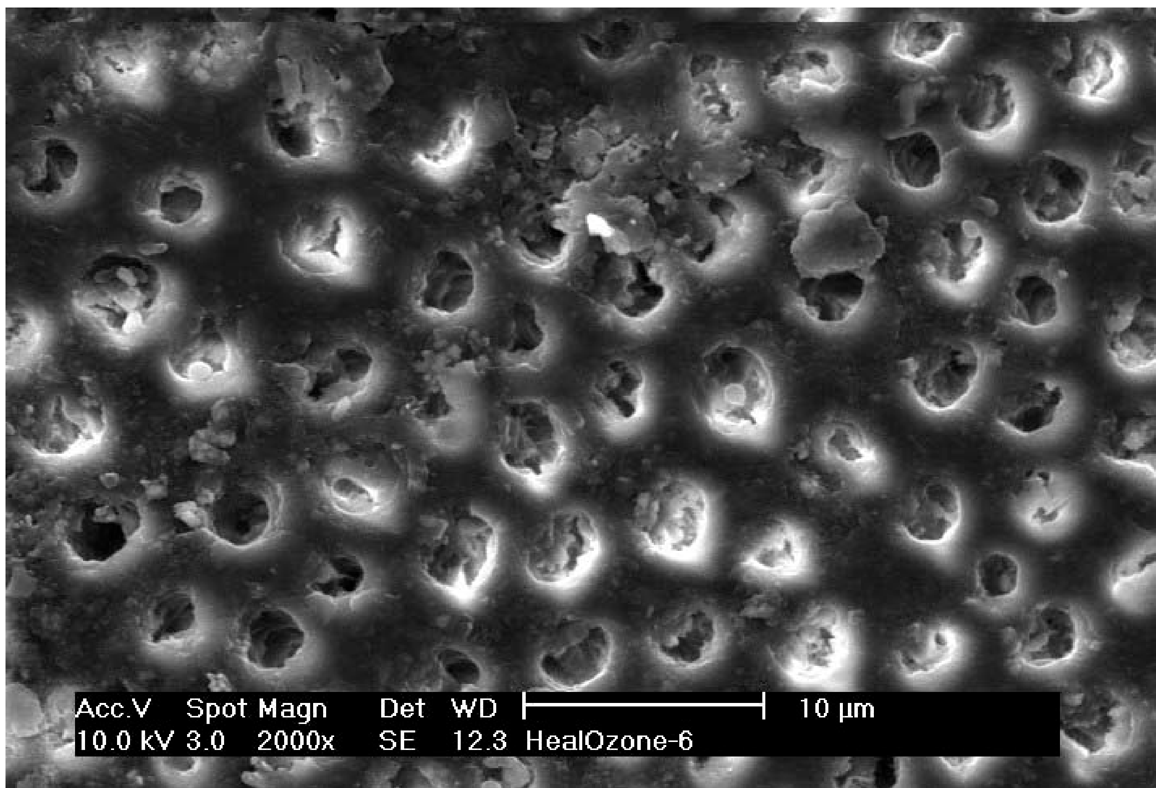
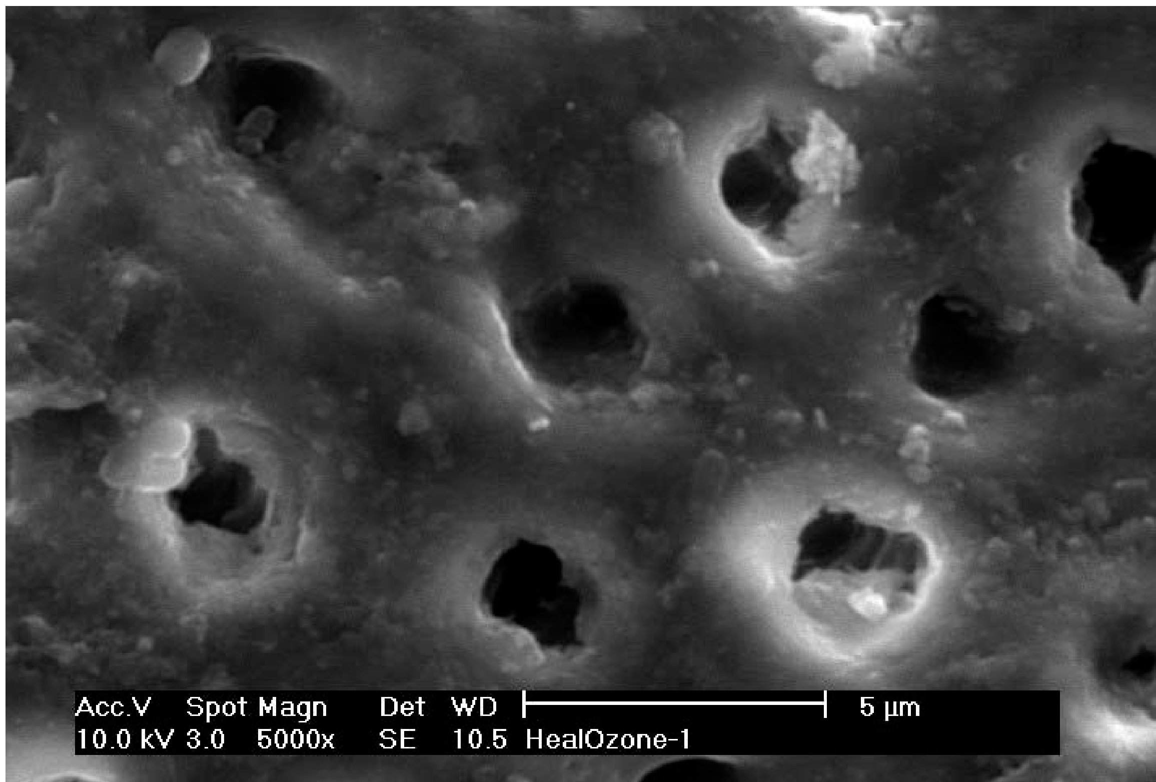


Figure 5. Higher magnification view of typical outer surface of ozonated sample after four weeks chemostat exposure. Only planktonic *Streptococcus mutans* and *Lactobacillus acidophilus* were present on the dentine surface.



DISCUSSION

Discussion of the cumulative data presented within the eight papers submitted as part of this dissertation will be segmented as follows:

1. An *in vitro* model to measure bacterial permeability;
2. Differences in permeability and growth between non demineralized dentine and demineralized dentine (silver fluoride and potassium iodide);
3. The effects on bacterial permeability and growth after pharmacological treatment of dentine (ozone);
4. The effects on biofilm formation and demineralization after pharmacological treatment of dentine (silver fluoride and potassium iodide);
5. The effects on biofilm formation after pharmacological treatment of dentine (ozone);
6. Ion exchange between selected adhesive restorative materials and dentine;
7. The effects on ion exchange of pretreating dentine with silver fluoride and potassium iodide prior to placing glass ionomer cement;
8. The in vitro effects of composite resin and glass ionomer cement on marginal dentine caries;
9. The effects of silver fluoride on the bond strengths of glass ionomer cement and dentine.

1. An *in vitro* model to measure bacterial permeability

The basis of developing the model was to compare the effects on biofilm formation with the ability to alter growth potential once the bacteria had travelled through the dentinal tubules into the nutrient solution. The topical application of a substance on the outer dentine surface could prevent biofilm

formation and prevent viable bacteria passing through the dentine or not affect biofilm formation yet prevent bacteria passing through the tubules. There are various combinations of results available with this model.

An interesting observation was that demineralized dentine did not prevent biofilm formation but was more effective in reducing bacterial growth than AgF/KI application onto non demineralized dentine prevented biofilm formation but had a limited effect on bacterial growth.

Similarly the topical application of ozone prevented biofilm formation but did not affect the growth potential of the bacterial once they had passed into the nutrient solution.

Thus the model assists in determining the relationship between biofilm formation and bacterial viability after various treatment modalities to dentine.

When EMPA analysis is carried out on the dentine samples there are further possibilities in determining the extent of demineralization and penetration levels of the topical agents applied to the dentinal surface.

This model may have useful applications in furthering the understanding of caries progression and management.

2. Differences in permeability and growth between non demineralized dentine and demineralized dentine (silver fluoride and potassium iodide)

The model demonstrated that *S. mutans* were able to migrate through all the samples in this study. The unencumbered migration of bacteria into a contained nutrient solution should result in a rapid propagation of bacteria over a relatively short period to completely deplete the nutrients available.

The lower optical density readings associated with some of the treated samples indicate that the applied agents had the ability to interfere with the growth of the migrated bacteria. Yamaga *et al*. (1972) 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 in the optical density results obtained with the demineralized control samples alone showed that some specimens seem to display a limited ability to inhibit growth in the migrated bacteria while others do not. This suggests that the process of demineralization may trigger a natural yet variable defence mechanism within dentine to bacterial assault that may warrant further investigation (Chaussain-Miller *et al.* 2006).

There was also a relatively wide range of readings obtained when non demineralized dentine was treated with AgF/KI. However the narrow band of results obtained when demineralized discs were treated with AgF/KI suggest that the combined effects of demineralization and medicament application may predictably decrease the growth potential of the organisms passing through these specimens. Further studies are required to determine if the application of AgF/KI to carious dentine inhibits cariogenic bacteria and what clinical significance this might have on caries management.

EPMA analyses showed that at the end of the experiment there were high levels of silver in the surface layers of the AgF and AgF/KI treated dentine discs. Whilst 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 *Staphylococcus aureas*

(Hall *et al*. 1987). This suggests that silver salts at this concentration act specifically as a growth inhibitor rather than a bactericide, although unspecified higher concentrations were found to be bactericidal (Li *et al*. 1994).

No attempt was made to determine the effects of lower concentrations of AgF/KI on reducing optical density by *S. mutans*, and this warrants further

investigation as does an *in vivo* study to clinically confirm the findings of this investigation.

3. The effects on bacterial permeability and growth after pharmacological treatment of dentine (ozone)

The ozone treatment of dentine failed to have a consistent effect on the optical density readings in the chamber of the diffusion apparatus. This is possibly not surprising in that the half-life of ozone is relatively short, especially so when dissolved in an aqueous medium. In the latter situation the half-life may be in the order of 20 minutes at 20^0C (Baysan *et al*. 2007). Consequently any ozone effect on bacterial viability would probably have dissipated early in the 4-week exposure of the dentine discs to the test organisms.

In regard to the anti-microbial properties of ozone, Müller *et al.* (2007) found no significant differences in numbers of viable bacteria between ozonated and non-ozonated infected dentine beneath demineralized enamel

4. The effects on biofilm formation and demineralization after pharmacological treatment of dentine (silver fluoride and potassium iodide)

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

Cellular polysaccharides are synthesized by glucosyltransferases enzymes produced by *S. mutans*. 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 (Tamesada *et al*. 2004). Metal cations have been shown to inhibit the

activity of several glucosyltransferases (Wunder *et al*. 1999). Consequently at sub-inhibitory concentrations silver ion release from the tooth surface may interfere with this enzyme activity.

It has been shown in numerous studies that fluoride can inhibit growth of *S. mutans* and other plaque bacteria (Li *et al*. 1994). In this study, at the end of the experimental period EPMA analyses showed the level of fluoride to a depth beyond 300µm was significantly higher in the demineralized discs treated with

AgF /KI. The role of the elevated fluoride levels in inhibiting biofilm formation in this study is not known.

The significantly lower levels of calcium and phosphorus in the control and KI treated discs compared to the AgF and AgF/KI treated discs suggest that the AgF based topical treatments had a discernable protective effect on the dentine surfaces. It is estimated that the surfaces of all sections were exposed to a pH of around 4.5 for the entire two weeks of the experiment.

Scanning electron micrographs of dentine 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. 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.

Whilst 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 either biofilm formation or the viability of planktonic organisms in groups showing no biofilm growth.

Silver fluoride has been used successfully clinically in deciduous teeth as a treatment of carious dentine prior to placing glass ionomer cement restorations (Gotjamanos 1996) and as a topical application to arrest existing caries (Chu *et al*. 2002). In both studies there were no reported systemic side effects.

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 discs of dentine that had been treated with AgF or AgF /KI, placed in a removable appliance and exposed to the oral environment.

While there was complete inhibition of biofilm on non demineralized and demineralized dentine discs treated with AgF/KI, the demineralized discs were better able to resist further demineralization than the non demineralized samples.

When the outer surfaces of the AgF/KI sections are examined under SEM it was evident that there was more reaction product present on the surface of the demineralized samples than the non demineralized ones.

Two factors that stood out in the EPMA data were the higher levels of silver deposited on the surface of the demineralized AgF/KI sections and the increased levels of fluoride present in the demineralized samples.

EPMA data showed that while fluoride levels on the surface of the discs were low there were significantly higher levels of fluoride within the demineralized discs that peaked at almost three per cent at a depth of 125µm and continued around two

per cent to a depth beyond 500µm. Fluoride levels within the non demineralized discs peaked a just over one per cent at a depth of 50µm then gradually fell away. While it may be a coincidence, the calcium and phosphorus levels in the non demineralized discs demonstrated a susceptibility to *in vitro* caries beyond 50µm whereas in the demineralized discs, the higher fluoride levels and penetration depths were associated with an ongoing resistance to *in vitro* caries demineralization. Higher fluoride levels in the AgF/KI treated demineralized discs may have had a minimal effect at the surface on biofilm formation they may certainly contributed to the improved *in vitro* caries resistance observed in the EMPA data and the low optical density readings.

The AgF/KI treated demineralized dentine had significantly higher levels of silver (16 per cent) precipitated than the AgF/KI treated non demineralized discs (two per cent). Both demineralized and non demineralized discs were able to completely inhibit biofilm formation suggesting that lower concentrations of AgF/KI may be effective in preventing biofilm formation in demineralized dentine. The penetration of silver into both demineralized and non demineralized AgF/KI treated dentine was around 50µm. This is the depth at which *in vitro* carious demineralization commenced in the AgF/KI treated non demineralized discs, suggesting the presence of silver may have inhibited *in vitro* caries

initiation. The much higher levels of silver on the surface of the AgF/KI treated demineralized discs may have adversely affected the viability of the *S. mutans* to the extent that *in vitro* caries initiation was unable to occur.

The application of AgF/KI onto non demineralized and demineralized dentine was able to significantly reduce *in vitro* caries progression and prevent biofilm formation. The ability of partly demineralized dentine to take up relatively large amounts of silver and fluoride suggests that this application is more effective as a means of arresting existing dentine caries, where the teeth have already undergone some demineralization, than preventing dentine caries initiation on sound dentine surfaces.

No attempt was made to determine the effects of lower concentrations of AgF/KI on biofilm formation or dentine demineralization by *S. mutans*, and this warrants further investigation as does an *in vivo* study to clinically confirm the findings of this investigation.

1. The effects on biofilm formation after pharmacological treatment of dentine (ozone)

There was no evidence of bacterial colonization on any of the ozonated dentine surfaces. In determining the possible mechanisms that may have operated to create this situation, the short half-life of ozone makes it highly improbable that it could have interfered with the synthesis of cellular polysaccharides produced by *S. mutans* by an inactivation of glucosyltransferases enzyme. 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 (Tamesada *et al*. 2004).

A more likely mechanism may have been that the ozone treatment changed the wettability of the dentine surfaces, making it more difficult for organisms to colonize. Glantz (1969) has shown that reducing the wettability of dentine surfaces can inhibit plaque formation. Ozone is a strong oxidizing agent that can react with almost every organic material. In the case of a dentine surface, the possibility exists that the treatment altered organic surface constituents, such as collagen, in some way. There is evidence for the ability of ozone to induce the release of selected low molecular mass salivary biomolecules from macromolecular binding-sites. This may have the effect of “smoothing” the

protein molecules thus affecting the wettability of the dentine surface (Grootveld *et al*. 2006). Additional studies are warranted to examine this aspect further.

The dentine surfaces used in this study were free of a bacterial biofilm prior to exposure to the test organisms. An examination of treatment protocols for root surface caries using ozone that suggests the removal of any bacterial biofilm prior to commencing ozone therapy is necessary for a successful clinical outcome of these procedures. Holmes was able to show a significant remineralization of root caries after cleaning the surfaces and applying ozone. It is suggested that as there was no biofilm forming over the surface of the lesions, the remineralization pH remained above the critical level for remineralization of the lesions to occur (Holmes 2003).

Whilst the results of this preliminary investigation point to a useful role for ozone in the inhibition of bacterial biofilm formation, no attempt was made to determine the effect of varying ozone infusion times on either biofilm formation or the effects on organism growth. Confocal laser scanning microscopy may be a useful tool to help examine some of these aspects further.

6. Ion exchange between selected adhesive restorative materials and dentine

Ionic exchange between tooth structure and an adhesive restorative material requires an aqueous environment in order for ion exchange to occur (Watson, 1999). Polyalkenoic acid from the cement is buffered by phosphate ions within the tooth and calcium and phosphate ions are dispersed from the hydroxyapatite into the unset cement. Ions are also able to disperse from the cement into the dentine resulting in an intermediate layer between the unset GIC and hydroxyapatite, referred to as the "ion exchange layer" (Tyas *et al*. 2004). Although the so called ion exchange layer appears no more than several micrometres wide (Ngo *et al*. 1997) when viewed using scanning electron microscopy (SEM), the penetration of ion exchange observed using EPMA within both the dentine and GIC extend beyond 50µm either side of the restorative interface.

Elements such as silicon were not measured and it would be of interest to see how many other elements transfer between the two materials. This further raises questions as to what these characteristics may have on the bond strength

and the anecdotal anti cariogenic properties of GIC's (Forsten *et al*. 1994). The fluoride levels in Ketac Molar (3M ESPE, Minnesota, USA) are greater than those measured in Fuji IX, Fuji VII (GC Corp. Tokyo, Japan) and Riva. It is surprising that the amount of fluoride ions transferred into the dentine is similar to the other

GIC's measured. As Ketac Molar has a calcium-containing glass filler, element transfer is less obvious to observe. There is, however, evidence of a slight spike occurring in both the calcium and phosphorus baselines close to the restorative interface that indicates calcium and phosphorus may have become incorporated from the dentine into the Ketac Molar.

Fuji VII is promoted as a high fluoride release GIC, yet the amount of fluoride released into the adjacent dentine is similar to that of Fuji IX. This suggests that although more fluoride ions are being released into the environment, there is a limit to the amount of fluoride that is capable of being deposited into dentine from auto cure GIC's.

The presence of Ca and P ions embedded in the composite resin is intriguing, as ion exchange can only occur in an aqueous environment. As SE Bond (J. Morita, Tokyo, Japan) incorporates a self etching primer, it can be expected that a small amount of calcium and phosphorus dissolved out from the dentine surface during the etching process becomes embedded into the composite resin. The extent that this may have on weakening the resin dentine bond is unknown.

The Sr and Al elements in dentine subjacent to the composite resin may have been derived from the inorganic filler fraction of the material. Studies using self etching primers for dentine bonding on extracted human teeth have shown extensive penetration of resin tags into the dentine tubules (Cal-Neto *et al*. 2004). Whether the Sr and Al elements followed such a pathway is not known.

These findings support the concept of ion exchange as a bonding mechanism between auto cure GIC and dentine. Using EPMA the penetration of elements from tooth structure and GIC were found to exceed well beyond the confines of the “ion exchange layer” observed in previous SEM studies.

No attempt was made to establish if ion exchange between the dentine and glass ionomer cement continues through time (Van Duinen *et al.* 2004) and what effects this may have on surface hardness of the dentine and glass ionomer cement, bond strength and increasing resistance to carious attack.
Further research on this topic is required.

7. The effects on ion exchange of pretreating dentine with silver fluoride and potassium iodide prior to placing glass ionomer cement

While there is evidence in clinical trials that AgF is able to reduce caries activity and the emergence of new lesions (Lo *et al*. 2001) although the mechanisms which cause this are uncertain.

It has been linked to fluoride uptake into the dentine, blocking the dentine tubules with silver precipitates or inactivation of cariogenic bacteria that come into contact with it (Gotjamanos 1996).

From this study it was found that silver iodide occurs in the dentinal tubules to a depth of over 100 microns and is a sparingly soluble salt that releases silver and iodine ions in small quantities. It can be postulated that this may add to the beneficial effects of AgF in controlling caries.

The application of AgF significantly increases the concentration and penetration depth of fluoride ions into the dentine, when compared to glass ionomer cement alone. Adding KI after AgF application does not appear to interfere with this mechanism.

There is clinical evidence that glass ionomer cements contribute to the remineralization of affected dentine by depositing strontium. Strontium is capable of replacing calcium and assisting with remineralization. Fluoride is also available to assist with the formation of fluor apatite (Mount *et al*. 2000). The application of AgF and KI to the surface of the dentine did not significantly interfere with the transfer of strontium into the dentine.

The penetration of fluoride ions into the dentine suggests the possibility of the formation of a fluor apatite layer from the demineralized carbonated apatite that should provide further protection from caries at the restorative interface.

This study has shown that the application of AgF/KI beneath a glass ionomer cement restoration placed on demineralized dentine is capable of significantly increasing the concentration and penetration depth of fluoride into dentine well beyond the demineralized zone.

The precipitation of silver iodide in the dentinal tubules may provide a synergistic effect in improving the ability of AgF to resist caries.

Further *in vivo* studies are required to establish the clinical benefits of this procedure.

8. The in vitro effects of composite resin and glass ionomer cement on marginal dentine caries

Surface loss of the dentine surrounding the composite resin restorations can be attributed to the demineralization of the surface dentine and collapse following dehydration of the specimens for SEM and EPMA analysis.

As the percentage weights of calcium and phosphorus adjacent to the GIC restorations were similar to sound dentine it can be assumed that surface collapse due to dehydration was less around the GIC restorations.

Some SEM's of Fuji VII restorations showed a slight subsurface break down. This may be attributed to the higher liquid powder ratio this material has compared to the other GIC's.

The *in vitro* caries model used in this study maintained pH levels of about 4.5, causing significant surface degradation of the GIC surfaces. This may explain clinically why GIC restorations appear to be lost more readily interproximally

where lower pH levels can be maintained over time due to bioload build up in the proximal spaces than restorations on occlusal, buccal or lingual surfaces subject to the effects of saliva flow where sustained drops in pH are less likely to occur.

The EPMA data shows that GIC's are capable of substantially reducing demineralization of dentine adjacent to the cavo restoration interface. Percentage weights of calcium and phosphorus adjacent to GIC's were similar to sound dentine yet percentage weights adjacent to bonded composite resin restorations were about half that. Furthermore, cavities restored with GIC have this protection extended out to beyond 130µm from the cavo restoration surface.

The release of fluoride from glass-ionomer cements is greater at lower pH than at higher pH (Gandolfi *et al*. 2006). The pH levels experienced by the specimens in this study (pH 4.5) would have been conducive to fluoride release. Other laboratory studies have confirmed the ability of fluoride to prevent or inhibit demineralization of dentine adjacent to glass-ionomer cement restorations (Tarn *et al*. 1997; Gilmour *et al.* 2002; Hara *et al.* 1997).

However, the effects of fluoride on preventing demineralization in this in vitro caries model are not clear cut. One glass-ionomer cement material tested, Riva

(Southern Dental Industries, Melbourne, Australia), was associated with lower fluoride uptake in the dentine than the other glass-ionomer cement materials, but was associated with quite high resistance to demineralization as evidenced by the high calcium and phosphorus levels in the adjacent dentine.

The possibility exists than other ionic species eluted from the material may have conferred additional protection. In addition, fluoride can inhibit biofilm formation by *S. mutans* and other bacterial species (Li *et al*. 1994). The extent of this happening in this study is not known.

Another protective factor associated with glass-ionomer cement is its excellent buffering capacity in an acidic environment (Nicholson *et al*. 2002; Czarnecka *et al*. 2000).

Irrespective of the causal factors protecting surrounding dentine from caries, these findings support the clinical impressions of Australian dentists who observed little caries activity associated with GIC restorations, (Forsten *et al.* 1994) although loss of a GIC restoration could be interpreted by a clinician as recurrent caries at that site.

The results of this *in vitro* study show that placing a bonded composite resin restoration into dentine affords little protection of the surrounding tooth from

caries attack although no destruction of the restorative surface occurs. Placing a glass ionomer cement restoration into dentine protects the surrounding tooth from caries but degradation of the restoration surface occurs. Further *in vivo* studies are required to confirm these findings.

9. The effects of silver fluoride on the bond strengths of glass ionomer cement and dentine

The application of KI to AgF, or the AgF/KI precipitate on the dentine surface, may have reduced the bond strength of dentine to GIC and limited the clinical applications of this procedure. While the bond strengths recorded for the conditioned and AgF/KI washed samples are similar to those found in other studies (Pereira 2002) it is evident that the reaction products of AgF and KI left to air dry on the dentine surface interfere with bond formation between GIC and dentine.

The standard protocol of conditioning dentine surfaces prior to bonding is to apply a ten per cent solution of poly acrylic acid (Mount 1994). Tay *et al.* (2001) have shown there is no significant difference in bond strengths between dentine that has

been conditioned or etched for up to 15 seconds. Unlike conditioning, phosphoric

acid removes the surface bioload as well as the smear layer and smear plugs and may facilitate the permeation of AgF and KI into the dentine.

GIC's have been shown to have a relatively low yet effective chemical bond to dentine. The long term maintenance of this bond is an integral part of the clinical application of GIC's.

This study has shown that dentine samples that had been etched with 37 per cent phosphoric acid for five seconds prior to the application of AgF and KI followed by washing away the precipitate and air drying the tooth produced bond strengths not significantly different to samples that had been treated with 10 per cent polyacrylic acid conditioner for ten seconds.

Washing away the reaction products and air drying is recommended as the clinical protocol for using AgF and KI on dentine surfaces prior to application of a GIC.

Conclusions

The two primary aims of this study were to firstly develop a number of *in vitro* experiments to investigate the ability of specific pharmacological agents and restorative materials to inhibit the bacterial degradation of dentine. The second aim was to determine if the pharmacological pretreatment of demineralized dentine improved remineralization under glass ionomer cement restorations and if this application adversely affected the adhesive properties of these restorative materials to dentine.

Under the conditions of these *in vitro* studies, the application of AgF/KI and ozone prevent the formation of a bacterial biofilm.

The application of AgF/KI also reduced in *vitro* caries development and reduced the growth potential of *S. mutans*. Reduction of caries development and bacterial growth potential was significantly more pronounced in the demineralized dentine samples.

Ozone application appeared to inhibit bacterial growth, however in this *in vitro* experiment there were no significant differences between ozonated and non ozonated samples.

By preventing biofilm formation is appears that the application of both ozone and AgF/KI may help protect teeth from caries initiation.

The application of AgF/KI to carious dentine also appears to prevent further caries progression and has a pronounced effect on bacterial growth. This suggests that AgF/KI may be useful in retarding existing caries.

The application of AgF/KI to demineralized dentine prior to placing a glass ionomer cement restoration improves fluoride penetration into the dentine and does not interfere with strontium transfer from the glass ionomer cement.

The application of AgF/KI did not interfere with the bond strengths between glass ionomer cement and dentine providing the reaction product between the silver fluoride and potassium iodide was washed from the surface of the dentine prior to placing the glass ionomer cement.

This suggests that AgF/KI may provide a therapeutic benefit protecting dentine from further carious progression and enhancing remineralization when applied prior to placing a glass ionomer cement restoration.

Further *in vivo* studies are required to confirm these findings.

Ionic transfer between dentine and glass ionomer cement was shown to extend well beyond the restorative interface suggesting that the therapeutic benefits of fluoride release and assistance with remineralization may penetrate deep into dentine and increase over time. No attempt was made to confirm this and requires confirmation by further research.

Glass ionomer cements prevented demineralization of dentine surrounding the restorations in experimental caries while composite resin offered no protection from demineralization. During the period of the study, there was a measurable amount of surface degradation in all the glass ionomer cement samples.
Clinical trials that have observed recurrent caries associated with glass ionomer cement may have confused the surface degradation in the glass ionomer cement. Further *in vivo* studies are required to confirm this hypothesis.

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