Research Portfolio

Vu Thanh My Anh

School of Dentistry

Faculty of Health Sciences

The University of Adelaide

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TABLE OF CONTENTS

OVERVIEW 3
DECLARATION 5
ACKNOWLEDGEMENTS 6
SECTION ONE 7
INTRODUCTION 8
LITERATURE REVIEW 10
Author Covering letter to the Australian Dental Journal (1) 13
MANUSCRIPT 1 14
Dental hard tissues 15
Enamel 16
Dentine 17
Dentinal tubules and dentinal fluid 19
Diffusion and filtration 19
Hydraulic conductance of dentine tubules 23
Dentinal fluid composition 24
Dental caries 25
Definition of dental caries 25
The demineralization and remineralization processes 26
Factors influencing demineralization and remineralization 27
Structure of carious dentine and its remineralization 28
Marginal fit and secondary caries 31
Luting cements 32
Biologic properties 32
Mechanical properties 32
Materials and methods used in this study 42
The method to be used is as follows: 42
The reason that Sinfony crowns and Fuji Cem cement were used as follow: 44
Fluid perfusion method: 44
Preparation of Simulated Dentinal Tubule Fluid (SDTF) 46
ORIGINAL STUDY 47
Author Covering letter to the Australian Dental Journal (2) 50

Manuscript 2 51
DISCUSSION AND CONCLUSION 74
REFERENCES 76
SECTION TWO: Other Scholarly Work 81
APPENDIX 84
Author Covering letter to the Australian Dental Journal (3) 85
Manuscript 3 86
Author Covering letter to the Australian Dental Journal (4) 99
Manuscript 4 100
Author Covering letter to the Australian Dental Journal (5) 133
Manuscript 5 134
Author Covering letter to the Australian Dental Journal (6) 160
Manuscript 6 161

NOTE:
Please refer to the list of presentations after page 81 for their availability online

OVERVIEW

This research portfolio is submitted as part of the requirements for the degree Doctor of Clinical Dentistry. It consists of:

Introduction

The section provides background information relating to the main research project

Literature review

This review summarises relevant information about dental hard tissues, dentinal tubules and fluid, cariology and the factors that influence recurrent caries associated with indirect restorations.

Original research

This section includes:

- An expanded version of the background to the reported work
- A manuscript ready for submission to an appropriate journal. The paper describes the effect of dentinal tubule fluid on demineralization and remineralization associated with fixed restorations in an *in vitro* experimental system.

Draft manuscripts of four additional papers reporting associated work completed previously but not yet published are presented as background to the present study in the Appendix (for reference only).

Discussion and conclusion

This section reviews the progress toward understanding the effect of dentinal tubule fluid on demineralization and remineralization and suggests areas for future investigation

Other scholarly work

This section includes electronic versions of all of the other scholarly work undertaken during the three years of the Doctor of Clinical Dentistry programme.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any other university or other 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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My Anh Vu Thanh

Dated this 4th day of February 2011

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SECTION ONE

Study to investigate

the remineralization potential of

demineralized dentine under crowns

INTRODUCTION

Recurrent caries is one of the factors leading to failure of crowns and bridges in prosthodontics. Generally it not only necessitates the removal of infected dentine but also leads to the replacement of crowns or bridges, or in more severe cases, endodontic treatment. Leakage is referred to as the passage of dissolving materials through gaps between prepared teeth and restorative materials. It is considered as one contributor to the development of recurrent caries (Pashley 1990).

It has been shown that crown preparation can expose up to two million dentinal tubules (Garberoglio and Brannstrom 1976). These opened tubules increase dentine permeability (Pashley 1985)and enable substances to pass to and from the dental pulp. There have been some previous studies investigating dentine permeability and its influence on micro-leakage. The permeability, microhardness and elasticity of dentine varies between different sites (Pashley 1990; Zheng et al. 2003). For example, the pulp horn sites are more permeable than sites nearer to the centre of the crown; the axial wall is more permeable than the pulpal wall. The thickness of dentine and the diameter of dentine tubules also influence dentine permeability. Pashley (1990) found greater potential of microleakage in the teeth which have more dentine exposure during tooth preparation. Ozok et al (2004) showed that dentinal fluid influenced the sealing of dental adhesives.

During crown preparation in carious teeth, some deminaralized dentine might remain. Caries may continue to develop especially where microleakage occurs. Hence, the

purpose of this investigationis to determine whether a luting cement which contain mineral ions can remineralize any demineralized dentine and consequently prevent caries from developing further.

Dentinal fluid has been studied since the 1960s. Larmas (1986) described the pressure and concentration of some minerals and electrolytes in the dentinal fluid. Shellis (1994) and Ozok et al.(2002; 2004)demonstrated that demineralization of dentine decreased in the presence of simulated dentinal fluid. A study by Vu (2008) found that dentinal fluid influenced remineralization of demineralized dentine. However, the remineralization process of demineralized dentine of a tooth prepared for crown placement needs to be further investigated.

The influence of simulated dentinal fluid in remineralization of demineralized dentine *in vitro* was shown in a previous study (Vu 2008). This study aims to investigate the interaction of simulated dentinal fluid and Fuji Cem Luting cement in the remineralization of remnant demineralized dentine in teeth prepared for crowns.

LITERATURE REVIEW

DENTAL HARD TISSUES AND FACTORS WHICH INFLUENCE THEIR DEMINERALIZATION AND REMINERALIZATION: A REVIEW OF LITERATURE

My Anh Vu Thanh

John McIntyre

Lindsay Richards

School of Dentistry, The University of Adelaide, Adelaide, SA 5005 Australia

STATEMENT OF AUTHORSHIP

DENTAL HARD TISSUES AND FACTORS WHICH INFLUENCE THEIR DEMINERALIZATION AND REMINERALIZATION: A REVIEW OF LITERATURE

VU THANH. MA (Candidate)

Carried out experiment, performed sample analysis, interpreted data, wrote manuscript and acted as corresponding author

I hereby certify that the statement of contribution is accurate

Signed *Date* *09/02/2011*

MCINTYRE. J

Contributed on manuscript outlines, provided feedback and edited manuscript

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the manuscript in the thesis

Signed *Date* *09/02/2011*

RICHARDS. L

Supervised study and provided manuscript evaluation

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the manuscript in the thesis

Signed *Date* *10/02/2011*

Author Covering letter to the Australian Dental Journal (1)

AUSTRALIAN DENTAL JOURNAL

In submitting the manuscript titled

DENTAL HARD TISSUES AND FACTORS WHICH INFLUENCE THEIR DEMINERALIZATION AND REMINERALIZATION: A REVIEW OF THE LITERATURE

to the *Australian Dental Journal* I/we declare that:

1. The results are original, not falsified or plagiarised form any source.

2. All people involved with this report and all grants and scholarships which supported this work are duly acknowledged.

3. Credit to authorship is only to those who have participated substantially in the research work and preparation of this manuscript.

4. This paper is not currently under consideration for publication elsewhere.

5. All financial and personal relationships which might bias the interpretation of the work described in this manuscript have been fully disclosed

My Anh Vu Thanh

Date 16/07/2011

MANUSCRIPT 1

Dental hard tissue and factors which influence their demineralization and remineralization: A review of the literature

My Anh Vu Thanh

John McIntyre

Lindsay Richards

School of Dentistry, The University of Adelaide, Adelaide, SA 5005 Australia

(Manuscript of a Review to be submitted to the Australian Dental Journal)

Abstract

This review summarises information about dental hard tissues, dentinal tubules and fluid, cariology and the factors that influence recurrent caries associated with indirect restorations. The understanding of how simulated dentinal fluid and a resin modified glass ionomer luting (RMGIC) cement affect the remineralization of demineralized dentine would provide clinicians with further information of how RMGIC luting cement works on vital prepared teeth for crowns.

Dental hard tissues

Studies on enamel and dentine have been carried out for more than 100 years and researchers now understand the caries mechanism more clearly. Enamel and dentine are highly mineralized tissues which can be described as carbonated hydroxyl-apatite but not pure hydroxyapatite (1). Normally, apatite has a high carbonate content and the natural tooth mineral may be represented by the following simplified formula; $Ca_{10}(PO_4)_6(OH)_2$ (2). The formulation of calcium apatite can also be illustrated as follows $\{Ca_{10-x}(Na)_x(PO_4)_{6-y}(CO_3)_z(OH_{2-u}(F)_u\}$ (3). From the Larsen and Featherstone formulas, the ratio between calcium and phosphorus varies from 1.6 to 2.2 depending on the amount of calcium and other elements in the formulation. The term Ca/P ratio refers to the ratio of Ca and P present in the tooth mineral. The P is presented at PO_4 ion and thus this term provides information on the likelihood of apatite or other calcium phosphate salts being present.

Studies on Ca/P ratios have been carried out since the 1960s. A study by Little et al. (4) demonstrated that the calcium and phosphorus concentrations by weight in normal dentine were 29.25% and 14.41% respectively and the Ca/P ratio was 2.03. According to Frazier (5), the Ca/P ratios measured by electron probe analysis showed a wide

variation from 1.42 to 2.27 (molar ratio). The excess of phosphorus leads to a low Ca/P ratio and vice versa. Derise et al. (6) compared two methods of analysis of mineral content and found that calcium, phosphorus, chlorine and potassium concentrations were greater in enamel than in dentine, but magnesium was greater in dentine. In enamel, concentrations of Ca and P by weight were 37.1% and 18.1% and in dentine were 26.7% and 13.6% respectively. The differences in Ca and P concentrations in dentine were influenced by age and sex.

According to a review by Russell et al. (7), the substitution of ions in apatite could occur either within the lattice or on the surface. Calcium deficiency is a product of the substitution process. Instead of substitution of $2H^+$ for one Ca^{2+}, one H^+ was added and one OH^- was deleted to produce mineral having the following formula $Ca_{10-x}H_x(PO_4)_6OH_{2-x}$, giving a molar Ca/P ratio below the theoretical value of 1.67. Moreno and Aoba (8) described the concentrations of Ca and P by weight percent as being 35.6±0.5% and 17.2 ±0.6% in enamel; 26.4±0.7% and 12.7± 0.3% in dentine. The Ca and P in hydroxyapatite (HA) in dentine is 38.3±0.2% and 18.9±0.7%, respectively and the Ca/P ratio was 1.65±0.02. The Ca/P ratio influences the hardness of enamel and dentine which will be discussed later.

Enamel

Composition

Enamel is a highly mineralized crystalline structure, containing 96% mineral, 1% protein/ lipid and 3% water by weight (2, 9).

The structure of enamel is a result of the combination of organic and inorganic components. Crystalline enamel is clustered into enamel rods which are about 4-5μ in diameter near the dentinal border, and about 8μ on the surface. The crystallites are

irregular in diameter and shape and formed by thousands of unit cells that have a highly ordered atomic arrangement. Filling the spaces between crystallites are protein, lipid and water which occupy about 13% by volume. The tiny spaces between crystals allow ions and other small molecules to diffuse in and out of the tooth. Taking advantage of this process, some studies attempted to slow down the stream of minerals coming out from the tooth surface or stimulated minerals from the oral environment coming into enamel (9).

Mineralization and solubility of enamel

Minerals are transported through small crystallite spaces in enamel because of its permeability and react with the structural proteins to form enamel crystallites. This process is termed mineralization. Enamel is quite highly mineralized but further calcium and phosphate continue to deposit in crystal defects after tooth eruption (10). Normal saliva is saturated with calcium, phosphate and fluoride ions. The substitution of carbonate by phosphate occurs progressively to form hydroxyl-apatite $Ca_{10}(PO_4)_6(OH)_2$ which is less soluble than carbonated apatite. The presence of elevated levels of fluoride ion in saliva results in replacement of some hydroxyl groups to form fluoridated or fluoride enriched apatite which is the least soluble. The permeability of enamel decreases with age and this is referred to as enamel maturation. However, ionic exchange of calcium, phosphate and fluoride between enamel and saliva (both inward and outward) occurs throughout the life of the tooth (10).

Dentine

Dentine is less highly mineralized and brittle than enamel and it is considered as supporting overlying enamel. Dentine contains 70% mineral, 20% protein/lipid and

10% water by weight (11). Hydroxyapatite crystallites are the primary constituent of dentine mineral. The crystallites of dentine are smaller and less systematically arranged than those of enamel.

The structure of dentine is different from that of enamel. Dentinal tubules extend from the pulp to the dentino-enamel junction. Lining the tubules is the peritubular dentine which is highly mineralized compared to the surrounding intertubular dentine. The odontoblast process extends from the outer surface of the pulp to at least one third of the length of the dentinal tubules (12-15). The initial shape of the tooth is formed by primary dentine. Dentine deposits incrementally until the external form of the tooth is completed. In a permanent tooth, the formation of the primary dentine is completed about three years after tooth eruption. The secondary dentine deposition continues from when the crown of the tooth is formed until the root formation is completed though at a slow rate. The secondary dentine is less mineralized than primary dentine. Tertiary dentine or reparative dentine is formed in response to caries or injury though is different in structure and content from secondary dentine. Mineral content in dentine increases with age resulting in the dentine becoming harder.

Mineralization of dentine occurs by globular calcification and depends on the rate of dentine formation. A review by Linde (16) pointed out that collagen by itself is not sufficient to influence mineralization *in vivo* and that the collagen matrix merely plays a role in the orientation and stable support for mineral crystals and non-collagenous proteins. Using $^{45}Ca^{2+}$, the transportation time of calcium from blood to the dentine mineral was shown to be from 10 to 15 minutes. However, little is known about the role of odontoblasts in calcium transportation.

Dentinal tubules and dentinal fluid

The odontoblast and its function

Some early reports proposed that the odontoblast process was limited in location to the inner one third of the dentine (12, 13). However, in other studies, the odontoblast process was demonstrated to extend throughout the whole length of the dentinal tubules to the dentino-enamel junction in sound teeth (14, 15, 17). In carious teeth, the odondoblasts extended to the inner layer of carious dentine (15).

The forming dentinal tubules are non-calcified tubules which extend peripherally from the dentino-enamel junction (DEJ) to the outer surface of the pulp chamber. The length of the dentinal tubule is about 5 mm. The tubule is tapered with a diameter of 2.9±0.22μ in the deeper layer and 2.65±0.19μ in the middle layer. The distribution of dentinal tubules in the deep layer is 21,343±7290 per mm^2 and the middle layer is about 18,781±5855 per mm^2 (18). The central part of dentine has more dentinal tubules than the pulp horn. The distance between the tubules is about 15 microns at the DEJ and 6 microns at the pulp (19, 20). The differences in number and diameter of dentinal tubules lead to the development of some rules which need to be followed when preparing samples for this study because the increased dentinal tubule diameter is responsible for an exponential increase in dentine permeability.

The permeability of dentine has been studied for more than 50 years and dentine permeability depends on the following several aspects:

Diffusion and filtration

The fluid in dentine tubules is termed dentinal fluid. The tubules are anatomical pathways available for the passage of solution either by flow or diffusion. Using a

simple method to study diffusion, Blake (21) demonstrated that mercuric chloride penetrated evenly throughout the dentine and filled the dentinal tubules and lateral branches completely. In 1962, Anderson and Ronning demonstrated diffusion of cresyl blue and methylene blue either toward the pulp or from the pulp (22). This study showed that molecular weight seemed not to be the main factor influencing the diffusion of dyes. In another study of dentinal fluid movement, Anderson et al. (23) determined the maximum flow rate obtained when calcium chloride solution diffused though dentine under normal hydrostatic pressure to be 4.8 nl/sec.

Dentine is a porous structure and the permeation of dentine also obeys filtration laws which are entirely different from those of diffusion. The flow rate in a cylindrical tube obeys Poiseuille's Law. However, according to Pashley (1985), the filtration of fluid across dentine (outward flux) obeys Poiseuille Hagen's equation which expresses the flow rate of the fluid through dentine (24).

$$J_V = \frac{\pi r^4 \Delta P}{8 L \eta}$$

In the equation, J_V= volume flow, µl min^{-1}

ΔP= hydrostatic pressure difference across dentine

η= viscosity

L= length of tubules

r= radius of tubule

Volume flow or flux flow will be proportional to the hydrostatic pressure of the dentinal fluid and the diameter of the tubules, but inversely proportional to the length

of the tubules (or dentine thickness) and the viscosity of the dentinal fluid. The driving force is pressure gradient (dP/dx =gradient pressure over a distance)

Diffusion obeys Fick's Law (1855) and the driving force is the concentration gradient (dc/dx= gradient concentration over a distance). The rate of diffusion was determined as follows (25):

$$J = DA \frac{dc}{dx}$$

Where J= solute flux

D= diffusion coefficient

A= diffusion surface area

$\frac{dc}{dx}$ = change in concentration over a distance, x

This equation showed the relationship between the flux and the change in concentration over the distance. A solute diffuses across dentine down a concentration gradient. The outward flux is also influenced by the distance from the pulp to the demineralized lesion. Some other factors also would influence the flux such as the diameter of the dentinal tubules and the deposition of minerals into the tubules (26).

The comparison of diffusion and filtration in a study by Merchant et al. (27) showed that acid etching facilitated filtration more than diffusion. According to Shellis (28), if the outer ends of the dentine tubules were occluded, diffusion might be the principal transport mechanism *in vivo* . When the tubules are open, the dentinal fluid will be slowly filtered through the tubules into the mouth (29).

Biological aspects: Blake (21) and (22) demonstrated an osmotic pressure experiment across sound enamel either from the pulp and dentine to the mouth or from the mouth through enamel to dentine, by various solutes. An experiment by Narhi (30) demonstrated that the mechanoreceptors in the pulp respond to the pressure gradient regardless of their orientation.

However, the tooth is a biological tissue which acts as a semi-permeable membrane (21) so the diffusion is generally controlled, which is different from diffusion within soil or in other materials.

Anatomical aspects: The number of tubules and the diameter of tubules influence dentine permeability. The inner dentine is expected to be more permeable than the outer dentine (31). This can be explained by the fact that tubular density is greater in the inner dentine and the diameter of dentinal tubules is larger in the inner dentine than in the outer dentine. Thick dentine is less permeable than thin dentine. According to Pashley (24), 1mm of dentine can diminish the concentration of an applied dental material between 100 to 1000 fold. Dentine near a pulp horn is more permeable than dentine in other sites. Moreover, any changes in structure within dentinal tubules will influence the permeability of dentine.

However, a study by Ozok et al. (32) which compared the mineral loss and lesion depth of low and high permeability samples on acidic challenge, found no difference in mineral loss and demineralized lesion depth.

Some pathological factors also influence dentine permeability. The dentine in caries lesions or under wear facets is less permeable than freshly cut surface dentine. The presence or absence of a smear layer also influences dentine permeability. Pashley et al. (33) showed that, dentine permeability increased 5 to 10 fold if the smear layer was

removed. This can be explained by the changes in structure of the tooth that results from the closure of the physiological channels in order to prevent the onset of caries, or the active establishment of barriers to the diffusion of harmful substances (21).

Hydraulic conductance of dentine tubules

The hydrodynamics of the dental tubules and of the pulp fluid was demonstrated in a number of studies (28, 34-37). The hydraulic conductance of the dentine tubules causes the movement of the fluid in the dentine tubules and this depends on several aspects. Changing temperature, air-blasting, removing parts of the contents of dentine and losing water in dentine, all increase the outward movement of the dentinal fluid (35). The maximum flow of fluid movement described in a study by Anderson et al. (23) was 4.8nl/s (per tubule) and the rate declined during the experiment. In some latter studies, the fluid flow rate at the outer end of the dentine tubules was 1.4 ±1.2 nl/s (26).

Vongsavan demonstrated that the hydrostatic pressure of cat dentine *in vivo* was 1.47kPa or 15cm of H_20 (37). Ciucchi et al. (38) demonstrated the pulpal tissue pressure in human teeth *invivo* was 14.1 cm H_2O in five teeth which was lower than pulpal pressure in cat. In another *in vitro* study, Shellis demonstrated that differences in the hydrostatic pressure of the reservoir did not show any influence on the formation of a caries-like lesion. However, the integrity of the odontoblast process may reduce the cross-sectional area of the dentine tubule and hence reduce the hydrophilic conductance of the dentine tubules (28). Shellis's model proved that simulated dentinal fluid penetrated about 1.5mm into dentine within two hours and through the full thickness within 20-24 hours.

Dentinal fluid composition

The composition of dentinal fluid has been studied since the 1970s. Dentinal fluid contains minerals, some albumin, and a wide variety of electrolytes. Coffey et al. (39) demonstrated the presence of potassium, sodium and chlorine in dentinal fluid. In a study by Larmas (40) calcium concentrations of 11 to 50 mmol/l, phosphate of 24-40 mmol/l, magnesium of 6-9 mmol/l and sodium of 105-153 mmol/l were reported. According to Larmas, dentinal fluid can also contain some dissolved products from the caries process. The calcium, phosphate and sodium concentrations of dentinal fluid in caries lesions were greater than those in sound dentine (40-42) demonstrated the presence of plasma protein in dentinal fluid from prepared cavities in young human teeth. The plasma protein complexes may be carried into the tubules with the outward fluid movement following injury (cavity preparation). Fibrinogen will deposit on the uncovered dentine as a protective response (37, 43, 44). The protein flux flows across three barriers: pulpal blood vessel, odontoblast and dentine. In the study of Knutsson et al. (42), albumin and IgA were found in all samples while fibrinogen was found occasionally in some samples. The fibrinogen level in dentinal fluid is about one fifteen times of albumin, and one ten to one fifteen of fibrinogen in human plasma.

A number of studies were carried out to investigate the role of dentinal fluid in demineralization and remineralization. Shellis (28) demonstrated the effect of saturated dentinal fluid with respect to the apatite which has a calcium concentration of about 1.1mM/L. The contents of Shellis' surrogate dentinal fluid per litre were:

- 7.149g HEPES Buffer
- 1.1ml 1.0mol/l H_3PO_4 stock solution
- 0.052g CaO, 0.024g MgO

- 4.54g NaCl

Shellis developed a model to test the effect of perfused surrogate dentinal fluid on the rate of demineralization of tooth root when exposed to an artificial demineralizing solution. Using the surrogate dentinal fluid, the depth of the caries-like lesion on root surfaces was consistently reduced with an artificial caries challenge. The supply of mineral ions in the dentinal fluid and buffers were considered to be responsible for the reduction of caries-like lesions.

Ozok et al. (45) also found that the dentinal fluid flow offered some protective effect against demineralization. This model differed from that of Shellis in that perfused surrogate dentinal fluid was diffused through a canula from the lingual side of the tooth and he looked at demineralization on the buccal surface. It was demonstrated that mineral loss was greater in the non-perfused samples than in the perfused samples. The lesion depth also was reduced. It was explained that the outward dentinal fluid flow decreased the mineral dissolution process. In another study (32), the lesion depth and mineral loss (by vol %) were greater in the samples perfused with water than those perfused with surrogate solution.

Dental caries

Definition of dental caries

Dental caries is defined as dissolution of tooth minerals due to the acids resulting from carbohydrate fermentation by bacteria. It may progress from micro to macro levels, from dissolution of minerals to degradation of the organic matrix. The chronic process usually progresses very slowly from loss of mineral at the microscopic level to total toothdestruction. The earliest carious lesion which can be seen in enamel is called a

'white spot' lesion (46). The final result of caries progress depends on the dynamic balance between demineralization and remineralization. If the lesion is detected early enough, the disease is reversible (3).

The demineralization and remineralization processes

In the past, dental caries was thought to be the result of a one-way progressive demineralization process (46). However, the recognition that caries has a multi-factorial aetiology and a dynamic nature improved our understanding of this disease. It is now referred to as the result of a prolonged imbalance of demineralization over remineralization. Demineralization and remineralization are ionic exchange processes that are affected by the pH of saliva among other factors. As described previously, the basic mineral component of mature enamel, dentine and cementum is hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. In the oral environment, when the pH of saliva drops below 5.5, the dissolution of the hydroxyapatite occurs from enamel. This process is called demineralization. When the pH is neutral and there are sufficient Ca^{2+} and PO_4^{3-} ions in the oral environment, the demineralization process can be reversed and remineralization occurs (47). The caries process is halted or reversed depending on the dynamic balance of demineralization and remineralization. Understanding this process provides the key to the effective investigation of dental caries. The process has been described as a delicate balance between protective factors (saliva, calcium, phosphate, fluoride) and pathological factors (bacteria and carbohydrates). This balance is easily tipped several times each day in a healthy mouth of most people from demineralization to remineralization and vice versa (48). After an initial lesion which can be seen only by the microscope is formed, a carious lesion often progresses slowly. The early enamel lesions can be reversed or arrested. However, when a cavity is

formed, the lesion cannot be reversed but still can be arrested (9).These concepts have been applied in the prevention of dental caries and are the basis of the “minimal intervention” principles for the treatment of dental caries.

Historically, an artificial demineralized lesion was first developed in 1904 and 1905 by Miller (49). Francis used different methods to quantify the carious lesion. Fluorescence, transmitted light, silver staining and micro-radiography were used. In 1972, when Fusayama studied the structure of artificially decalcified dentine, he found stainable and un-stainable layers by using fuchsin staining techniques (50, 51).

Factors influencing demineralization and remineralization

Based on the above understanding, many studies have been carried out to investigate the role of external protective factors such as the quality and quantity of saliva (flow rate, pH, protein, ions Ca, P, F) (52). Bacteria and carbohydrate types have also been shown to influence both demineralization and remineralization (53, 54). The use of mouth-rinses, dentifrices and water fluoridation influence caries control (55, 56); (57); (58, 59). A low flow rate of saliva, lack of protein to form pellicle, low pH and lack of Ca, PO_4 and F in saliva contribute to demineralization. If the saliva flow rate is enhanced by chewing gum and/or fluoride is supplied by mouth rinse or tooth-pastes, the demineralization process can be reversed (60). An increased focus of investigation currently is on the internal protective factors within tooth and dentinal tubular fluid that play a part in halting or reversing dental caries (32). The role of dentinal fluid in supplying nutrients and minerals to dentine and enamel is well understood while its role in delivering minerals to the site needing remineralization is relatively un-investigated.

Structure of carious dentine and its remineralization

When caries invades dentine, there will be some changes in its physiology, biology and chemistry. Physiologically, the color of dentine may change from light brown to dark brown; the micro-hardness also changes with softening occurring (61, 62). Biologically, the invasion of the bacteria into dentinal tubules occurs causing some changes in the dentinal tubules and odontoblasts (15, 63). Chemically, there are some changes in the contents of the carious dentine such as pH and variation in the content of the dentinal fluid and the hydration of carious dentine (4, 64, 65). Physically, dentinal tubules in the transparent layer are occluded with new crystals such as whitelockite, which has a lower hardness and calcium content compared to that of apatites (66).

Carious dentine contains two discernible layers which have been demonstrated in a number of studies (61) (51, 63, 67). In a study in 1972, using a variety of dyes, Fusayama and Terachima differentiated these two layers (51) which can be clearly distinguished both in artificially demineralized and carious dentine (62). According to Fusayama, a basic fuchsin propylene glycol solution can stain two layers of carious dentine distinctly differently, the outer layer was stainable and the inner layer was not (51).

The outer layer or stainable layer is often totally demineralized and according to Fusayama, it cannot be remineralized. The color of this layer is changed to dark brown, brown, brown yellow, yellow or light yellow. The color is lighter in acute caries and darker in chronic caries. Using the fuchsin technique, the staining was clear in acute caries and faint in chronic caries (63). Under the electron microscope, the leaf-like crystals of apatite were scattered irregularly. The peritubular dentine and the odontoblasts disappeared and the tubules were filled with bacteria (63). As a result

this layer was called infected dentine. In this layer the intertubular dentine was decalcified and the micro-hardness decreased and was softer than the inner layer (61). Yamada also found that the odontoblast process had collapsed and disappeared at the border between the inner and outer layer and there was no odontoblast process observed in the dentinal tubules of the outer layer of carious dentine (15).

The inner or un-stainable layer is not infected and consequently was described as "affected" dentine. This layer is partly demineralized but still vital and can be remineralized to the level of normal dentine in living teeth if there are sufficient time and protection factors (50, 62). The remineralization occurs even in devitalized teeth (66). Miyauchi first confirmed the remineralization of the inner carious dentine (68). Fusayama also found that the calcium content and the hardness in the inner layer of carious dentine increased markedly and even reached the normal level (62).

Under the electron microscope, the inorganic substance of peritubular dentine is homogeneous. The needlelike crystals were shorter than those in normal dentine because of its dissolution. Odontoblast processes were found to extend to the inner carious dentine as defined by TEM (15, 63). The Scan Electron Microscopy (SEM) images showed that the odontoblast processes extend continuously to the boundary between the inner and outer layer, which confirmed the observation by Ohgushi. The odontoblast processes regained their smooth surface so there was no hole or depression seen on the odontoblast process in this layer (15). The intertubular dentine was not completely digested (62).

The transparent and sub-transparent zones were recognized and viewed under electron microscopy. These two layers are not stainable. Some researchers claimed the transparency of this layer to be the results of deposition of the crystal minerals and

believed that it increased in hardness. However, according to Ogawa et al. the hardness of the inner carious dentine increased towards the subtransparent layer and normal dentine. The transparent layer was a soft part of carious dentine and not the hardest layer (69). The odontoblast processes in the sub-transparent layer had minute holes and depressions. The odontoblast processes in the transparent layer were observed to be rougher and the holes and depressions increased in size (15). The dentinal tubule structure was apparent in this layer and more collagen remained surrounding the process. The dentine tubules showed no change in the translucent band in some samples and were partly occluded in the other samples but not every tubule was affected (28).

According to Ogawa et al. (69) the hardness decreased gradually through sub-transparent and transparent layers. The transparent layer was much softer than the normal dentine despite the mineral deposition in the dentine tubules. This study confirmed that the transparent layer is not the hardest layer as was stated in some other studies. The dissolution of the peritubular and intertubular dentine in the sub-transparent and transparent layers was quite apparent in this study. The deposition of apatite inside the peritubular dentine subjacent to the caries lesion was considered as a vital reaction. Therefore, the transparent layer is not a sclerotic layer.

There have been some conflicting arguments on the transparent layer in respect to its ability to be remineralized. The study of Yamada et al. in 1983 focused on whether it is possible for physiological remineralization through the transparent layer (15). The odontoblast was considered to be responsible for this vital reaction by the transportation of ions in remineralization. In 1986, Larmas found that the sclerotic dentine is a hypermineralized layer and the electron microscope showed a marked

increase in peritubular dentine (70). A study of Kinney et al. (71) was different from previous studies in terms of measuring the hardness of peritubular and intertubular dentine separately which enabled a determination of dentine constituents of peritubular dentine and intertubular dentine matrix. The results showed greater nano-hardness of the intertubular dentine near the dentino enamel junction than near the pulp. The hardness of the peritubular dentine was four times more than that of the intertubular dentine regardless of the site (72). The decrease in micro-hardness proximal to the pulp was explained in this study by the decrease in micro-hardness of the intertubular dentine (72) but not by the increase in number of the dentine tubules as previously suggested (15, 24). Later studies confirmed that the nano-hardness of the peritubular dentine in the transparent layer was significantly less than that of normal dentine (65, 73).

Marginal fit and secondary caries

Marginal integrity and durable adhesion are the two most important factors in preventing secondary caries developing under crowns (74). A study by Kidd et al. (1995) showed that wide ditched margins harboured significantly more bacteria than did clinically intact margins and margins with narrow ditches (75, 76).

Luting cements are used to fill the space between the prepared tooth surface and internal surface of crown and secure the crown in place by flowing into the irregular surfaces of the tooth and crown. According to Ayad, (77) adhesive resin cement showed the lowest discrepancy value which was 19±17μm when tooth preparation was refined with a finishing bur.

Resin modified glass ionomer cements are thought to have a combination of the good properties of both resin and glass ionomer cements. Fluoride release is important in

inhibiting secondary caries especially in patients who are at high caries risk (78). Studies (74, 78) demonstrated that whether the demineralization was controlled depends on the fluoride release ability of the luting cements.

Luting cements

Cementation is critical to the success of fixed prostheses. Dental cements are used to establish a barrier to prevent microbial leakage, sealing the interface between the internal surface of the crown and the surface of the tooth and holding them together. An ideal luting cement should have the following characteristics:

Biologic properties

- Biocompatible: Dental luting cement should be biocompatible, causing little or no interaction with tooth structure, be non toxic and low allergic potential.
- Caries and plaque inhibition: Dental luting cements should prevent demineralization at the tooth luting cement interface. It has been shown that fluoride release from GIC luting cement enhances remineralization around a crown (79).
- Microleakage: Dental luting cements should provide a resistant to microleakage as microleakage may cause pulpal irritation and hence reduced restoration longevity (80-82). Resin cements have became popular because of their resistance to microleakage *in vitro* and *in vivo* testing (83-85).

Mechanical properties

- Provide a durable bond. Resin cements shows the highest compressive strength followed by glass ionomer cements and resin modified glass ionomer cements (86). The unfilled resin and traditional cements exhibit lower values in comparison to

resin cements (86, 87). Luting cement should also be able to wet the prepared surface of the tooth and the internal surface of the crown to provide a good bond.

- Low solubility and water sorption: luting cement should be resistant to moisture and less susceptible to water resorption (88, 89). A crown preparation carried out on a vital tooth need to take the dentinal fluid flow into consideration. According to Ozok (90), dentinal fluid flow may have an adverse effect on the adhesion of dental adhesive. However, it depends on the type of the dental adhesive was used. This has an implication on the cementation procedure where the total etch technique is applied.
- Exhibit adequate film thickness and viscosity; have adequate working and setting time to ensure complete seating. Rely X® and MaxCem®have been shown to meet the standard requirements (25μm) and Fuji Cem has film thickness of 28μm with three minutes working time. This allows sufficient time for seating a single unit crown (88)

Summary of literature review

This session has addressed the knowledge related to the understanding of the nature, structure and concentrations of calcium apatite in enamel and dentine. This knowledge helps in the development of methods to evaluate demineralization and remineralization of artificial caries. The factors affecting remineralization of remnant demineralized dentine could be either external sources such as fluoride (91, 92) and glass ionomer cements (93) or internal source (28); (32). However, the combination of both external and internal factors has not been investigated. The knowledge of the effect of simulated dentinal fluid and Fuji Cem luting cement on the remineralization of demineralized dentine would provide the clinicians with some understanding of how

Fuji Cem luting cement works on vital prepared teeth for crowns in helping to arrest and remineralize any remnant or recurrent dentinal caries.

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Materials and methods used in this study

The method to be used is as follows:

Twelve sound teeth were selected for the experiment. These teeth were collected under the conditions laid down by the Committee for the Ethics of Human Experimentation, Adelaide University. The teeth were cleaned to remove the remaining soft and hard tissue and stored in Thymol 0.2% solution in the refrigerator until being used.

This small number were used as the technique for delivery of SDTF to demineralized dentine is very difficult and time consuming, and the time available for the research project did not permit more samples to be examined at this time. The teeth were prepared as for placement of a porcelain bonded to metal crown (PBM). Each prepared specimen was submerged in a container containing 40ml of demineralizing solution at 37^0C, four teeth each group. The demineralizing solution (ten Cate and Duijsters 1982)contained 2.2mmol $CaHPO_4$ (0.2993g), 2.85ml of concentrated glacial acetic acid in one litre of solution adjusted to pH 4.3 using 10% NaOH. Thymol crystals were used to make a 0.2% concentration to prevent growth of mould. Three groups were assigned to experience one, two or three weeks of demineralization.

Half of the prepared demineralized dentine was painted with nail varnish, which would act as a control side. The other half thus became the test side, where both the cement and SDTF were to be in contact with the demineralized dentine. The roots then were sectioned 1mm below the cemento-enamel junction (CEJ). Honigum® light and mono

(DMG, Germany) impression materials were used to take impressions to construct laboratory processed resin crowns. (Sinfony, 3MESPE) and the crowns were cemented using Fuji Cem (GC America Inc). After cementation, the specimens then were connected to a reservoir under a hydrostatic pressure of 15cm above the pulp chambers. The reservoir supplied Simulated Dentine Tubule Fluid (SDTF) to simulate hydrostatic pressure in dentinal tubules for six weeks. This perfusion system enables the ionic exchange process between luting cements and dentine. The crowns were submerged in remineralizing solution consisting of 10ppm fluoride ion (as NaF), 1.5mM calcium, 0.9mM phosphate and 20mM HEPES Buffer at pH 7.0, at 37^0C (Arendset al. 1989).

The crowns were detached, sectioned and polished and carbon coated for Electron Probe Micro Analysis (EPMA). In this study, the EPMA data was acquired using a Cameca SX51 Electron Microprobe with wavelength dispersive spectrometer (WDS). The analysis was carried out across the samples' surfaces. The first spot was selected just beyond the demineralized dentine so that the element values could start from zero. The iDFIX program was used to determine point zero if it would be necessary. An interval of 5μ was set between points and 10μ between lines which allowed an analysis of two distinct points. 71 points of each line, three lines on the test and three on the control side of the lesions were analyzed. On the test side, the analysis was made 100 microns within the luting material to search for migrated elements. The calibration was made by comparing the peak and background intensity with Astimex 13($SrSO_4$), Fluorite and Camesa Apatite Standard ($CaPO_4$).

The relative weight percent of Ca, Sr, P and F in dentine on both test and control sides were determined.Areas under the mineral profile curves were charted against the

lesion depths.One way ANOVA test was used to test the differences between these area under the curve readings between each half of the samples. Images of the bonding interface between luting cement and dentine were observed and compared between the test and the control side.

The reason that Sinfony crowns and Fuji Cem cement were used as follow:

- Sinfony crowns can be used as provisional restorations in some full mouth rehabilitation cases.
- Sinfony crowns are cemented in Fuji Cemwhich is resin modified glass ionomer luting cement. The diffusion of Sr from Fuji IX (GC, Japan) was demonstrated in previous studies (Ngo 2005, Vu 2008). Fuji Cem has Sr in its content which can be exchanged with calcium from the dentinal fluid. The ion exchange enables a comparison of mineral levels in tooth structure as well as in luting material hence, the assessment of increased mineral levels could be made.

Fluid perfusion method:

The method to deliver the fluid into the pulp chambers of tooth crowns was described in a previous study (Vu 2008). Perspex containers were manufactured with floor dimensions of 3 x 4.5 cm^2 and 3 mm thick with Perspex bonded sides and having a depth of 1.5 cm. Six holes with the diameter of 1.1 mm were drilled through the base, the holes were 1.5cm from each other. Six stainless steel tubes 1.1 mm in external diameter, 0.9mm internal diameter and 12 mm in length were inserted through the holes of the Perspex base. A length of 1.5mm of the tubing was projected into the container designed to be placed within the pulp chamber of a sectioned tooth crown. Sticky wax was used to seal the tubing to the Perspex base which prevented no leaking

during the experiments. Figure 1 illustrates the chamber with a piece of the stainless steel tubing ready to be placed in the holes, and a gate enabling silicone tubing to be attached to each piece of stainless steel tubing in the chamber.

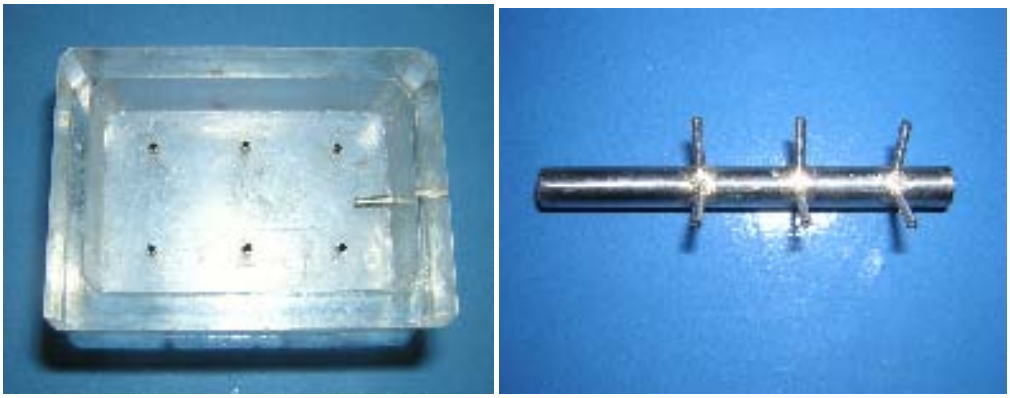

Figure 1: The manufactured container and gates

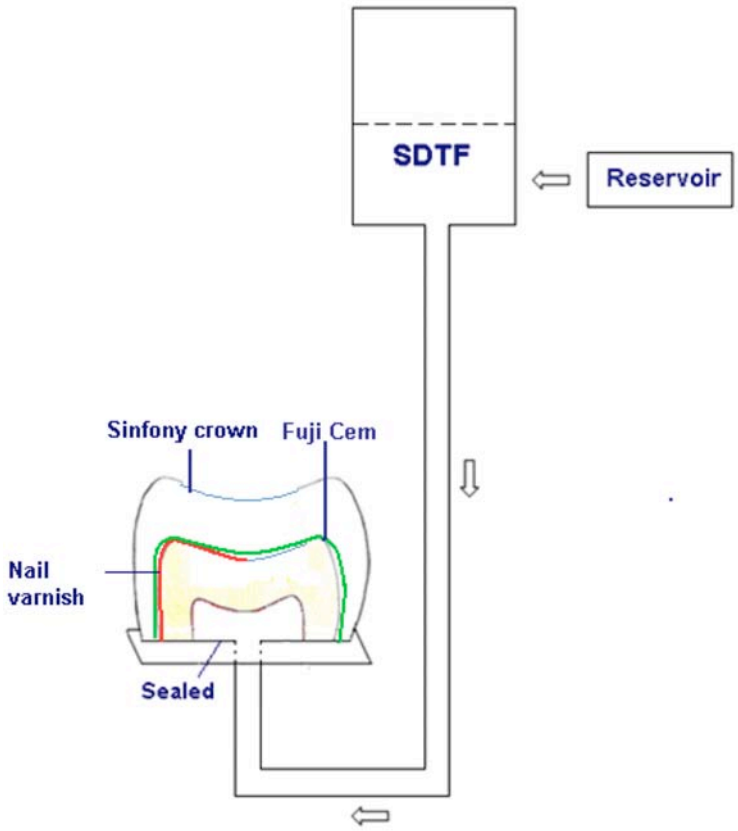

Figure 2: Perfusion system to supply the SDTF to the pulp chamber of the crown

A reservoir of perfusing solution with silicone tubing attached through the lid of a sealed bottle was elevated to a prescribed height above the pulp chamber (As illustrated in Figure 2). This enabled the fluid to enter the chamber through the stainless steel tubing under hydrostatic pressure, and thus into the pulp chamber of a tooth crown which had been placed over each piece of steel tubing and sealed to the chamber base. The intention was that the reservoir fluid would then flow into the dentine tubules.

Preparation of Simulated Dentinal Tubule Fluid (SDTF)

Human dentine tubular fluid contains several electrolytes such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, P^{2-}, CO_3^{2-}, proteins, mucoproteins and some immuno-globulins (Larmas 1986). The simulated dentinal tubular fluid was developed in a previous study (Vu 2008) having representative concentrations of Ca and PO_4 ions as present in dentinal tubular fluid, which permitted selective analysis of the role of calcium on the remineralization of remnant demineralized dentine.

The SDTF formula selected contained

- 7.149g HEPES buffer
- 1.1ml of 1 M H_3PO_4
- 0.123g CaO or 0.163g $Ca(OH)_2$
- 1.54g NaCl
- 1g Thymol per one litre with the pH adjusted to 7.

Large quantities of SDTF were prepared to ensure the same batch was used for each experiment, and stored at $4^{o}C$.

ORIGINAL STUDY

A PILOT IN VITRO STUDY TO INVESTIGATE THE INFLUENCE OF DENTINAL FLUID ON REMINERALIZATION OF DEMINERALIZED DENTINE UNDER CROWNS

My Anh Vu Thanh

John McIntyre

Lindsay Richards

School of Dentistry, The University of Adelaide, Adelaide, SA 5005 Australia

STATEMENT OF AUTHORSHIP

A PILOT IN VITRO STUDY TO INVESTIGATE THE INFLUENCE OF DENTINAL FLUID ON REMINERALIZATION OF DEMINERALIZED DENTINE UNDER CROWNS

VU THANH. MA (Candidate)

Carried out experiment, performed sample analysis, interpreted data, wrote manuscript and acted as corresponding author

I hereby certify that the statement of contribution is accurate

Signed *Date* *09/02/2011*

MCINTYRE. J

Contributed on manuscript outlines, provided feedback and edited manuscript

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the manuscript in the thesis

Signed *Date* *09/02/2011*

RICHARDS. L

Supervised study and provided manuscript evaluation

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the manuscript in the thesis

Signed *Date* *10/02/2011*

Author Covering letter to the Australian Dental Journal (2)

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to the *Australian Dental Journal* I/we declare that:

1. The results are original, not falsified or plagiarised form any source.

2. All people involved with this report and all grants and scholarships which supported this work are duly acknowledged.

3. Credit to authorship is only to those who have participated substantially in the research work and preparation of this manuscript.

4. This paper is not currently under consideration for publication elsewhere.

5. All financial and personal relationships which might bias the interpretation of the work described in this manuscript have been fully disclosed

My Anh Vu Thanh

Date 16/07/2011

MANUSCRIPT 2

A pilot *in vitro* study to investigate the influence of dentinal fluid on remineralization of demineralized dentine under crowns

My Anh Vu Thanh

John McIntyre

Lindsay Richards

School of Dentistry, The University of Adelaide, Adelaide, SA 5005 Australia

(Manuscript of a Review to be submitted to the Australian Dental Journal)

Abstract

Introduction: Recurrent caries is one of the factors leading to failure of crowns in prosthodontics. A previous study showed that dentinal fluid could be delivered under a certain hydrostatic pressure into demineralized dentine. However, how dentinal fluid influences recurrent caries needs to be further investigated. Also the study investigated whether a glass Ionomer based cementing agent would contribute to any remineralization in terms of ionic diffusion.

Materials and methods: Twelve extracted sound third molar teeth were selected and had crown preparations carried out which would be required for PBM crowns. The cut dentine surfaces on each sample of four teeth in each group were then artificially demineralized to simulate the presence of remnant dentinal caries. Half of the exposed dentine was covered by varnish, creating a control section where there was no direct contact between the cement and the demineralized dentine, but it was exposed to simulated dentinal tubule fluid (SDTF). The other half thus became the test side with direct contact of the demineralized dentine with SDTF and the cement. Sinfony crowns were made and cemented on to prepared teeth using Fuji Cem. The crowns were connected to a reservoir supplying SDTF for six weeks then removed from the system and prepared for Electron Probe Micro Analysis (EPMA). EPMA provided profiles of element concentrations across the demineralized dentine from control and test sections of the prepared dentinal surface exposed to SDTF and the luting cement. From this data area under the profile curves in both test and control sectors of the same tooth could be calculated and compared. Statistical analysis of differences was estimated using a one way ANOVA analysis.

Result: There were increased strontium and fluorine levels on the test sides compare with the control sides. Calcium was present in the luting cement which could be considered as evidence of the perfusion of selected ions from dentinal fluid into the luting cement.

Conclusion: This *in vitro* study showed that there was an uptake of strontium into the demineralized dentine. There was an ion exchange between dentinal fluid and the luting cement with the evidence of calcium presented in Fuji Cem luting cement on the test sides. These molecules are known to enhance remineralization of demineralized dentine, and thus to prevent reactivation of remnant caries.

Introduction

Recurrent caries is one of the factors leading to crown and bridge failure in prosthodontics. Generally it not only necessitates the removal of the infected dentine but also leads to the replacement of crowns or bridges, or endodontic treatment in more severe cases.

Crown preparation requires removal of enamel and dentine. A crown preparation of a molar tooth can expose up to two million dentine tubules leading to sensitivity and increased dentine permeability (1) which may result in later micoleakage (2). Leakage is considered as a contributor to the development of recurrent caries (3). Pashley (4) found greater potential for microleakage in teeth which have more dentine exposed during tooth preparation. Cementation of the crown was intended to seal these routes hence decreases dentine permeability as well as sensitivity.

In crown preparation, the intention is that the infected dentine will be removed and it is possible that some affected dentine will be retained. It is expected that minerals from a GIC restoration will be taken up into the dentine resulting in remineralization as was demonstrated in previous studies (5, 6). The dentinal fluid from sound dentine plays a role in the remineralization process, which was also demonstrated in previous studies (6). However, it has not been demonstrated that remineralization of any remnant demineralized dentine under crowns is achievable using a GIC based luting cement in conjunction with the presence of diffused SDTF.

Objective

To investigate the effect of Simulated Dentine Tubular Fluid (SDTF) and Fuji Cem (GC America Inc) cement on remineralization of artificially demineralized dentine under crowns.

Materials and methods

Twelve sound teeth were selected for the experiment. These teeth were collected under the conditions laid down by the Committee for the Ethics of Human Experimentation, Adelaide University. The teeth were cleaned to remove the remaining soft and hard tissue and stored in Thymol 0.2% solution in the refrigerator until being used.

This small number were used as the technique for delivery of SDTF to demineralized dentine is very difficult and time consuming, and the time available for the research project did not permit more samples to be examined at this time. Teeth were embedded into dental plaster then primary impressions were taken. The Crown preparation was carried out as for Porcelain Bonded to Metal (PBM) crowns (Fig 1). Secondary impressions were taken using Honigum® light and mono (DMG, Germany) impression materials and poured up for the master casts. Vacuum formed moulds (Erkodur C ERKODENT Erich Kopp GmbH) and special trays (Light Tray, Ivoclar Vivadent AG) were made.

The teeth were removed from the plaster mould. The roots were painted with nail varnish (Fig 2). Each tooth then was placed in a plastic container containing 40ml of demineralizing solution (7) and the containers were placed in an incubator at 37^{o}C. Groups of four teeth were taken out after one, two and three weeks after artificially demineralized dentine was generated (Table 1).

Sinfony (3M ESPE) crowns were fabricated using the vacuum formed moulds and master casts. The Sinfony crowns have two layers of spacer to allow for the luting cement between the crowns and the prepared teeth.

Half of each prepared dentine surface, exposed by crown preparation on each tooth sample, was painted with nail varnish (Fig 3) providing a control section of demineralized dentine where no contact between the luting cement and the SDTF could take place. The remainder of the demineralized dentine served as the test section of the tooth. Sinfony crowns after polishing and fitting were cemented using Fuji Cem (Fig 3). These teeth containing crowns were sectioned 2mm below the CEJ and connected to the system delivering SDTF (Fig 3). A reservoir of SDTF was set up at 15cm above the pulpal plane (2, 8, 9) and SDTF was supplied to each pulp chamber (Fig 3). After six weeks, the crowns were detached from the system. Each crown was sectioned sagittally, mesio-distally to a thickness of 1mm. Samples were dehydrated in ethanol (Merick Pty Limited, Australia). The samples were sectioned, embedded into epoxy resin then polished and carbon coated for Electron Probe Micro Analysis (EPMA) (Fig 4). In this study, the EPMA data was acquired using a Cameca SX51 Electron Microprobe with wavelength dispersive spectrometer (WDS). The analysis was carried out across the samples' surfaces. The first spot was selected just beyond the demineralized dentine so that the element values could start from zero. The iDFIX program was used to determine point zero if it would be necessary. An interval of 5µ was set between points and 10µ between lines which allowed an analysis of two distinct points. 71 points of each line, three lines on the test and three on the control side of the lesions were analyzed. On the test side, the analysis was made 100 microns within the luting material to search for migrated elements. The calibration was made

by comparing the peak and background intensity with Astimex 13($SrSO_4$), Fluorite and Camesa Apatite Standard ($CaPO_4$).

Determination of mineral profiles

Mineral profiles were determined across demineralized dentine both in weight percent and atomic weight percent quantities using EPMA (Fig 5). The test and control sides of the samples were analysed separately. Means of the three lines on the test and three lines on the control side were calculated and charted against lesion depth (Fig 7,8).

In Ngo's studies (5, 10, 11), the symbol delta Z was used to denote the final profile of mineral loss area as shown in A area (Fig 6). In our study, we used the area under the curve (Fig 6), represents the remaining mineral (B area). The black line represents the mineral level in the samples after the demineralization. The green line in the diagram represents the mineral level after remineralization. The difference between these two lines is the mineral gain area (D) (Fig 6). The area under the mineral profile curves were calculated and the level of difference between test and control sides of each sample determiner as explained above.

Statistical analysis of the data was undertaken using One way ANOVA test. Significance in differences required P values < 0.05.

Results

In most of the samples, there is a trend of an increase in calcium and phosphorus levels on the test sides compared with those on the control sides (Table 3). There is also a trend of greater remaining mineral on the test sides compared with those on the control sides (Table 2). One way ANOVA test was used to test the differences between two halves of the samples. The differences in strontium and fluorine levels on the test sides compared to the control sides are significant ($p<0.001$ for both elements)

Also, calcium was found in the luting cement even though calcium is not a composition of Fuji Cem (Fig 7). Samples four, sixteen and three have greater calcium levels on the control sides associated with high levels of strontium on the test sides (Table 3).

Discussion

In the presence of both SDTF and Fuji Cem, there was significant increase of fluoride and strontium ions in dentine in the test sides compared with the control sides. This finding agreed with the findings in previous studies (6, 12).

The increase in calcium and phosphorous levels on the test sides was not statistically significant as shown in previous studies (5, 6). This could be explained partly by the biologic variability between teeth. Because extracted teeth had an unknown history, the mineral levels at the baseline could be different. Also, each sample responded to the remineralization process differently leading to a different amount of gained minerals. The use of the test and control in the same tooth enable a true comparison between the treated and non-treated dentine. This method minimizes the biological variation issue.

The greater calcium level on the control side of one sample was associated with an increase of strontium on the test side. This could be explained by the fact that the valence of calcium is similar to that of strontium. Due to the greater molecular weight of strontium, the relative weight percent of calcium decreased with the uptake of strontium. However, the absolute weight of calcium could remain the same or even be increased. This phenomenon is complex and needs a further investigation.

The presence of calcium in Fuji Cem luting cement was observed on the test sides of all samples, whilst not being observed on the control sides. Noting that the dentine on the control sides was separated from direct contact with the luting cement and the

dentinal fluid. This demonstrated that there was an ion exchange between the dentinal fluid and the Fuji Cem luting cement on the test side. It also demonstrated that calcium diffused throughout the demineralized dentine and penetrated into Fuji Cem luting cement to a depth of about 50μ.

Conclusion

There was a trend of increased mineral profiles on the test sides. Calcium and other minerals from SDTF and Fuji Cem were taken up into the artificially demineralized dentine. The presence of calcium in Fuji Cem showed that there was a mineral ion exchange process between dentinal fluid and luting cement.

However, the study has a limitation of a small sample size. Further study with more samples is needed to confirm this initial result. Other material such as resin cement can be used to provide a comparison between the influences of resin cement and Fuji Cem luting cement on remineralization, from which a stronger conclusion can be drawn.

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Legends

Figure 1: Teeth embedded in dental plaster and prepared as for PBM crown 62

Figure 2: Roots were painted leaving dentine exposed for remineralization 63

Figure 3: Perfusion system 64

Figure 4: Sample prepared for EPMA 65

Figure 5: EMPA image 66

Figure 6: Mineral profile diagram.....67

Figure 7: Mineral profile sample 1- test side.....68

Figure 8: Mineral profile sample 1- control side 69

Figure 1: Teeth embedded in dental plaster and prepared as for PBM crown

Figure 2: Roots were paintedleaving dentine exposed for remineralization

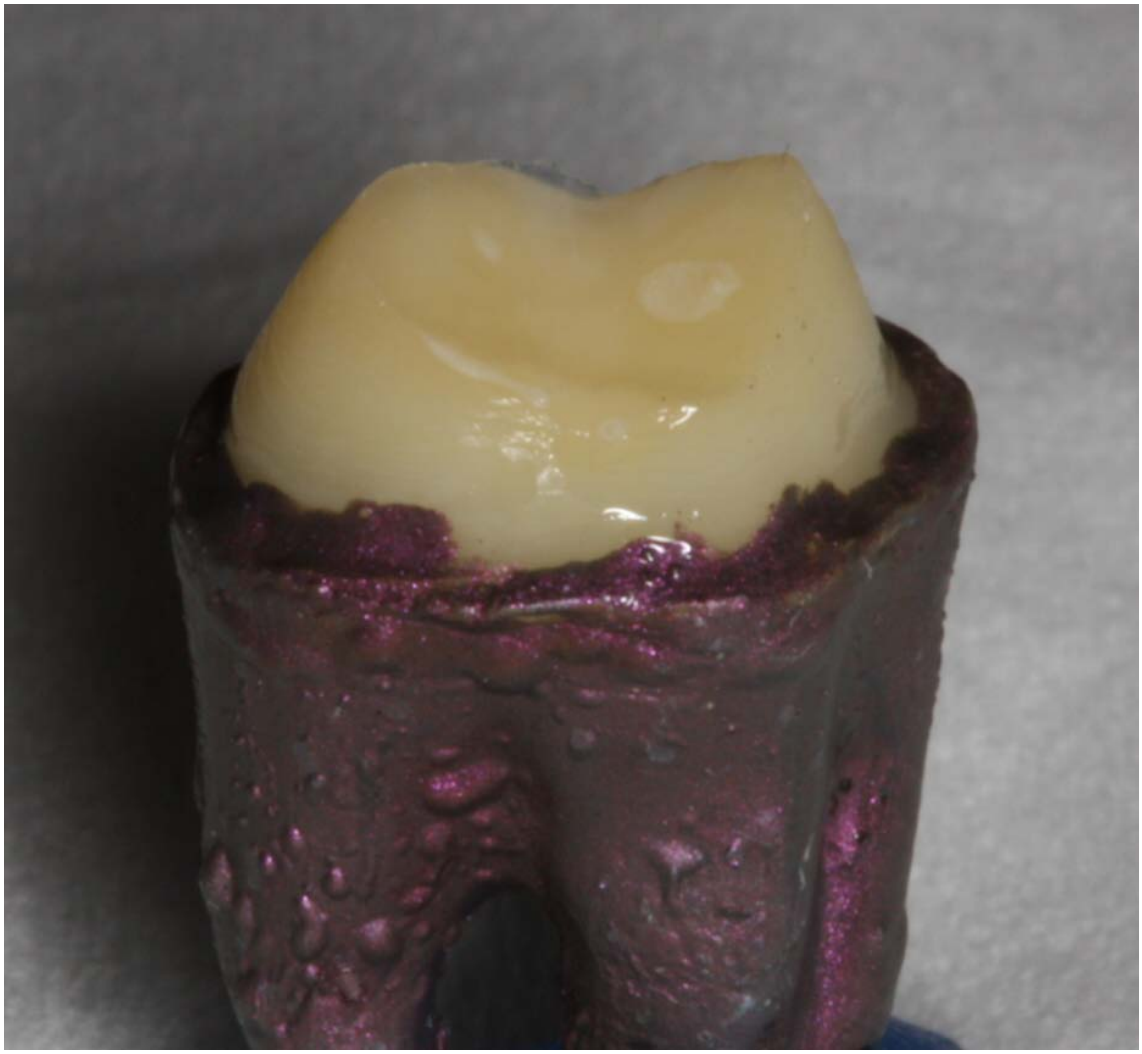


Figure 3: Perfusion system

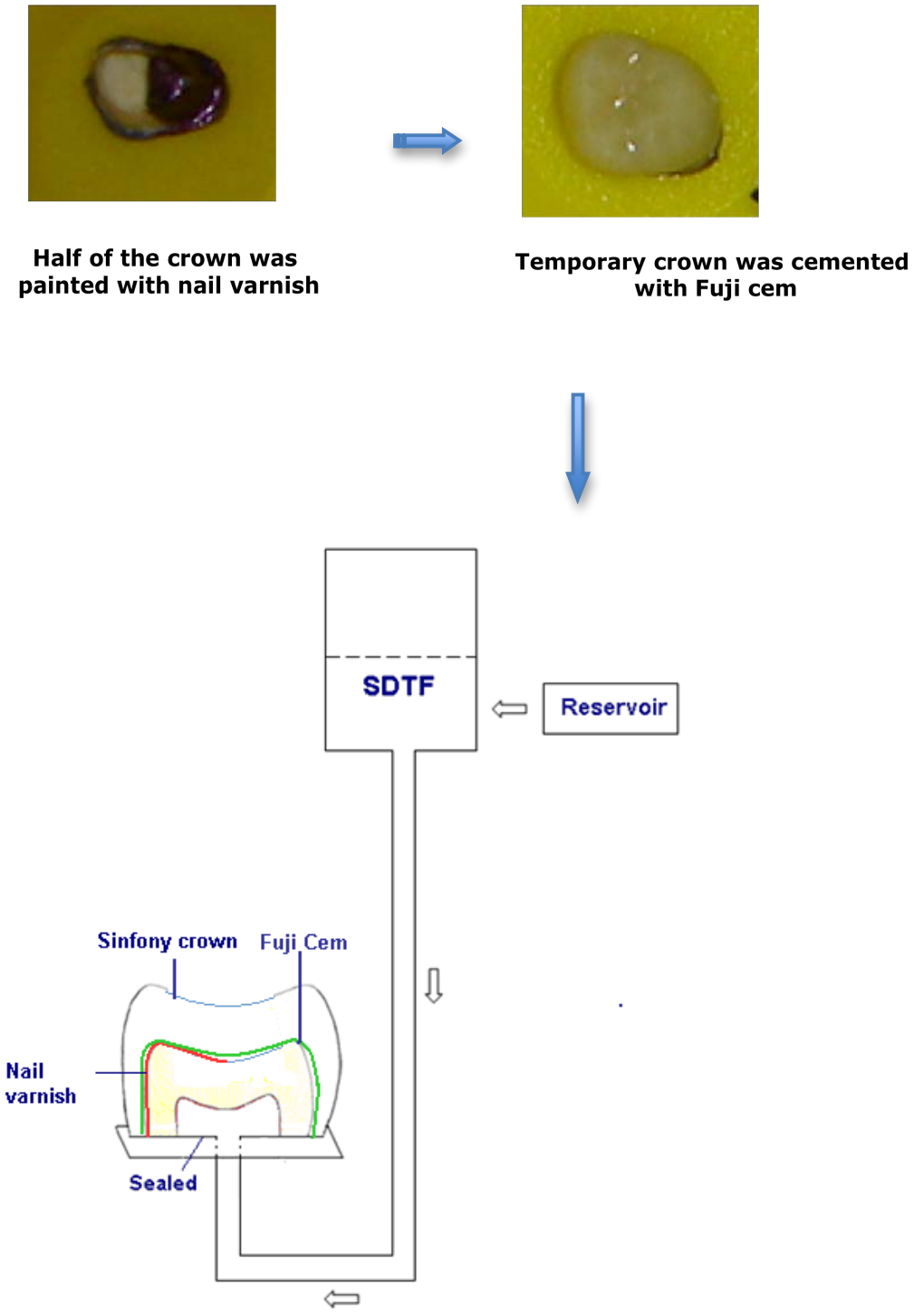


Figure 4: Sample prepared for EPMA

Figure 5: EMPA image

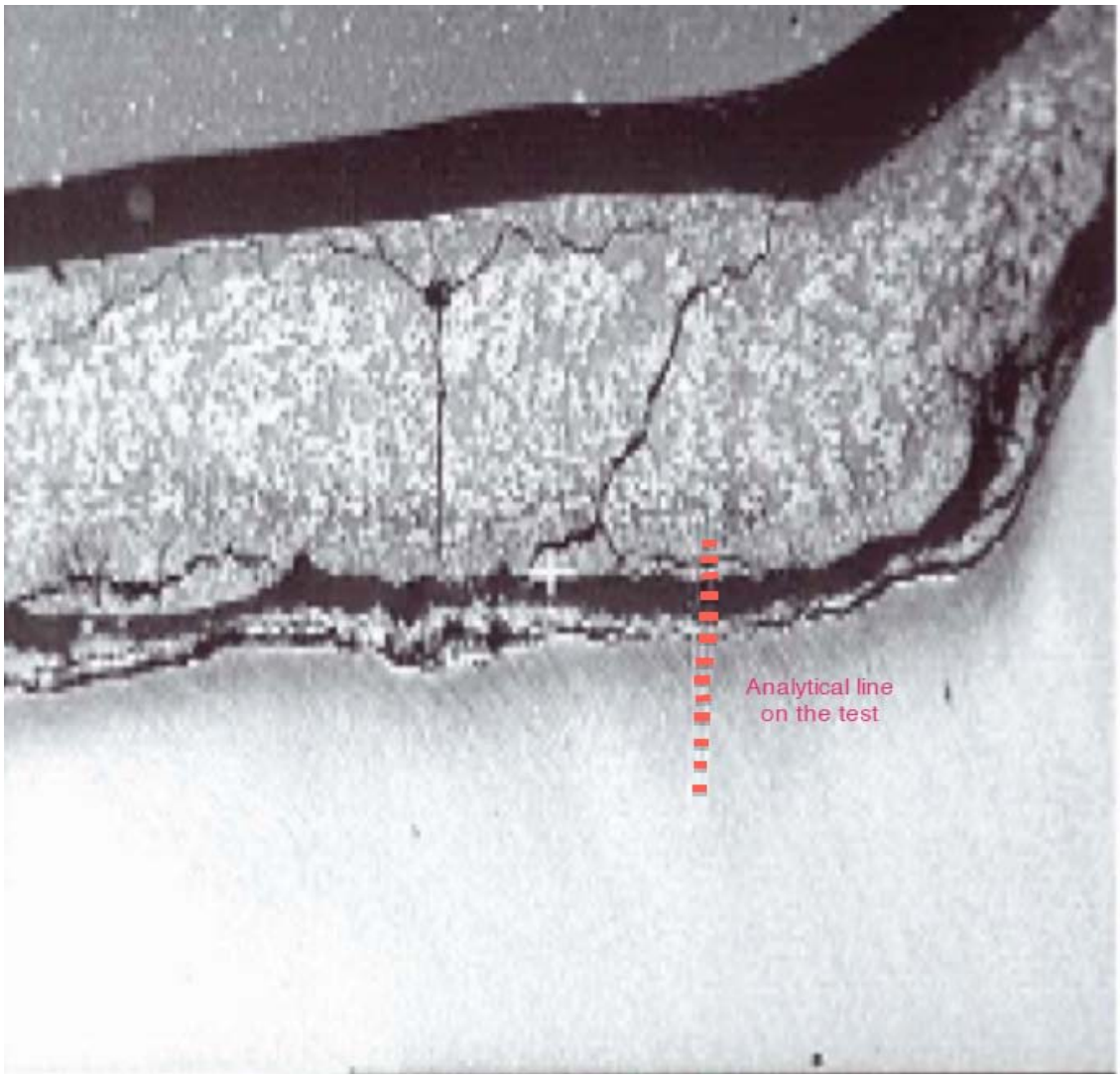
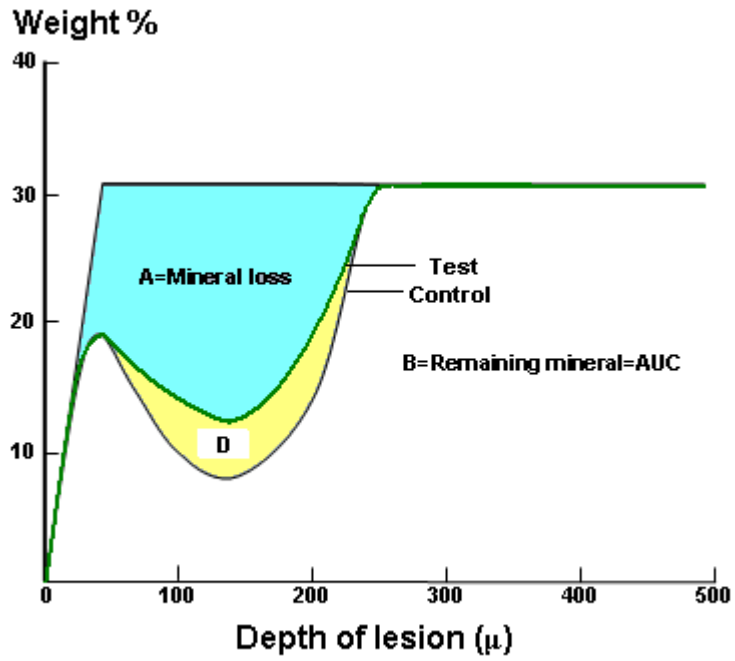


Figure 6: Mineral profile diagram



Zone A=mineral loss area

Zone B= Remaining mineral area=AUC

Zone B- Zone A= Zone D= Amount of remineralization

Figure 7: Mineral profile sample 1- test side

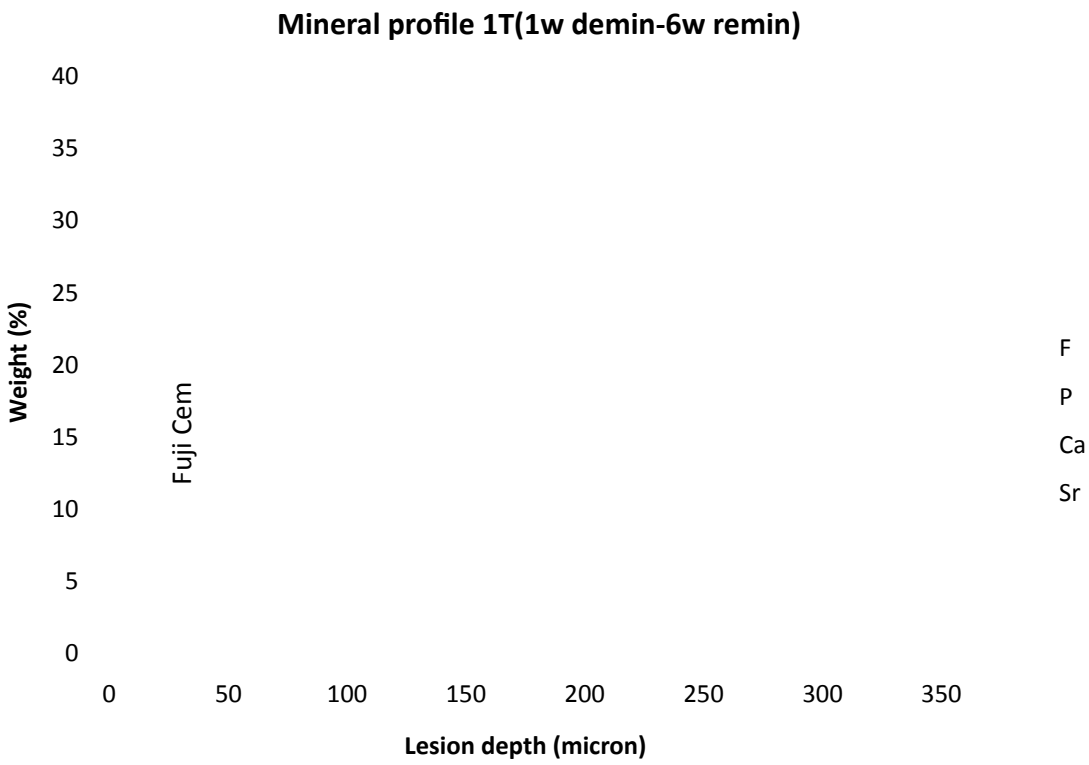
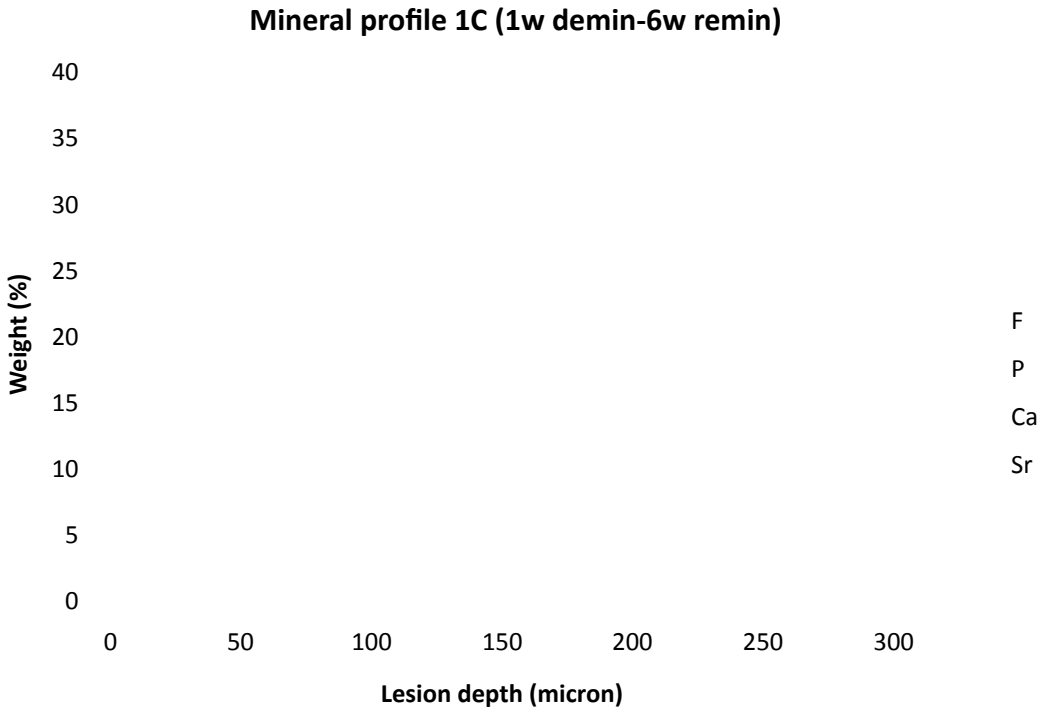


Figure 8: Mineral profile sample 1- control side



List of tables

Table 1: Samples and treatment methods..... 71

Table 2: Remaining mineral values (AUC) 72

Table 3: Comparison of the test and control sides for individual element..... 73

Table 1: Samples and treatment methods

Sample name	Demineralising time
1, 4, 6, 15	One week of demineralization, six weeks of remineralization (SDTF+Fuji Cem on the test side; SDTF only on the control side)
9,13, 16,18	Two weeks of demineralization, six weeks of remineralization (SDTF+Fuji Cem on the test side; SDTF only on the control side)
3, 5, 10, 11	Three weeks of demineralization, six weeks of remineralization (SDTF+Fuji Cem on the test side; SDTF only on the control side)

Table 2: Remaining mineral values (AUC)

Remaining minerals	AUC Test	AUC Control	Absolute diff
1 week	9295	7182	2113
2 weeks	9319	7007	2312
3 weeks	7013	6648	365

Table 3: Comparison of the test and control sides for individual element

Sample	F		P		Ca		Sr	
	Test	Control	Test	Control	Test	Control	Test	Control
1	88	47	3818	3783	7872	7615	40	13
4	231	58	3339	3305	6798	6919	100	8
6	474	157	2483	2281	4896	4565	424	85
15	174	170	3339	3305	7673	7025	116	97
9	458	102	3184	3068	6415	6127	367	60
13	481	89	2771	2596	5485	5229	452	40
16	334	180	2924	3472	5899	7117	248	102
18	316	192	2887	2738	5682	5492	378	175
3	257	66	3222	3284	6493	6819	199	32
5	196	221	3611	3469	7225	6866	265	128
10	525	92	3113	3017	6214	5961	595	28
11	512	86	3025	3026	5922	5975	548	21
P	0.00088		0.60039		0.61231		0.00077	

DISCUSSION AND CONCLUSION

As demonstrated in a previousstudy (Vu 2008), in the presence of simulated dentinal fluid, minerals from GIC restorations are taken up into artificially demineralized dentine.

This study aimed to investigate whether the simulated dentinal fluid and Fuji Cem (a strontium-based luting cement) influenced the remineralization of demineralized dentine.

The results of this study showed an increase of strontium and fluoride on the test side of the samples which confirmed the results from previous studies (Ngo 2005, Vu 2008). There were greater calcium levels on the test sides compare with those of the control sides. In one sample, the lower level of calcium on the test side coincided with increased strontium on the test sides. These findings are also in agreement with those from the previous studies.

However, there are some limitations in study design, the choice of material and data analysis. Due to limitation in time and budget, only one luting cement was used resulting in no data for comparison with other luting cements. Sinfony crowns were used instead of PBM crowns to reduce the laboratory cost and time and to simplify the process for sectioning and sample analysis. However, the interpretation of adhesion between Sinfony crown and teeth needs to be considered cautiously. Under these experimental conditions no occlusal loads were applied and the issue of adhesion

between Fuji Cem and Sinfony crowns is not a clinical problem as these materials are rarely used together.

The use of half of the tooth as a control side enables a true comparison between the tests and control sides. This limits the confounding effects of biological variation between teeth which is largely overcome by allowing the comparison between halves of the same tooth.

In the data analysis, relative amounts of mineral rather than absolute amounts were reported due to the analytical methodology, suggesting that further study is needed to precisely quantify mineral concentrations.

Despite these limitations, the findings from this study suggested that remnant demineralized dentine, if left behind during the crown preparation, could be remineralized. Understanding the potential for remineralization of artificially demineralized dentine under Sinfony crowns in the presence of simulated dentinal fluid contributes to our current knowledge of how to control recurrent caries. The clinical implication of these findings are that Fuji Cem luting cement may help affected dentine to be remineralized under crowns in the presence of the dentinal fluid.

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SECTION TWO

Other Scholarly Work

This section includes electronic versions of all of the other scholarly work undertaken during the three-years of the Doctor of Clinical Dentistry programme.

To access this information open the MS word file “Other_Scholarly_Work” on the enclosed CD and open any of the presentations by selecting the appropriate hyperlink.

NOTE – SECTION TWO – OTHER SCHOLARLY WORK PRESENTATIONS

Presentation files containing copyrighted material are contained on the CD included with the print copy of the thesis held in the University of Adelaide Library.

Presentation files included on the CD are highlighted in – BLUE

Presentation files containing copyrighted material have – Please refer to CD

Implant

1 Implant/Cantilever implants.ppt – Please refer to CD
2 Implant/Temporary cements for implant crowns.pptx – Please refer to CD
3 Implant/Comparing different implant systems.ppt – Please refer to CD
4 Implant/Implants in radiated sites.pptx
5 Implant/Methods to evaluate implant stability.pptx – Please refer to CD
6 Implant/Implant overdenture fabrication.pptx – Please refer to CD
7 Implant/Precision attachments.pptx – Please refer to CD
8 Implant/Retention of implant prosthesis.ppt – Please refer to CD
9 Implant/Tooth VS implant.pptx – Please refer to CD
10 Implant/Torque wrenches.pptx – Please refer to CD
11 Implant/Partially and completely edentulous patients.ppt – Please refer to CD

Dental materials

12 Dental materials/Ceramics in Dentistry.pptx – Please refer to CD
13 Dental materials/Ceramic classifications.ppt – Please refer to CD
14 Dental materials/Glass Ceramic.ppt – Please refer to CD
15 Dental materials/Clinical applications of glass ceramics.ppt – Please refer to CD
16 Dental materials/Dental luting cements.ppt – Please refer to CD
17 Dental materials/Composite Resins Nov 09.ppt – Please refer to CD

Removable Prosthodontics

18 Removable Pros/Partial denture with distal extension.ppt – Please refer to CD
19 Removable Pros/Neutral zone impression technique.pptx – Please refer to CD
20 Removable Pros/Retention of complete deture.ppt

Caries

21 Caries/Dental caries.ppt – Please refer to CD
22 Caries/Diagnosis of dental caries .ppt – Please refer to CD
23 Caries/Dental caries from Prosthodontics' view.ppt
24 Caries/Treatment of dental caries.ppt – Please refer to CD

Interdisciplinary treatment

25 Interdisciplinary treatment/Complications in periodontically compromised patients.pptx – Please refer to CD
26 Interdisciplinary treatment/Cracked tooth syndrome.pptx – Please refer to CD
27 Interdisciplinary treatment/Esthetic and anterior tooth position.ppt – Please refer to CD
28 Interdisciplinary treatment/Gingival recession.ppt – Please refer to CD
29 Interdisciplinary treatment/History taking.ppt
30 Interdisciplinary treatment/Periodontal complications in prosthodontics.ppt – Please refer to CD
31 Interdisciplinary treatment/Biomechanics of masticatory system.ppt – Please refer to CD
32 Interdisciplinary treatment/Tooth discoloration.ppt – Please refer to CD

Fixed Prosthodontics

33 Fixed Pros/Posterior tooth wear.ppt – Please refer to CD
34 Fixed Pros/Resin bonded bridges.ppt – Please refer to CD

Others

35 Others:Colgate Clinical Day 07/2009_Tardive dyskinesia.ppt – Please refer to CD
36 Others/Colgate research day_The effect of dentinal fluid on remineralisation.pptx – Please refer to CD
37 Others/DClinDent_pulp's story.ppt – Please refer to CD

Seminar presentations during D Clin Dent training (2008-2010)

Vu Thanh My Anh, School of Dentistry, The University of Adelaide

	Implant
1	Implant/Cantilever implants.ppt
2	Implant/Temporary cements for implant crowns.pptx
3	Implant/Comparing different implant systems.ppt
4	Implant/Implants in radiated sites.pptx
5	Implant/Methods to evaluate implant stability.pptx
6	Implant/Implant overdenture fabrication.pptx
7	Implant/Precision attachments.pptx
8	Implant/Retention of implant prosthesis.ppt
9	Implant/Tooth VS implant.pptx
10	Implant/Torque wrenches.pptx
11	Implant/Partially and completely edentulous patients.ppt
	Dental materials
12	Dental materials/Ceramics in Dentistry.pptx
13	Dental materials/Ceramic classifications.ppt
14	Dental materials/Glass Ceramic.ppt
15	Dental materials/Clinical applications of glass ceramics.ppt
16	Dental materials/Dental luting cements.ppt
17	Dental materials/Composite Resins Nov 09.ppt
	Removable Prosthodontics
18	Removable Pros/Partial denture with distal extension.ppt
19	Removable Pros/Neutral zone impression technique.pptx (Presented in Colgate Clinical Day 2010)
20	Removable Pros/Retention of complete deture.ppt
	Caries
21	Caries/Dental caries.ppt
22	Caries/Diagnosis of dental caries .ppt
23	Caries/Dental caries from Prosthodontics' view.ppt
24	Caries/Treatment of dental caries.ppt
	Interdisciplinary treatment
25	Interdisciplinary treatment/Complications in periodontically compromised patients.pptx
26	Interdisciplinary treatment/Cracked tooth syndrome.pptx
27	Interdisciplinary treatment/Esthetic and anterior tooth position.ppt
28	Interdisciplinary treatment/Gingival recession.ppt

29	Interdisciplinary treatment/History taking.ppt
30	Interdisciplinary treatment/Periodontal complications in prosthodontics.ppt
31	Interdisciplinary treatment/Biomechanics of masticatory system.ppt
32	Interdisciplinary treatment/Tooth discoloration.ppt
	Fixed Prosthodontics
33	Fixed Pros/Posterior tooth wear.ppt
34	Fixed Pros/Resin bonded bridges.ppt
	Others
35	Others:Colgate Clinical Day 07/2009_Tardive dyskinesia.ppt
36	Others/Colgate research day_The effect of dentinal fluid on remineralisation.pptx(D Clin Dent Research)
37	Others/DClinDent_pulp's story.ppt (Research Proposal)

These presentations were carried out as literature review requirements during three years of D Clin Dent training to supervisors and colleagues otherwise specified.

APPENDIX

This section includes an author covering letter to ADJ and draft manuscripts of four additional papers reporting associated work completed previously but not yet published as background to the present study.

Author Covering letter to the Australian Dental Journal (3)

AUSTRALIAN DENTAL JOURNAL

In submitting the manuscript titled

VALIDATION OF THE INFUSION MODEL

to the *Australian Dental Journal* I/we declare that:

1. The results are original, not falsified or plagiarised form any source.

2. All people involved with this report and all grants and scholarships which supported this work are duly acknowledged.

3. Credit to authorship is only to those who have participated substantially in the research work and preparation of this manuscript.

4. This paper is not currently under consideration for publication elsewhere.

5. All financial and personal relationships which might bias the interpretation of the work described in this manuscript have been fully disclosed

My Anh Vu Thanh

Date 16/07/2011

MANUSCRIPT 3

Validation of the infusion model

My Anh Vu Thanh

John McIntyre

Lindsay Richards

School of Dentistry, The University of Adelaide, Adelaide, SA 5005 Australia

(Manuscript of a Review to be submitted to the Australian Dental Journal)

Abstract

The objective of this study is to determine whether Simulated Dentine Tubule Fluid (SDTF) will enhance remineralisation of demineralised dentine. In this study, class one cavities of similar dimension were cut into the occlusal surface of twelve extracted, intact third molar teeth. These teeth were painted with varnish leaving the cavity floors exposed for generation of artificial caries (4 teeth each for 7 days, 14 days and 21 days demineralization). Half of the demineralised cavity floor was protected with nail varnish (control side) and perfused with either simulated dentinal tubular fluid (SDTF) or double distilled water (DDW). The crowns were placed in demineralising solution. In this study, a reservoir was connected to each tooth to supply SDTF under recognized hydrostatic pressure to each pulp chamber for 21 days during which the perfusion of SDTF or DDW occur in the presence of demineralizing solution. After three weeks, teeth were detached from the system, sectioned and prepare for analysis by Electron Probe to determine profiles of Ca, P, F and Sr.

Results: It was demonstrated that alizarin red perfused throughout and present at the lesion front. There was a reduction of demineralized lesion depth, greater in sample perfused with STDF compared to that of sample perfused with DDW. The EPMA results showed an increase in calcium levels on the test side compared to that of the control side in the samples perfused with SDTF.

Conclusion: The perfusion model supplied dye throughout dentine and the SDTF reduced lesion depth of the demineralized dentine.

Introduction

This study was to validate the infusion model's suitability for the analysis of the effects of SDTF on demineralization and remineralization capabilities within exposed dentine. This model was built based on Anderson's model (1, 2).

The infusion model was use to infuse Alizarin red into dentine (2) to demonstrate that the apparatus worked. This was evidence of an effective hydrostatic pressure which enabled the diffusion of Alizarin red into the dentine tubules. In this stage, the first analytical tests using the infusion system were carried out on a small number of teeth that had occlusal cavities with standard dimensions using in all experiments.

The objective of these experiments was to gain more direct evidence that diffusing SDTF could impact on both demineralization and remineralization within dentine.

Materials and methods

Twelve intact third molar teeth were collected (with Adelaide University Human Ethics Committee approval). It was preferred to use those teeth which were previously un-erupted to ensure dentine tubules remained patent. The teeth were cleaned to remove the remaining soft and hard tissue and stored in Thymol 0.2% solution in the refrigerator until being used. Occlusal Class 1 cavities of dimension 5mm mesio-distally, 4mm bucco-lingually with occlusal depth of 3mm ending in dentine were prepared using high speed hand-pieces with water-cooled diamond burs. Care was taken to ensure the dentine was not dehydrated during cavity preparation. A bench stereomicroscope (Leitz, Germany) was used to check the floor of the cavities to ensure there was no enamel remaining as well as no irregularities present. These teeth were then painted with nail varnish to cover all enamel, roots and the enamel wall in the cavity leaving the dentine floors exposed for the generation of artificial caries. The

reason for covering the enamel wall was to prevent the enamel from dissolving when it was in contact with demineralizing solution. Dissolved products of the enamel may have altered the saturation level of the demineralizing solution thus causing some variation in the depth of the demineralized dentine lesion.

The teeth were divided into two groups of six, each group being used for separate tests. For the first test, the dentine remained intact. For the second, six teeth had demineralized dentine generated by immersing each tooth in 40mls of demineralizing solution at 37^0C. The teeth were divided into three groups and each group was exposed for 7, 14 and 21 days of demineralization. This provided three different grades and depth of demineralized dentine for later investigation of the potential for different susceptibilities to remineralization.

In the first experiment, the objective was to expose the six teeth with the sound dentine floors to an artificial demineralizing solution (3) while they were infused in three cases with either SDTF (3 samples) or DDW (3 samples).

To achieve this, the teeth were sectioned 2mm from the CEJ to remove the roots as described previously then sealed onto the Perspex containers to ensure the penetration of a steel tube into the pulp chamber. The crowns were then connected to the system which supplied SDTF/DDW under a hydrostatic pressure of 15cm from the pulp chambers. When the six teeth had all been satisfactorily connected to the reservoirs of SDTF or DDW, the Perspex container had 40mls of demineralizing solution placed in it. Afterwards, the containers were covered with lids to ensure the demineralizing solution would not evaporate. The whole system was placed in an incubator for one, two and three weeks and regular checks were made to ensure the height and pressure of SDTF remained constant and no leaking occurred. At each time-

point two crowns (one from the SDTF and one from the DDW group) were detached from the system for analysis. To maintain the required pressure in the system, the crowns removed for analysis were replaced with “non-experimental” crowns.

The samples which experienced one week of demineralization were coded D1D with the DDW supplied sample, and D1S where the SDTF was supplied. Similarly, D2D following two weeks exposure with the DDW supplied, and D2S with SDTF supplied; D3D following two weeks exposure with the DDW supplied, and D3S with SDTF supplied. The samples were prepared for EPMA.

In the second experiment, teeth with pre-demineralized dentine were to be all subjected to a remineralizing solution for three weeks. Three teeth (with one, two, three week pre-demineralized dentine) were assigned into a separate experiment either with DDW or SDTF.

After removing the roots, a slot was cut in the middle of the dentine floor using a half round bur. Half of each cavity floor was painted with nail varnish to produce a control side, the other half becoming the test side which was directly in contact with the remineralizing solution. The crowns were sealed onto individual containers. The metal tubings penetrated through the base of the containers which supplied the SDTF to three teeth and DDW to the other three under a hydrostatic pressure of 15cm high from the pulp chambers. Once the crowns were sealed to a Perspex container base, 40mls of remineralizing solution (4) was placed in each container so that the crowns were completely covered. Afterwards, the teeth were supplied with either SDTF or DDW for three weeks at 37^0C in an incubator. The crowns were then detached from the system and samples were prepared for EPMA. Samples which were supplied with DDW were coded as 1D, 2D, 3D and 1S, 2S, 3S depending whether they had previously

been exposed to demineralizing solution for on one, two or three weeks of pre-demineralized dentine respectively.

Results

Comparison of calcium profiles between the samples supplied with SDTF/DDW

Figure 1 shows the comparison of calcium levels between the samples supplied with DDW and SDTF after demineralization for one week (D1D, D1S), 2 weeks (D2D, D2S) and 3 weeks (D3D, D3S), respectively. The first chart shows greater calcium levels on the samples supplied with SDTF compared to that of the samples supplied with DDW. In the second and third chart, the calcium levels between samples supplied with SDTF and with DDW are quite similar. As this was a pilot study with a small number of samples, statistical evaluation of AUC changes were not carried out.

The influence of SDTF on remineralization of pre-demineralized dentine

Figure 2 shows the calcium profile of pre-demineralized samples which were exposed to a specific remineralizing solution for three weeks with either SDTF or DDW supplied to the pulp chamber. The charts on the left illustrate the comparison of calcium levels between the test and control side of individual samples which had previously experienced one, two and three weeks of remineralization with supplied DDW. The right side charts illustrate the comparison of calcium levels between the test and control side of samples which experienced one, two and three weeks of demineralization and were infused with SDTF. As shown in the charts, the calcium levels on the test side were greater than those of the control sides in most of the samples. Again, the difference reduced with increase levels of period pre-demineralization.

Figure 3 shows the comparison of calcium levels between samples. Calcium levels on the test and control sides of the samples which experienced one week of

demineralization and three weeks of remineralization were greater than those of the samples which experienced two and three weeks of demineralization and three weeks of remineralization.

Discussion and conclusion

The purpose of these pilot tests was to ensure that the SDTF did react with the dentine in a readily detectable manner. As only one sample was involved in each category of test, it was not intended to obtain a significant result in terms of the effect of SDTF on demineralization and remineralization. The results of this stage demonstrated that the calcium levels in dentine increased in the presence of SDTF in both demineralization and remineralization. This validated the model and hence supported its use for the main experiments. However, it did point clearly to the need to have a test and control sample of demineralized dentine within one tooth, and to have a sufficient number of samples as to permit statistical analysis of the results.

A major requirement of the project was the development of the apparatus and methodology to allow the tests to be carried out. As described in methodology, the diffusion model which was used in this study was based on that developed by Anderson (1, 2). Using this model, SDTF was supplied into the pulp chamber causing the fluid to flow into the dentine tubules. This differed from other models where the surrogate dentinal fluid was supplied through a window on the lingual surface of a tooth to the pulp chamber and from there was supplied to the dentine window on the buccal surface (5, 6). Due to the fluid supply and location of the tested dentine, the perfusion rate in these previous studies might be different compared to those of our study. This relates also to the initial pilot studies where the effect of SDTF on demineralization of sound dentine and remineralization of the artificial demineralized dentine were investigated. The reduction of demineralized lesion depth in our study

differed from those in Shellis (5) and Ozok's (6, 7) studies. This may have been because the perfusion rate of buccal dentine (Shellis and Ozok's studies (5-7)) and occlusal dentine (in our study) is different (8), other aspects of the both studies also varied. These latter test results will be discussed in more detail later.

The dye diffusion experiment demonstrated that Alizarin red with a small molecular weight can diffuse into artificial demineralized dentine and from its entry through the pulp chamber to be present in the outer layers of carious dentine. In other experiments, the finding that SDTF began to leak through even previously demineralized dentine into the cavity floor when the SDTF reservoir was connected to the crown demonstrated that this fluid was at least being transported to this lesion.

The presence of either Alizarin red or calcium in the demineralized dentine can be explained by both diffusion and filtration force, as described in literature review. The dentinal fluid flowed through dentine tubules and as it filled all available spaces, relied on diffusion gradients for ion transport. It would also be partly filtered in peri-tubular dentine (9) as the hydrostatic pressure of delivery provided sufficient filtration pressure. The observation of the penetration of Alizarin red and calcium through carious dentine in this study suggested that, even though the dentine tubule in carious dentine may look occluded microscopically, dentine is a relatively porous tissue with small channels interconnecting tubules which allow the diffusion of small molecules such as calcium and Alizarin red. This agreed with Merchant's explanation in his study (10).

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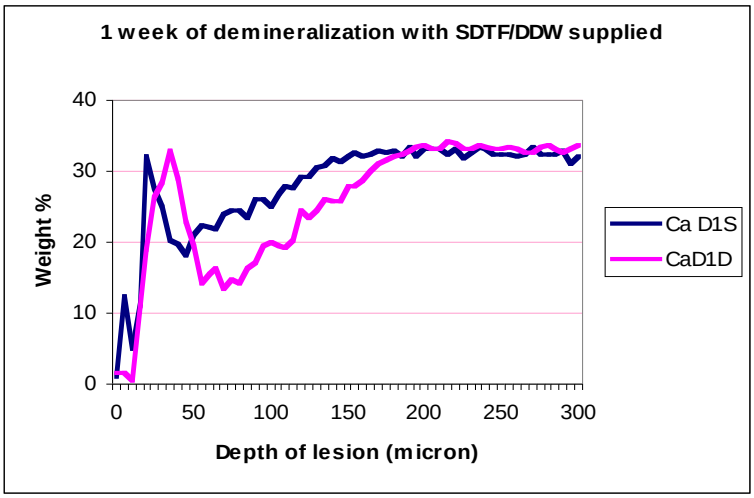
Legends

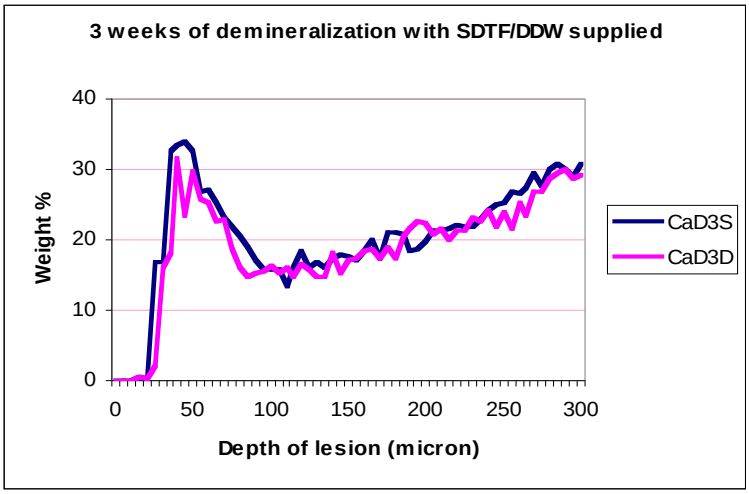
Figure 1: Comparison of calcium levels between the test and control sides of demineralized samples 96

Figure 2: Comparison of calcium levels between the test and control sides of remineralized samples 97

Figure 3: Comparison of calcium levels between samples 98

Figure 1: Comparison of calcium levels between the test and control sides of demineralized samples





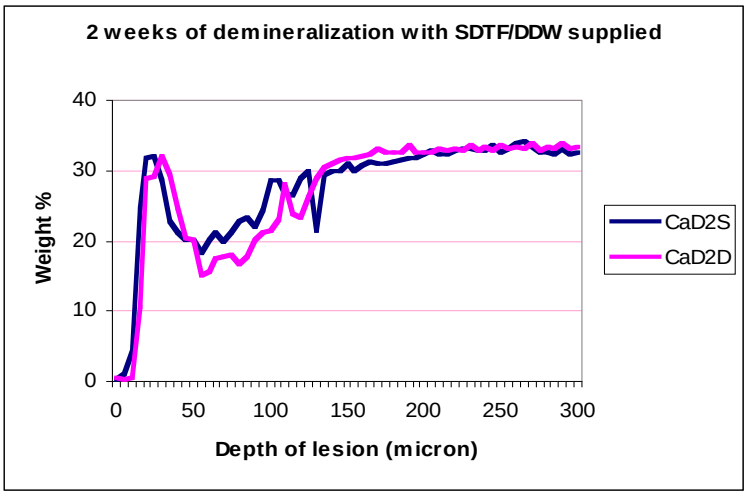


Figure 2: Comparison of calcium levels between the test and control sides of remineralized samples

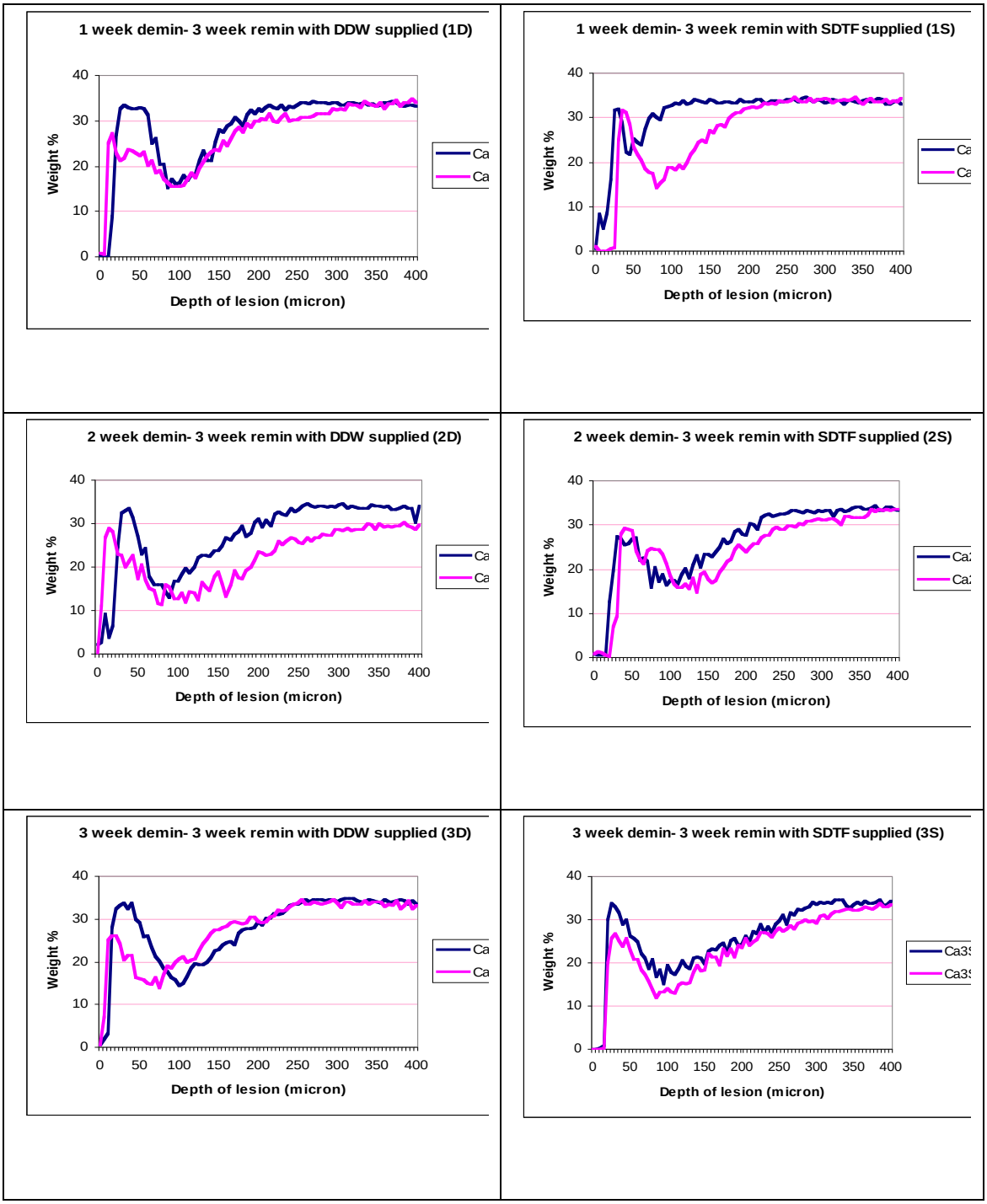
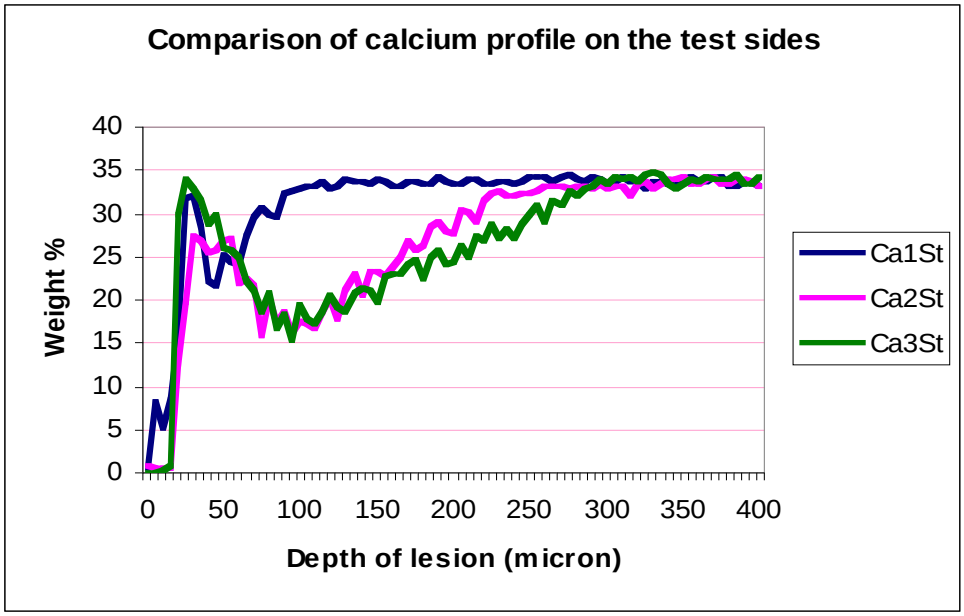
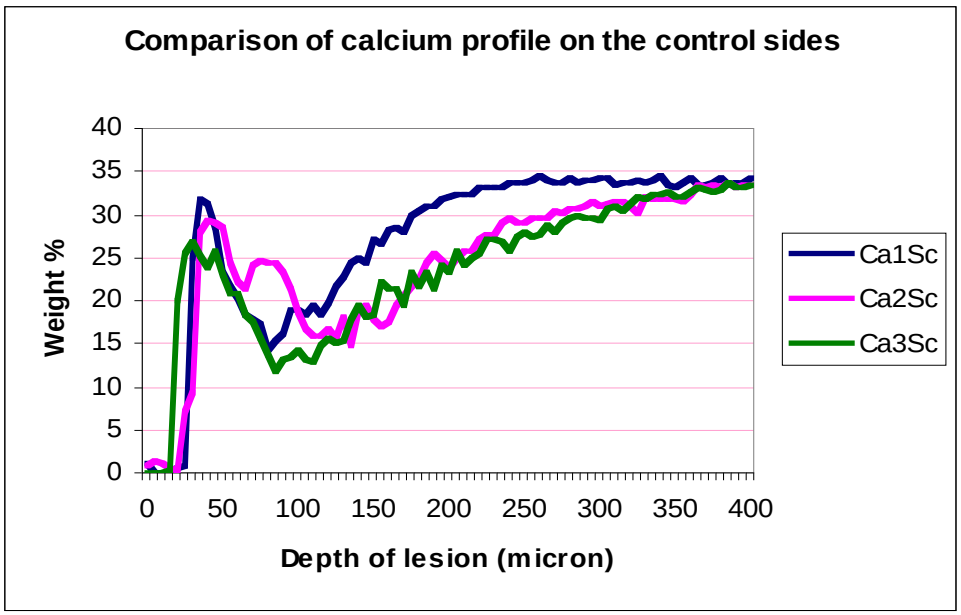


Figure 3: Comparison of calcium levels between samples





Author Covering letter to the Australian Dental Journal (4)

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to the *Australian Dental Journal* I/we declare that:

1. The results are original, not falsified or plagiarised form any source.

2. All people involved with this report and all grants and scholarships which supported this work are duly acknowledged.

3. Credit to authorship is only to those who have participated substantially in the research work and preparation of this manuscript.

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5. All financial and personal relationships which might bias the interpretation of the work described in this manuscript have been fully disclosed

My Anh Vu Thanh

Date 16/07/2011

MANUSCRIPT 4

Effect of dentinal fluid on remineralization under Fuji-IX restorations

My Anh Vu Thanh

John McIntyre

Lindsay Richards

School of Dentistry, The University of Adelaide, Adelaide, SA 5005 Australia

(Manuscript of a Review to be submitted to the Australian Dental Journal)

Abstract

Objective: To determine whether Simulated Dentine Tubule Fluid (SDTF) will enhance remineralization of demineralized dentine using an in vitro Atraumatic Restorative Technique (ART) simulation method.

Method: Class one cavities of similar dimension were cut into the occlusal surface of eighteen extracted, intact third molar teeth. These teeth were painted with varnish leaving the cavity floors exposed for generation of artificial caries (4 teeth each for 7 days, 14 days and 21 days demineralization). Half of the demineralized cavity floor was protected with nail varnish (control side) and Fuji IX restorations placed. In this study, a reservoir was connected to each tooth to supply SDTF under recognized hydrostatic pressure to each pulp chamber for 21 days during which ion exchange was proceeding between GIC and demineralized dentine. After three weeks, teeth were detached from the system, sectioned and prepare for analysis by Electron Probe to determine profiles of Ca, P, F and Sr.

Results: Relative weight percent of calcium and phosphorus on the test side were greater than those on the control side in most cases (significant at 5% level). In two cases, calcium and phosphorus on the test side were smaller than those on the control side though this coincided with high levels of strontium on the test side. Strontium and fluorine concentrations were high both at the surface of the lesion and at the lesion front.

Conclusion: The increase in calcium and other elements (strontium, fluorine, phosphorus) showed a positive trend of remineralization of the demineralized dentine. Comparing this data with that from previous in vitro studies, it was concluded that SDTF enhanced the remineralization process.

Introduction

Validation of infusion model confirmed the diffusion of calcium from the SDTF to demineralized dentine and showed a decrease in the demineralization of the lesion in the presence of SDTF. Also, in the remineralization experiment, there was an increase of calcium in the presence of SDTF. This evidence supported progression to the next stage of experiment in which the basic *in vitro* model was developed for quantitative evaluation of the level of element gain or loss.

The objective of this stage was to supply simulated dentinal tubular fluid (SDTF) into dentine tubules and to investigate the role of SDTF in the remineralization process.

Materials and method

Preparation of the basic in vitro model

The basic *in vitro* model was a combination of a diffusion model and perfusion model (1, 2). The basic *in vitro* model was designed as described below.

Eighteen intact third molar teeth were collected (with Adelaide University Human Ethics Committee approval). Teeth with Class 1 cavities experienced a generation of demineralized dentine by immersing each tooth in 40mls of demineralizing solution (3) at 37^0C. The teeth were divided into three groups and each group was exposed for 7, 14 and 21 days of demineralization. This provided three different grades and depth of demineralized dentine for later investigation of the potential for different susceptibilities to remineralization.

The teeth were then sectioned 2mm below the cementum enamel junction to remove the root using an IsoMet® Slow Speed Saw (Buehler USA) with a Diamond Watering Blade (Van Moppes, England). A slot was cut in the middle of the cavity which divided the cavity in two equal halves. Prior to placing a restoration, half of the demineralized

cavity floor was painted with nail varnish to provide a control side while the other half remained unpainted (test side). This allowed for a contact between demineralized dentine and the restoration on the test side, enabling ion exchange to occur between restoration and demineralized dentine. Following previous evidence of substantial ion exchange between GICs and the demineralized dentine *in vitro*(4), Fuji IX (GC Corporation, Tokyo, Japan) was selected in this study as the restorative material to seal the cavity. In order to more closely simulate the ART method of restoration, the powder/liquid manual mix version of Fuji IX was used.

Just before placing restorative material, the test half of the cavity was conditioned for ten seconds using 10% polyacrylic acid (Dentine Conditioner, GC Corporation, Tokyo, Japan). The cavity was then washed for 20 seconds and dried for 5-10 seconds but not desiccated. Fuji IX was mixed using one level scoop of powder to 1 drop of liquid on a mixing pad. A plastic spatula was used to mix half of the powder to the liquid in 10 seconds then the other half was incorporated and mixed thoroughly for 20 seconds. A plastic instrument was used to place the material into the cavity. The crowns were stored in a humidor for one hour to permit initial setting of the GIC to take place. The restored crowns were then sealed onto the Perspex base using sticky wax, with the stainless steel tubing penetrating into the pulp chamber.

A reservoir of SDTF as described above was connected to the metal tubing which supplied SDTF to each pulp chamber (Fig 1). Once SDTF began to flow into the pulp chamber, it was ascertained that no air bubble was caught within the tubing and the pulp chamber. The experiment was proceeded at 37^0C with care being taken that the SDTF hydrostatic pressure was maintained as required for the duration of the

experiment. The following diagram describes the basic *in vitro* model and the experimental method.

Three sets of the same experiment were set up with six teeth in each. A reservoirs of SDTF were connected to each tooth crown under a hydrostatic pressure of 15cm (5, 6) of water for three weeks.

In order to simulate the oral environment more closely, an "artificial saliva" solution comprising 2.2mM $CaHPO_4$, 0.05M acetate at pH 7 was placed in the chamber surrounding the restored cavities. The chambers were placed in a 37^oC incubator, static, for three weeks.

After three weeks, the crowns were detached from the system and prepared for EPMA. The Camesa SX51 Electron Microprobe (Cameca, Corbevoie, France) was used with the standard set up voltage of 15kV, current beam of 20mA and the beam perpendicular to the flat surface of the sample. Counting time was 10s on the peak and 5s on each of the backgrounds for both standard known composition (during calibration) and restorative materials/dentine (during analysis). Wavelength Dispersive Spectroscopy was used with the SAMx software performed with the iDFIX program. In this study, the EPMA data was acquired using a Cameca SX51 Electron Microprobe with WDS. The advantage of the WDS over the EDS is the resolution of X-ray energies which is much smaller for WDS (10eV) compared with EDS (150eV). There is a strong overlap between the Strontium Ka X-ray line and a second order Calcium X-ray line which can be resolved by the WDS whilst the EDS cannot. Furthermore, WDS enables a determination of heavy elements which are of interest in this study.

The analysis was carried out across the samples' surfaces. The first spot was selected just beyond the demineralized dentine so that the element values could start from

zero. The iDFIX program was used to determine point zero if it would be necessary. In setting up EPMA, a centre point was recorded using X and Y axes. The lines on the test side were chosen with the same distance to the centre point compare to that of the control lines. This allows an accurate comparison between the test and control side later. An interval of 5 microns was set between points which allowed an analysis of two distinct points. 101 points of each line, three lines on the test and three on the control side of the lesion were analyzed. Under certaincircumstances, the analysis was made 100 microns within material to search for migrated elements. The calibration was made by comparing the peak and background intensity with Astimex 13($SrSO_4$), Fluorite and Camesa Apatite Standard ($CaPO_4$).

Samples were coded as shown in table 1.

Results

Lesion depth of samples

Table 2 provides data describing the lesion depths (Ld) of the samples which experienced different demineralizing durations (one week, two weeks and three weeks) and three weeks of remineralization. The first column shows the demineralizing duration. The second and third columns show means and SD values of each group of samples. The third and fourth columns show the data on lesion depth on the test and the control sides. The fifth and sixth columns show data on calcium levels in normal dentine. In this experiment, there was no difference in the lesion depth and calcium level between the test and control side.

Strontium and fluorine levels on the test sides

Fuji IX is strontium based glass-ionomer cement and rich in fluorine. In this experiment, strontium and fluorine from Fuji IX restorations diffused into

demineralized dentine. As illustrated in Figure 2, strontium and fluorine levels were high at both the surface of the lesion and the lesion advancing front on the test sides.

Mineral profiles on the test and control sides

Examples of element profiles in a representative sample

Mineral profiles of four measured elements, calcium, phosphorus, fluorine, strontium were charted as shown in Figure 3. Oxygen profile was excluded to simplify both the chart and subsequent calculations.

In this experiment, sample 7a experienced one week of demineralization and three weeks of remineralization. The left chart illustrates mineral profiles of the test side and the right chart illustrates mineral profiles of the control side. The vertical axis indicates the weight percent of minerals whilst the horizontal axis indicates the lesion depth (the distance from the lesion front to the sound dentine).

The test side showed greater levels of calcium, phosphorus, fluorine and strontium compared to the control side. On the control side, the AUC values of calcium and lesion depth were greater than those of the test side. The calcium peak on the test side was 30.4 w% whilst that of the control side was 27.5 w%. The strontium peak was 7.6 w% on the test side whilst that of the control side was almost nil. Fluoride was at peak at 10microns depth with 4% in weight on the test side while the control side was peak at 1.6 w%. Lesion depth of the test side was 165 microns while that of the control side was 180 microns.

Aggregate Ca + P+ Sr+ F AUC results amongst all samples

Table 3 provides data on the aggregate Ca, P, Sr and F levels for this experiment. The first column identifies the samples in the experiment. The second and third columns show the “area under the curve” (AUC) aggregated values of calcium, phosphorus,

fluoride, strontium of the test and control side, respectively. The fourth column shows the absolute difference between the AUC aggregate values of all minerals of the test and the control side.

Generally, the absolute difference shows the tendency for relative weight percents of calcium, phosphorus, strontium and fluorine on the test side to be greater than those on the control side. However, the difference in the AUC values between the test and control sides is not significant. Table 3 illustrates the aggregate AUC values of Ca+P+F+Sr.

Changes in calcium levels on the test and control sides

Calcium levels increased on the test sides

The bar chart in Figure 4 below shows the difference in weight percent of calcium between the test and control side of each sample. Samples 7ab, 8ab, 9ab represented three groups which experienced different demineralization. Samples 7a and 7b had experienced one week, samples 8a and 8b two weeks and samples 9a and 9b three weeks of demineralization. They were all subjected to three weeks of remineralization with Fuji IX and the SDTF exposure.

Figure 5 also shows the difference in calcium levels between the test and control side by comparing the calcium level between the test and control side of a representative sample. Calcium level of the test side was slightly greater than that of the control side.

The AUC values of calcium were calculated and compared between the test and control sides. Table 4 shows the AUC of calcium levels on the test and control side of all samples. The first column indicates the names of samples. The second and third columns indicate the AUC values of the test and control sides. The last column

indicates the difference in the AUC value between the test and control side. Generally, the calcium levels of the test are greater than those of the control sides (P=0.78).

Increase of calcium levels on the control side in some cases

In five out of 18 samples described in Table 4, calcium and phosphorus levels on the test sides were smaller than those on the control sides though this coincided with high levels of strontium on the test sides. As shown in Figure 6, the area of mineral loss on the test side of sample 7b was greater than that on the control side. The strontium level on the test side of sample 7b was observed to be 1.5 w% at the depth of 150 microns compared to 0.6 w% at the respective depth of sample 7a (Figure 3). The calcium level on the test side of sample 7b was observed to be 24.4 w% compared to 32w% for sample 7a(Figure 3)at the same lesion depth. In comparison, the calcium level on the control side of sample 7b (Figure 5) was 32.84 w% at the depth of 150 microns compared to 31.3 w% at the respective depth of sample 7a (Figure 3).

Comparison of calcium levels between the test and control sides of representative samples

Figure 7 illustrates the difference in calcium levels between the test and control sides of sample 7b. The calcium level of the control side is clearly greater than that of the test side. In other words, the calcium gain of the control side is greater than that of the test side. This figure also shows evidence of a deeper lesion on the test side than the control side.

However, when fluorine and strontium w% concentrations were added to calcium and compared between the test and control side, the curve of aggregate Ca+F+Sr on the test side was equal or greater than those on the control side as illustrated in Figure 8.

Influence of demineralization period on remineralization

Figure 9 shows the differences in AUC of calcium between some representative samples. The samples which experienced one week of demineralization followed by three weeks ofremineralization shows higher levels of remineralization. These figure illustrates a greater calcium level in sample 7a (which experienced one week of demineralization) compared to those of samples 8a, 9a (which experienced two or three weeks of demineralization). In one -week pre-demineralized samples, calcium level on the control side was greater than those of the test sides ($p<0.001$). In two and three week pre-demineralized samples, calcium levels on the test sides were greater than those of the control sides ($p <0.001$).

Table 5 shows the AUC of calcium of one, two and three week demineralization respectively. The AUC value of the one week demineralized sample was greatest following by AUC calcium values of three week and two week demineralized samples on both test and control sides. The AUC value of calcium on the control side of the one week demineralized group was greater than that of the test side. The AUC calcium values on the test sides of two and three week demineralized samples were greater than that of the control sides. However, the difference between samples was the greatest in two week pre-demineralized group.

The data presented in Table 5 shows a significant difference in calcium levels between one week and two week of demineralization and three week of remineralization ($p=0.01$) but no difference between week 1 and 3 ($p=0.31$) or week 2 and 3 ($p=0.32$).

The presence of calcium in Fuji IX restorations

The areas of the Fuji IX restoration which contacted the demineralized dentine on the test side of some representative samples were analyzed using the EPMA for all four

elements. In the Table 6, the first column indicates the depth to which calcium penetrated from the dentine into the restorations. The first row indicates the name of the representative sample; "t" means test and "c" means control. The values under 1bt, 3bt, 4at, 5bt, 9at, 9bt indicate the calcium levels in weight % in the Fuji IX restorations at different depths. The last column under 1bc indicates the calcium values on the control side of sample 1b. The red bold letters indicate the increased calcium values. The green bold letters indicate the calcium in dentine and the black letters indicate the calcium out side either dentine or Fuji IX. The starting point of sample 1bt, 3bt and 5bt are 55, 85 and 80 microns with calcium values 1.09%, 1.5 and 1.2 respectively.

Calcium was found at a low level in the first 100 microns of the Fuji IX restoration on the test sides of some samples as is shown in Table 6.

Mean Δz of calcium and phosphorus concentrations

In this analysis, the Δz area represents the amount of mineral loss of a lesion. Table 7 shows the mean of Δz Ca, Δz P, Δz Ca+P and SD values of three groups of samples with different demineralization time regarding to the test and the control side. In general, the calcium and phosphorus loss on the test side was not significantly different from that of the control side.

Comparison of Δz of current study to previous study

Table 8 compares Δz of samples which were demineralized for one, two and three weeks and remineralized for three weeks under Fuji IX restorations and supplied SDTF with data from Ngo's (2006) study. The mean Δz Ca of one week pre-demineralized and three week remineralized samples was greater in Ngo's study ($p=0.001$). However, mean Δz Ca of two and three week pre-demineralized and three week remineralized

samples was greater in the current study compared to those of Ngo's with p= 0.031 and p=0.025, respectively.

Discussion and conclusion

Following the pilot studies designed to demonstrate that the diffusion system worked adequately, the basic *in vitro* model was developed to carry out the initial experiments.

This experiment looked at the effectiveness of the diffusion and filtration of calcium from SDTF into demineralized dentine; the diffusion of minerals from GIC restoration into demineralized dentine and the diffusion of calcium from demineralized dentine into Fuji IX restoration. Hence, this model involved a three-way ion-exchange which differed from the original diffusion model. This model provided the advantage of having the test and control sections within the same tooth. The test side was exposed to Fuji IX restoration and the SDTF diffused into both test and control sides.

However, it was soon realized that it also had the disadvantage that the results were difficult to interpret because any change could not be interpreted in relation to these minerals originally present. The changes in profiles of calcium and phosphorus could not be assessed in relation to those originally present in the initial demineralized dentine. The increase of calcium on both test and control sides in some cases using the basic model suggested a need to use the same tooth providing two extra control and test segments. This would provide before and after element profiles. The enhanced model was developed to provide this additional information.

Despite the need for this new enhanced model, the basic model was able to provide crucial data relating to the interaction between GIC and SDTF and demineralized dentine. Even so, the results showed a reasonably high degree of variability between

teeth, indicating that the potential for remineralization was very strongly subject to minor changes in affecting variables.

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Legends

Figure 1: Diagram of the basic *in vitro* model.....115

Figure 2: Mineral profiles in demineralized dentine and in Fuji IX 116

Figure 3: Mineral profiles of the test and control sides of sample 7a 117

Figure 4: Calcium levels of test and control sides (7a7b, 8a8b, 9a9b) 118

Figure 5: Comparison of calcium levels between the test and control sides of 7a119

Figure 6: Comparison of calcium levels between the test and control sides of sample 7b 120

Figure 7: Mineral profiles on the test and control sides of sample 7b.....121

Figure 8: Aggregate Ca, F, Sr uptake on the test and control sides.....122

Figure 9: Comparison of calcium levels of samples 7a, 8a, 9a.....123

Figure 1: Diagram of the basic *in vitro* model

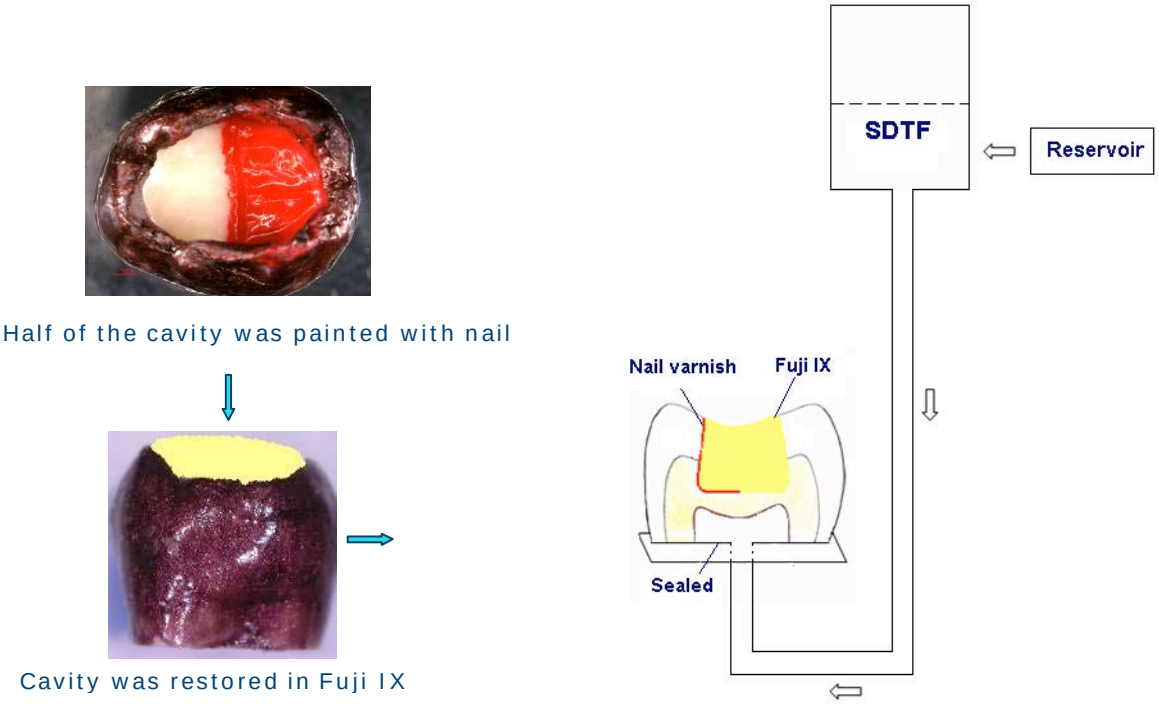
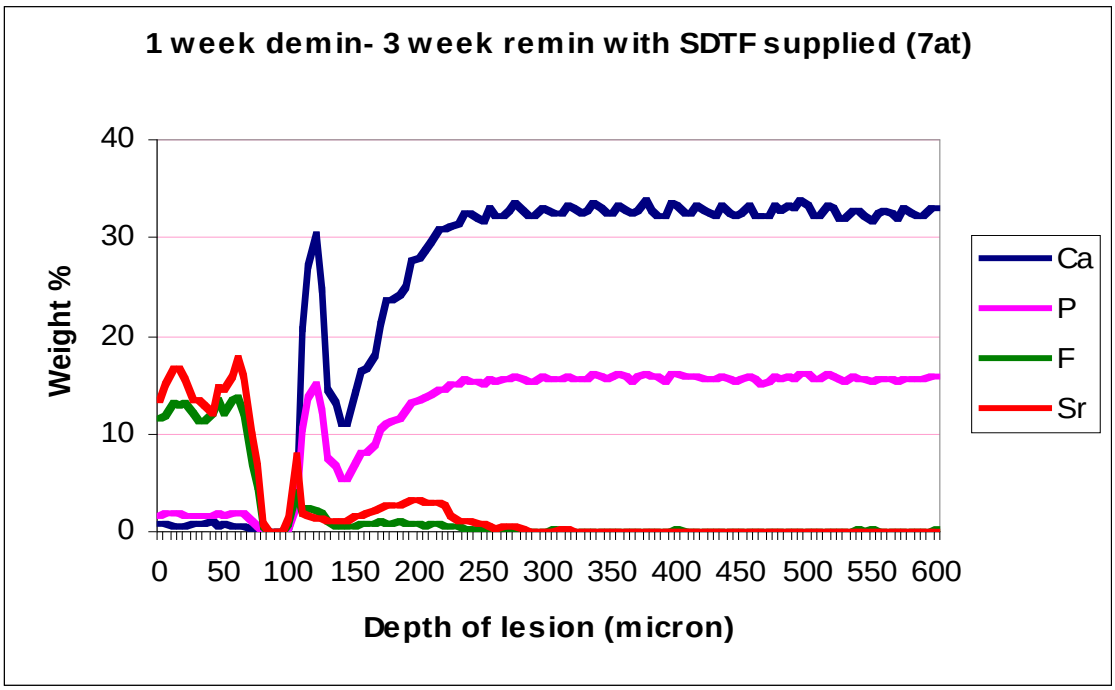


Figure 2: Mineral profiles in demineralized dentine and in Fuji IX



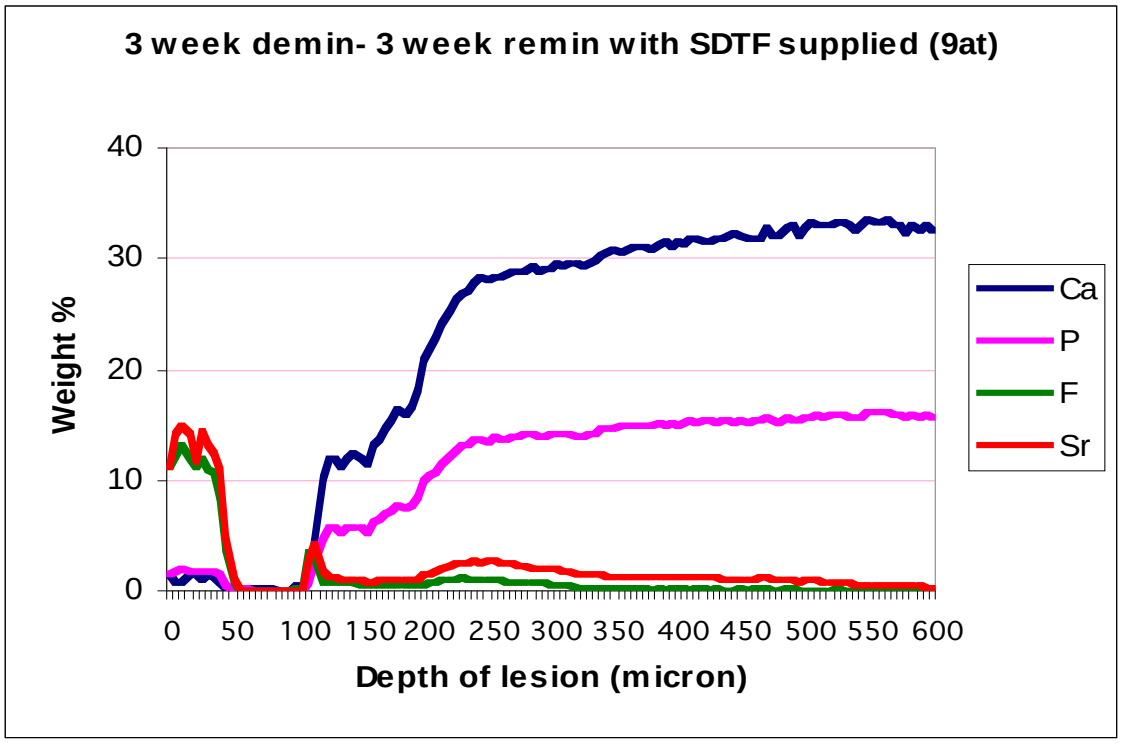
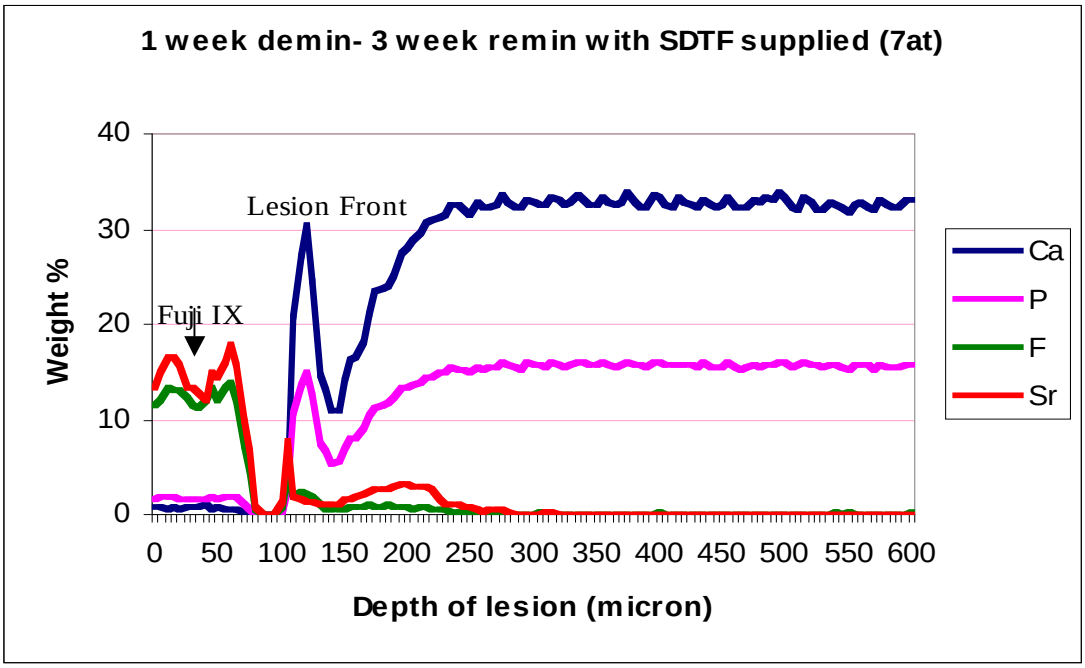


Figure 3: Mineral profiles of the test and control sides of sample 7a



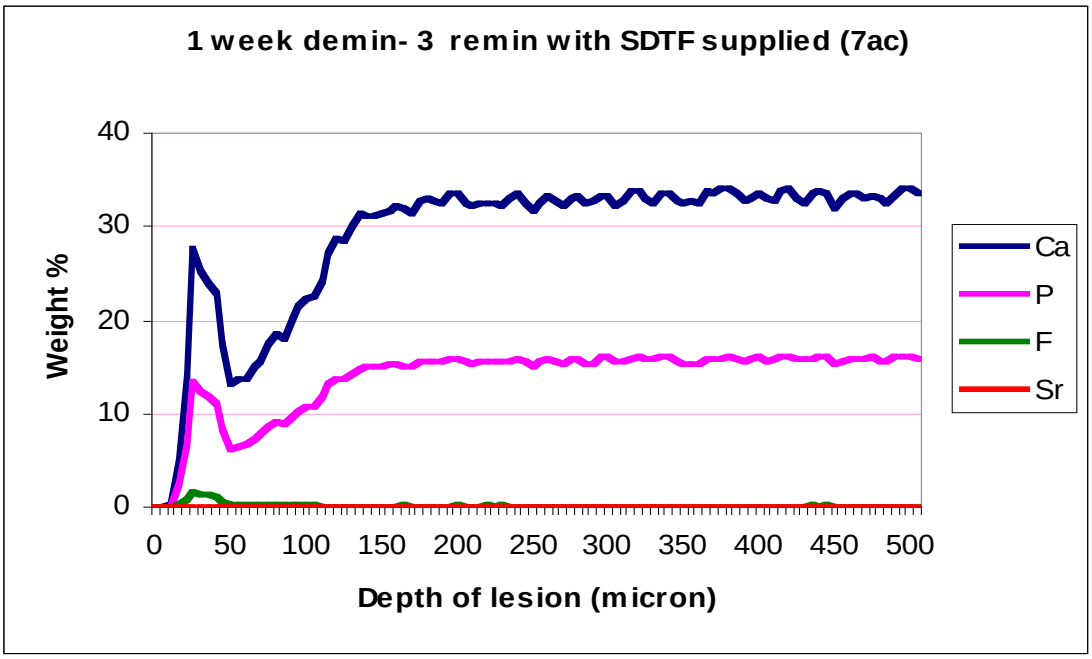
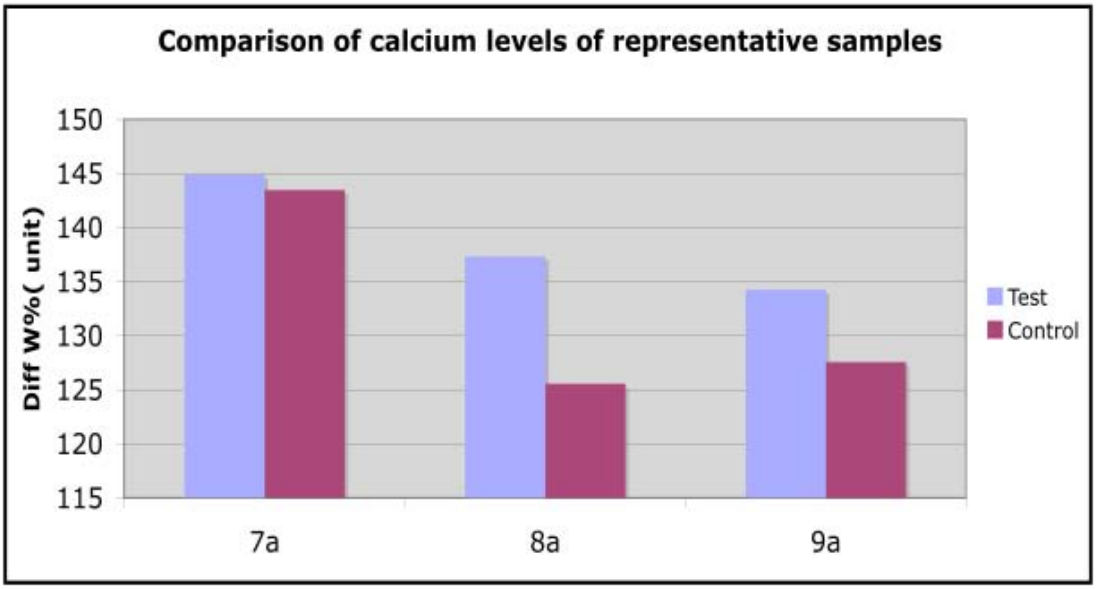


Figure 4: Calcium levels of test and control sides (7a7b, 8a8b, 9a9b)



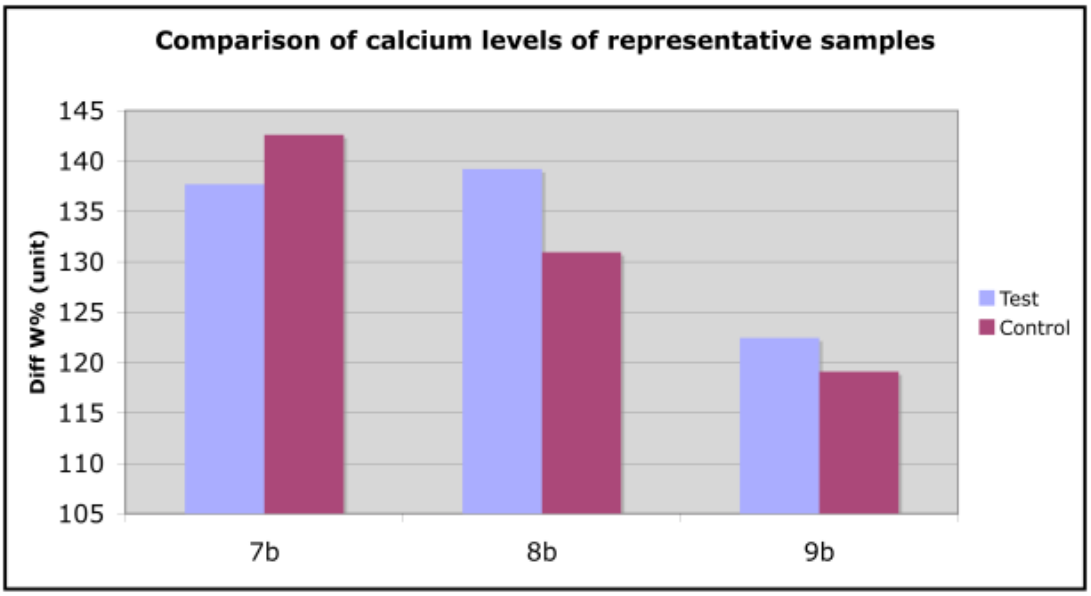


Figure 5: Comparison of calcium levels between the test and control sides of sample 7a

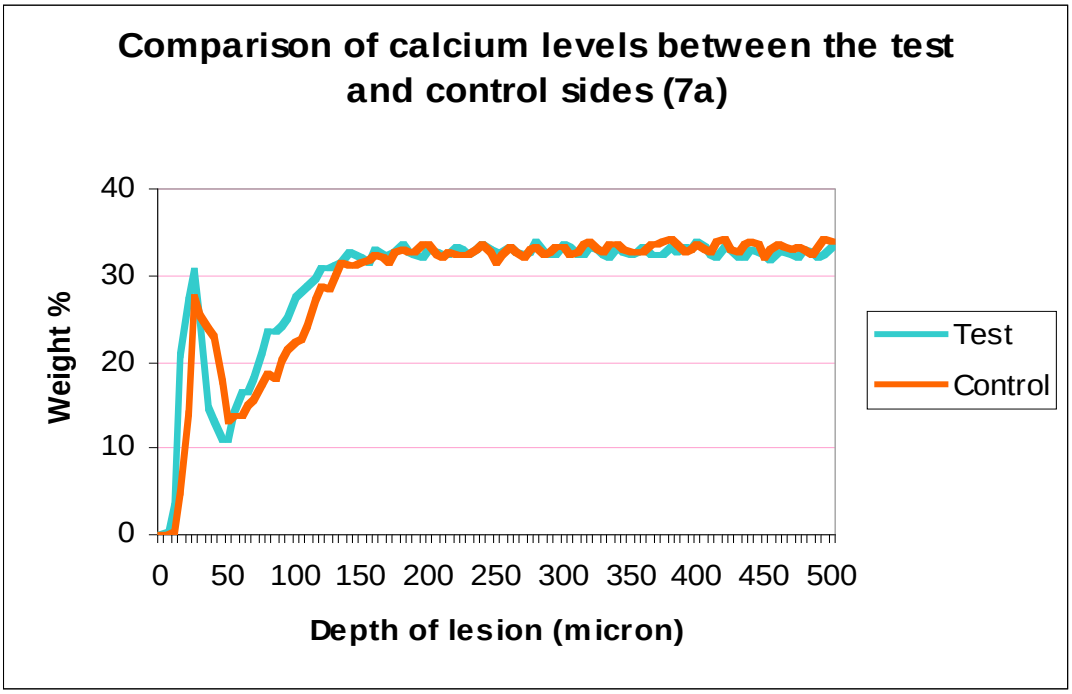


Figure 6: Comparison of calcium levels between the test and control sides of sample 7b

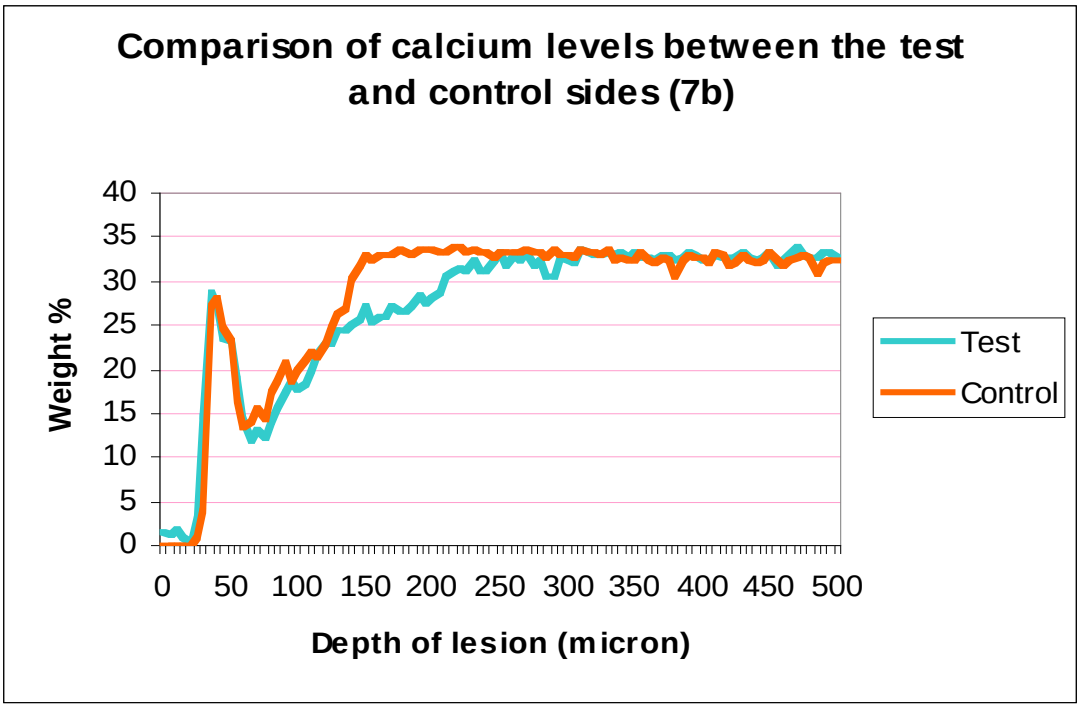
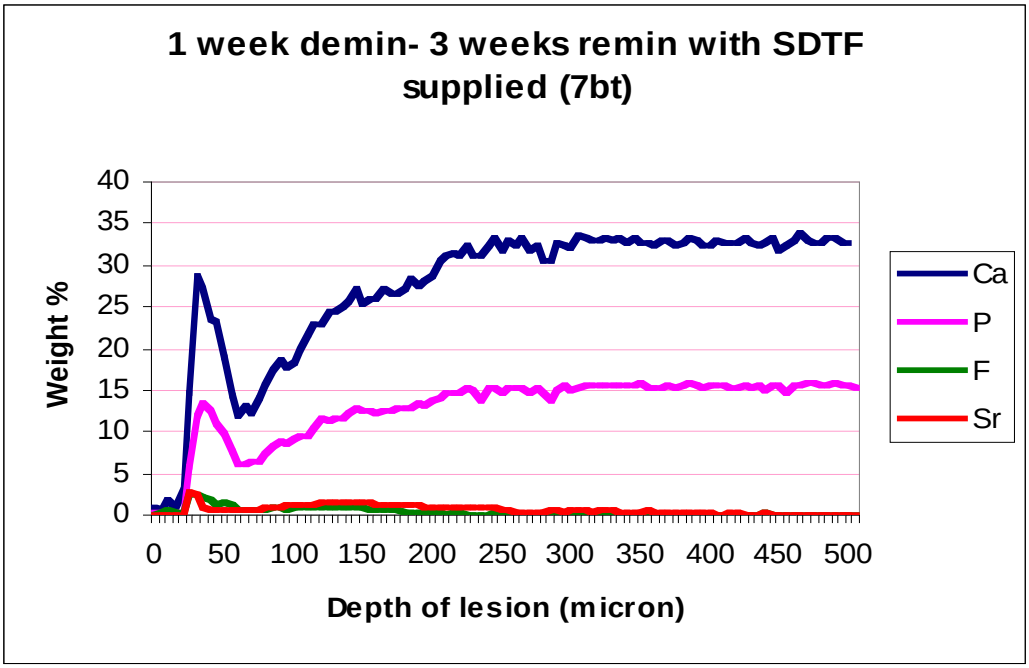


Figure 7: Mineral profiles on the test and control sides of sample 7b



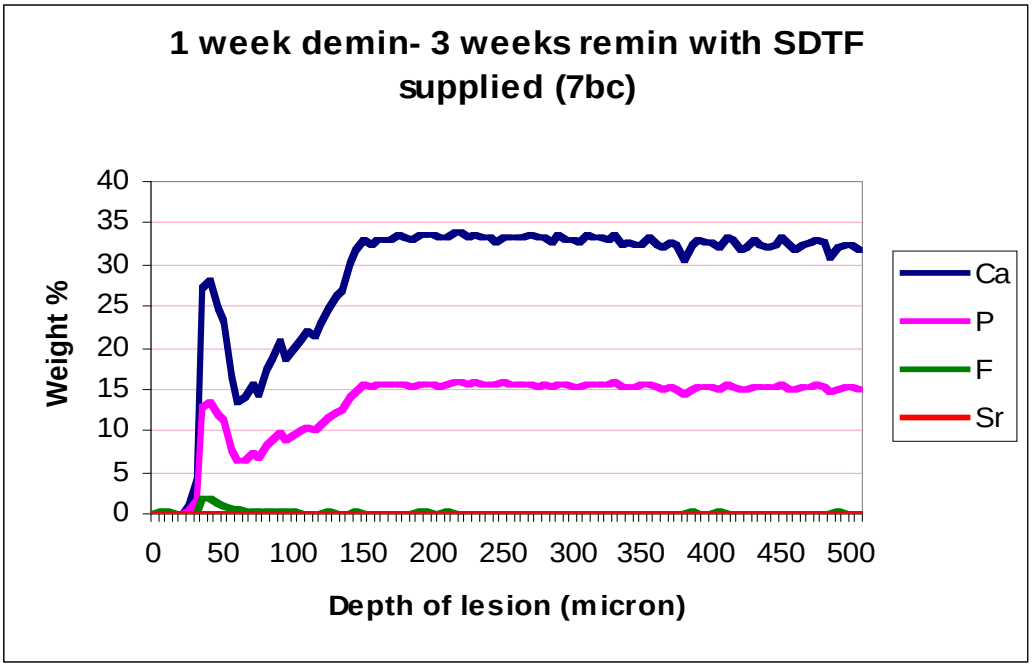
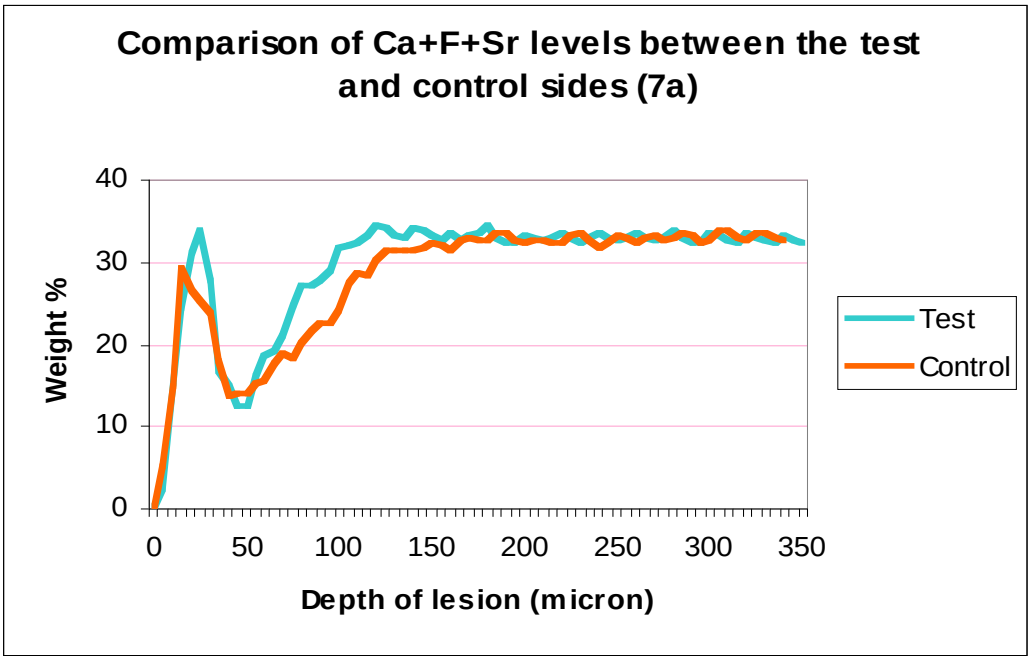


Figure 8: Aggregate Ca, F, Sr uptake on the test and control sides



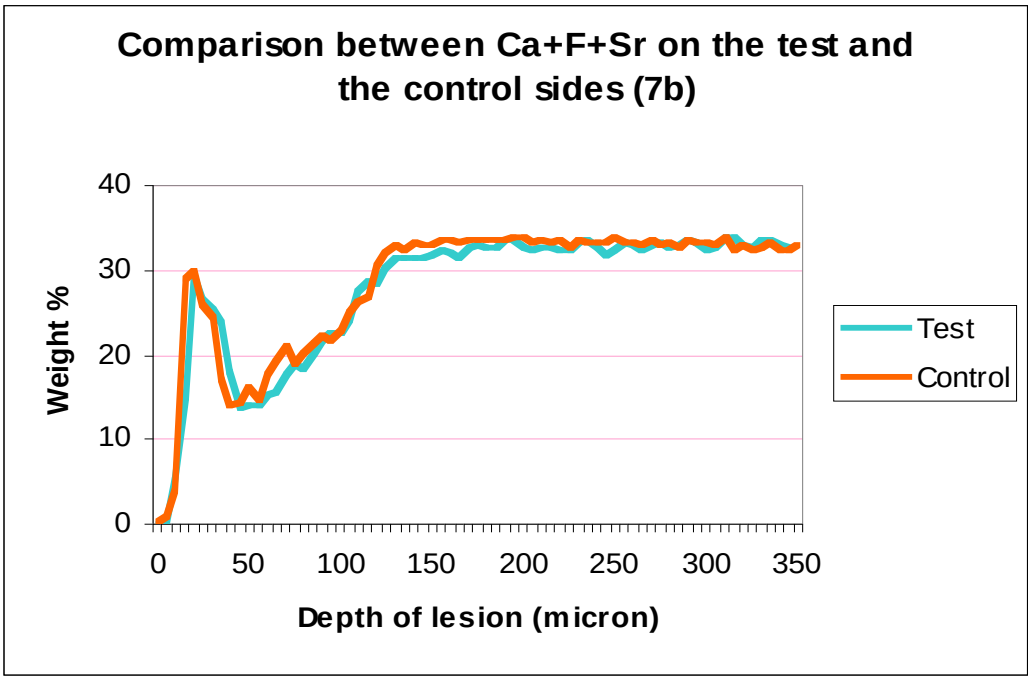
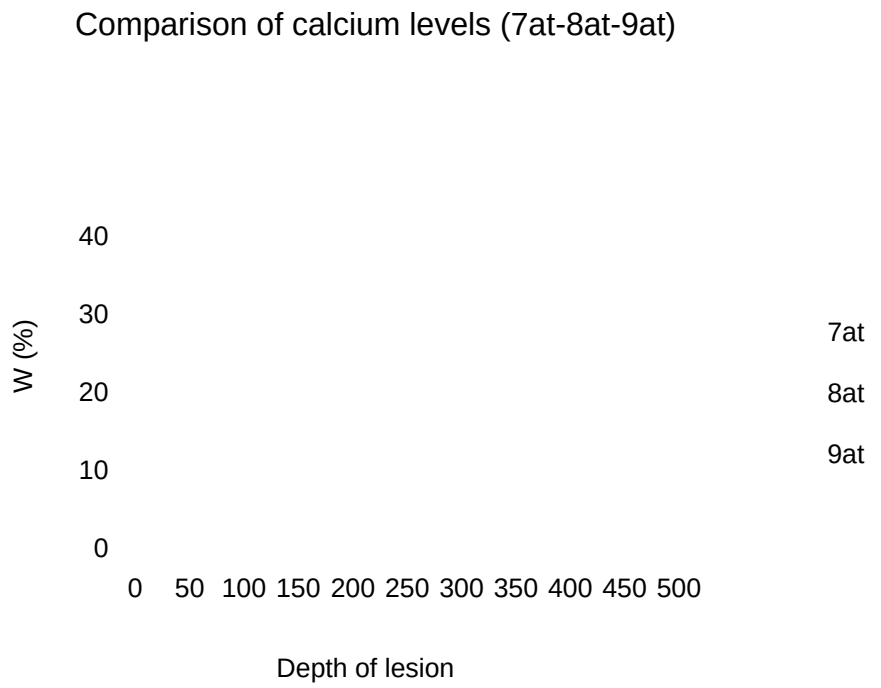
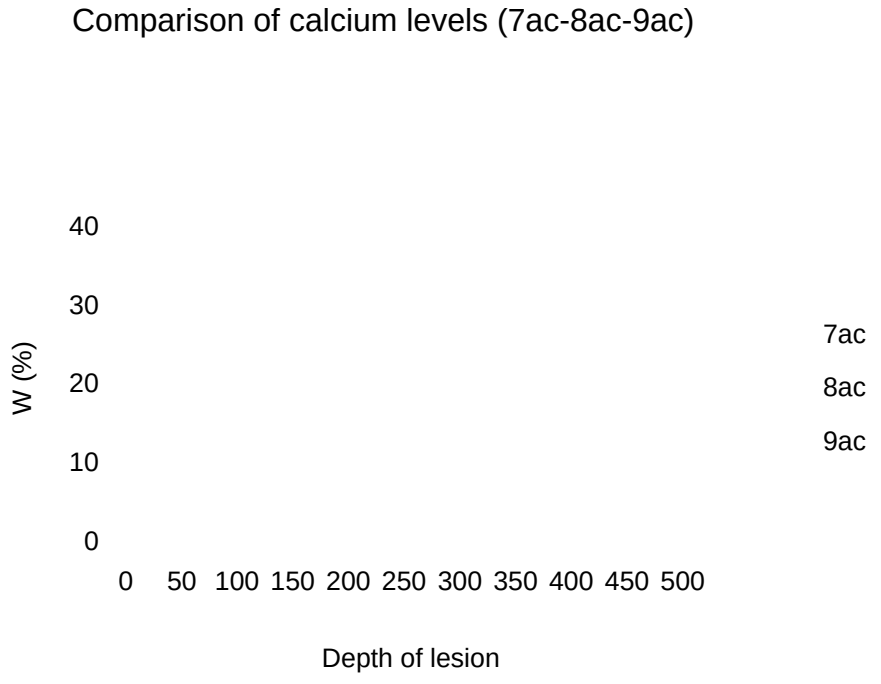


Figure 9: Comparison of calcium levels of samples 7a, 8a, 9a





List of tables

Table 1: Sample codes 125

Table 2: Means of lesion depth and calcium 126

Table 3: Aggregate mineral uptake of all samples 127

Table 4: AUC of calcium on test and control sides of all samples 128

Table 5: Mean of AUC of calcium of different demineralized time 129

Table 6: Perfusion of calcium in Fuji IX 130

Table 7: Δz Ca of three groups of remineralization relating to different demineralization time 131

Table 8: Comparison of Δz Ca from current study to that of a previous study 132

Table 1: Sample codes

Pre-demineralization time	Sample codes
1 week	1a, 1b, 4a, 4b, 7a, 7b
2 weeks	2a, 2b, 5a, 5b, 8a, 8b
3 weeks	3a, 3b, 6a, 6b, 9a, 9b

(NB: All samples within each group had similar treatment. Numbers 1 to 9 represented code numbers; letter “a” and “b” were code letters. The numbers and letter were used to name samples.)

Table 2: Means of lesion depth and calcium

Pre-demin	Ld test (µ)	Ld control(µ)	P values	Ca test (W%)	Ca control (W%)	P values
1 week (n=6)	155(32.7)	128.3(24.6)	**0.2**	30.6(0.3)	30.2(0.3)	**0.1**
2weeks (n=6)	195.8(67.4)	183.3(41.3)	**0.7**	30.3(0.3)	30.4(0.2)	**0.4**
3weeks (n=6)	206.6(62.4)	221.6(109.6)	**0.7**	29.9(1.0)	29.8(0.8)	**0.7**

Table 3: Aggregate mineral uptake of all samples

Samples (n=18)	Test	Control	Absolute diff
1a	13477.9	15004.8	-1526.9
1b	13566.2	13162.1	404.0
4a	14715.6	14173.4	542.3
4b	13931.2	14715.2	-784.0
7a	15209.8	14277.2	932.6
7b	13538.0	14731.9	-1194.0
2a	14815.5	11941.4	2874.1
2b	11617.3	11320.9	296.4
5a	14617.1	14149.2	468.0
5b	11824.4	13493.9	-1669.5
8a	13855.4	11803.1	2052.3
8b	13867.1	12828.5	1038.6
3a	13138.3	14790.1	-1651.8
3b	15711.2	14187.9	1523.2
6a	12502.4	11162.3	1340.1
6b	14644.8	13622.4	1022.4
9a	13697.7	12191.1	1506.6
9b	13056.4	10815.4	2240.9
Mean (SD)	13765 (1097)	13242(1386)	523

P=0.12

Table 4: AUC of calcium on test and control sides of all samples

Sample (n=18)	Test	Control	Absolute difference
1a	8800.893	10106.85	-1305.96
1b	9122.771	8877.056	245.7144
2a	9806.896	9566.174	240.722
2b	9078.285	9932.637	-854.352
3a	9999.889	9690.749	309.1396
3b	8881.78	9975.311	-1093.53
4a	9053.233	8016.874	1036.359
4b	6850.28	7615.874	-765.594
5a	9493.766	9548.779	-55.0128
5b	6996.058	8922.817	-1926.76
6a	9232.664	8097.562	1135.101
6b	8945.109	8488.383	456.7261
7a	8026.56	9914.686	-1888.13
7b	9994.116	9626.279	367.837
8a	8020.663	7063.554	957.109
8b	9773.607	9080.484	693.123
9a	8824.264	8215.362	608.9024
9b	8126.411	7440.844	685.5677
Mean (SD)	8835 (922)	8899(969)	-64

Table 5: Mean of AUC of calcium of different demineralized time

Demineralized time	Test	Control	Absolute diff
1w	9282(500)	9691(455)	-409.0
2w	8429(1181)	8448(637)	-19.0
3w	8794(898)	8556(1170)	238.0

Table 6: Perfusion of calcium in Fuji IX

Penetration depth (μ)	1bt	3bt	4at	5bt	9at	9bt	1bc
0	**2.06**	0.00	0.18	0.52	**1.40**	0.44	0.00
5	**1.85**	0.01	0.23	0.52	**0.80**	**1.32**	0.24
10	**1.99**	0.20	0.23	0.48	**0.74**	**1.40**	0.24
15	**1.49**	0.47	0.19	0.46	**1.44**	**1.47**	0.28
20	0.06	**1.26**	0.21	0.50	**1.61**	**1.29**	0.70
25	0.04	**0.80**	0.27	0.29	**0.96**	**1.41**	0.10
30	0.03	**0.71**	0.34	**1.07**	**1.39**	**1.21**	0.03
35	0.04	**1.49**	0.34	0.87	**1.29**	**1.14**	0.02
40	0.05	**1.78**	0.36	0.07	**0.85**	**1.43**	0.03
45	0.05	**1.30**	0.46	**1.29**	0.33	**0.74**	0.02
50	0.04	**0.92**	0.60	**1.69**	0.20	0.13	0.03
55	**1.09**	**1.04**	**0.81**	0.39	0.27	0.08	0.02
60	**8.50**	0.93	**0.62**	0.30	0.32	0.08	0.02
65	**9.07**	**1.31**	**0.69**	0.09	0.18	1.07	0.03
70	**10.40**	**1.64**	**0.95**	0.07	0.13	0.73	0.03
75	**9.90**	**1.18**	**1.33**	0.21	0.19	0.48	0.03
80	**11.10**	**0.21**	**1.27**	**1.20**	0.22	0.66	0.03
85	**10.69**	**1.50**	**1.61**	**4.95**	0.09	0.49	0.02
90	**11.57**	**8.86**	0.09	**3.28**	0.03	0.34	0.02
95	**11.53**	**17.77**	0.06	**9.56**	0.11	0.16	0.04
100	**15.18**	**16.12**	0.07	**9.68**	0.49	0.10	0.03

There was no statistical analysis for this table.

Table7: Δz Ca of three groups of remineralization relating to different demineralization time

Pre-demineralization		Δz Ca	Δz P	Δz Ca+P
1 week	Test	1474.4(408.1)	676.4(214.5)	2160(608.0)
	Control	1330.3(445.3)	717.4(167.5)	2048(578.4)
2 weeks	Test	1854.8(1013.3)	895.7(377.3)	2750(1382.2)
	Control	1944.8(608.2)	942.8(356.9)	2993(802.2)
3 weeks	Test	1399.3(492.6)	854.6(564.7)	2254(822.4)
	Control	1643.7(665.8)	856.8(300.5)	2500(939.3)

Table 7 shows no difference in Δz Ca between the test and control sides of one, two and three weeks of pre-demineralization and three weeks of remineralization with p=0.36, p= 0.78 and p= 0.25, respectively.

Table 8: Comparison of Δz Ca from current study to that of a previous study

	1 week		2 weeks		3 weeks	
	Test	Control	Test	Control	Test	Control
Ngo's results (n=5)	678(490)	1219(827)	4964(1508)	4496(3396)	4818(2201)	5335(3090)
Current results (n=6)	2160(608)	2048(578)	2750(1382)	2993(802)	2254(822)	2500(939)

Author Covering letter to the Australian Dental Journal (5)

AUSTRALIAN DENTAL JOURNAL

In submitting the manuscript titled

CHANGED CALCIUM PROFILES IN DEMINERALIZED DENTINE FOLLOWING INDIRECT PULP CAPPING TECHNIQUE

to the *Australian Dental Journal* I/we declare that:

1. The results are original, not falsified or plagiarised form any source.

2. All people involved with this report and all grants and scholarships which supported this work are duly acknowledged.

3. Credit to authorship is only to those who have participated substantially in the research work and preparation of this manuscript.

4. This paper is not currently under consideration for publication elsewhere.

5. All financial and personal relationships which might bias the interpretation of the work described in this manuscript have been fully disclosed

My Anh Vu Thanh

Date 16/07/2011

MANUSCRIPT5

Changed calcium profiles in demineralised dentine following indirect pulp capping

My Anh Vu Thanh

John McIntyre

Lindsay Richards

School of Dentistry, The University of Adelaide, Adelaide, SA 5005 Australia

(Manuscript of a Review to be submitted to the Australian Dental Journal)

Abstract

In previous studies (1), class one cavities with artificially generated dentinal caries were restored in Fuji IX and supplied with Simulated Dentinal Tubule Fluid (SDTF) via the pulp chamber. Increased calcium levels on both control and test side in some samples indicated a need for a model modification to gain further information on calcium baselines in dentine.

Objective: To determine calcium profiles in demineralized dentine under different experimental conditions.

Materials and methods: Demineralized dentine was generated into occlusal cavities in 12 intact extracted third molar teeth which were sectioned 1mm below CEJ to remove the root, then hemi-sectioned mesio-distally. Each half of the tooth was restored in GIC in one experiment and composite resin in the other, and afterward sealed onto a Perspex base. The restored halves were connected to a system supplied with SDTF or De-ionized Distilled Water (DDW) under specific hydrostatic pressure for 21 days, then detached from the system and prepared for Electron Probe Microscope Analysis (EPMA). Mineral profiles in weight percent were measured in dentine. Calcium and strontium profiles were compared between samples.

Results: Calcium levels increased significantly in demineralized dentine under composite and GIC restorations supplied with SDTF while there was no increase in calcium levels in those supplied with DDW (significant at 5%). Diffusion of strontium into demineralized dentine under GIC restorations was observed at a low level. Discussion and conclusion: The model enabled a comparison of calcium profiles between two halves of the tooth. Calcium profiles in samples supplied with DDW could be considered as calcium baseline. Increased calcium in samples supplied with SDTF

suggested that SDTF has the potential to increase calcium concentration in demineralized dentine in relation to those initially present. This would explain the effectiveness of the indirect pulp capping technique in achieving better remineralization of remnant demineralised dentine.

Introduction

The results from using the basic *in vitro* model described above showed that change in calcium levels occurred on both test and control sides. This made it difficult to interpret the degree of increase in calcium and phosphorus above that present in the initial lesion. This leads to a need of modification of the initial model to provide a more comprehensive set of baseline controls. In this enhanced model, the initial tooth preparation (generation of demineralized dentine) was the same. The main changes are described below.

Materials and methods

In this experiment, two halves of the tooth were restored in the same material, but supplied with different solution (SDTF or DDW). This provided four different experimental samples:

- Additional series of specimens were restored in composite resin (rather than with Fuji IX as was the case in the previous series of experiments) and supplied with DDW to demonstrate a "calcium baseline" as neither composite resin nor DDW supplied any additional mineral.
- A second additional test included specimens restored in composite resin and supplied with SDTF where calcium uptake from the SDTF (2) only is possible.
- Samples were restored in Fuji IX and supplied with DDW, demonstrating the ion-uptake from Fuji IX into demineralized dentine (as in the previous series of experiments)
- Samples restored in Fuji IX and supplied with SDTF could demonstrate the effect of the interaction between the Fuji IX and SDTF on the demineralized dentine.

Table 1 illustrates these combinations.

The placement of restorations

The restorative materials Fuji IX and resin composite Z100 were used in this experiment.

Six teeth with pre-demineralized cavities which were prepared previously were selected into this experiment. Each tooth was sectioned labial-lingually. Six halves from three teeth were included in the experiment using Fuji IX as the restorative material. The other six halves from the other three teeth were include in another experiment using composite resin as the restorative material. A groove was cut in the middle of the cavity of each half. One side of the cavity including the groove was painted with nail varnish providing the control and the test side.

In the first experiment, each half of the tooth was sealed onto a Perspex bases using opaque unfilled resin (Denton, Densply). To provide a micro-mechanical bonding and ensure a good seal between the Perspex bases and the tooth halves, sand blasting was carried out on the Perspex base individually. When Fuji IX was used, the procedure was applied as described in methodology. After being restored, the samples were connected into the system which supplied the SDTF or DDW under a prescribed hydrostatic pressure (2, 3), in an incubator of 37^0 C for 21 days.

Figure 1 shows each half of the tooth being sealed onto a Perspex base and was ready for individual experiment. Sample 1AFS was restored in Fuji IX and supplied with SDTF whilst sample 1AFD was restored in Fuji IX and supplied with DDW.

In the second experiment, sectioned teeth were also sealed onto a Perspex base as described in the previous experiment. When composite Z100 was used, the unpainted part of the cavity was etched using acid etch gel (SDI, Melnbourne, Australia)

containing 37% orthophosphoric acid for 10 seconds to remove the debris. SE bond system was used in this experiment. Primer (Clearfil SE Bond, Kukaray Medical Inc, Okayama, Japan) was applied onto the etched surface for 20 seconds. Adhesive resin (Clearfil SE Bond, Kukaray Medical Inc, Okayama, Japan) was applied for 10 seconds and light cured for 20 seconds.

After being restored, the samples were connected to the system which supplied the SDTF or DDW under a prescribed hydrostatic pressure, in an incubator of 37^0 C for 21 days.

In order to simulate the oral environment more closely, an "artificial saliva" solution comprising 2.2mM $CaHPO_4$, 0.05M acetate at pH 7 was placed in the chamber surrounding the restored cavities.

The samples from experiments were prepared for EPMA as described above. The AUC values were calculated and compared between samples. Samples were coded as shown in Table 2.

Results

Calcium levels on the test and control sides

As shown in the Table 3, in the presence of SDTF, calcium levels on the test sides were not different compared to those of the control side p= 0.26 (samples restored in Fuji IX).

Figure 2 illustrates one example of the data from Table 14. Sample 1AFS (Fuji IX restoration and SDTF perfusion) and 1AFD (Fuji IX restoration and DDW perfusion) are representative samples. As shown in the left chart, the calcium level on the test side (in blue colour) is equal to that on the control side (in pink colour). In the right chart, calcium level on the control side (pink) is significantly greater than that on the test

side. Calcium levels of other samples also were charted and can be seen in the Appendix 4 in Vu's thesis (1).

The experimental design in this series of experiments allowed other pairwise comparisons. Calcium levels on the test sides of the samples restored with Fuji IX demonstrated significantly higher levels for specimens perfused with SDTF comparing to that of specimens perfused with DDW (Table 5).

Figure 3 illustrates the difference in calcium level (on test side only) between samples restored with Fuji IX and supplied with either SDTF (1AFST) or DDW (1AFDT).

For the series of specimens restored with resin composite (i.e. no mineral available from the restorative material) and supplied with SDTF the calcium levels tended to be greater on the test sides than on the control sides (Table 6).

In the specimens restored with CR and supplied with DDW (no mineral available from either the restoration of the perfusing solution), there is no difference in calcium level between the test and the control sides (Table 7).

Figure 4 illustrates the trends in the data presented in table 7 and 8. The left chart illustrates the calcium levels of sample restored in CR and supplied with SDTF (1ACS). As indicated in the left chart, calcium level on the test is greater than that on the control side. Calcium levels of the sample was restored in CR and supplied with DDW is illustrated in the right chart. There is no difference in calcium levels between the test and control sides of this sample.

Table 8 includes data comparing of calcium levels on the test sides of the CR/SDTF and CR/DDW group, there was no significant difference in calcium levels between those groups.

Figure 5 compares the calcium levels between the test and control sides of sample restored in CR and supplied with either SDTF (1ACST) or DDW (1ACDT). There was a slight difference in calcium levels between two samples but not significantly as stated above.

Ca/P ratio

In Table 9, the first column indicates the depth of the lesion in microns (made at 5microns intervals). The other columns indicate the calcium/phosphorus ratios of six representative samples. Both samples restored in Fuji IX (AFS series) and CR (ACS series) have calcium/phosphorus ratios increased towards the ratio of hydroxylapatite.

Discussion and conclusion

The aim of this paper was to investigate changes in calcium levels compared with controlled baseline levels. The findings of this stage were:

- There was no significant difference in calcium levels on the test and control sides of the samples restored in CR and supplied with DDW. The calcium level on the test sides of samples restored in CR and supplied with SDTF tended to be greater than that on the control sides but the differences were not statistically significant.
- The calcium levels on the test sides of the samples restored in Fuji IX and supplied with SDTF tended to be greater than those on the control sides (not significant). The calcium levels on the control sides of the samples restored in Fuji IX and supplied with DDW were significantly greater than those on the test side. This could be explained by the uptake of strontium and fluorine which changed the concentration of calcium. However, calcium levels of the samples

supplied with SDTF were greater than those of the samples supplied with DDW.

The results from this stage confirmed the diffusion of SDTF into demineralized dentine and provided data on baseline levels of calcium in demineralized dentine. The question for the next stage is whether other factors influence the remineralization process. This question will be addressed in the next paper.

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Legends

Figure 1: Halves of the tooth being sealed onto Perspex bases..... 145

Figure 2: The test and control sides of representative samples supplied wih SDTF/DDW 146

Figure 3: Comparison of calcium levels between the SDTF and DDW supplied samples 147

Figure 4: The test and control sides of representative samples supplied with SDTF/DDW..... 148

Figure 5: Comparison of calcium levels of samples restored in CR and supplied with SDTF/DDW..... 149

Figure 1: Halves of the tooth being sealed onto Perspex bases

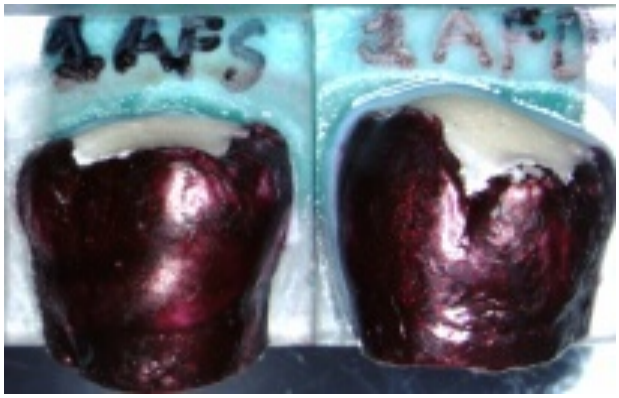
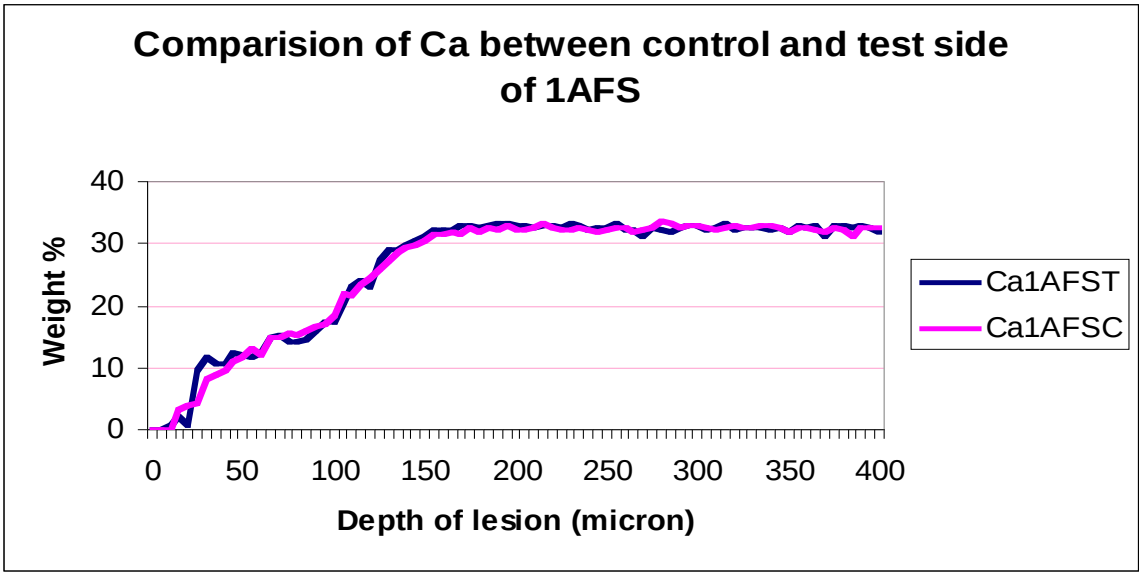


Figure 2: The test and control sides of representative samples supplied wih SDTF/DDW



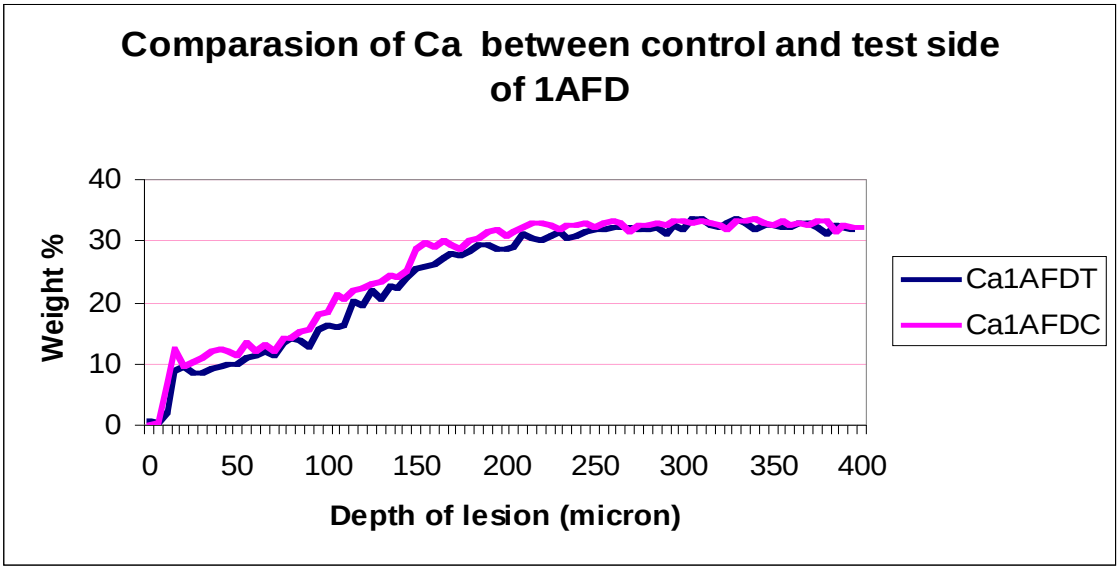


Figure 3: Comparison of calcium levels between the SDTF and DDW supplied samples

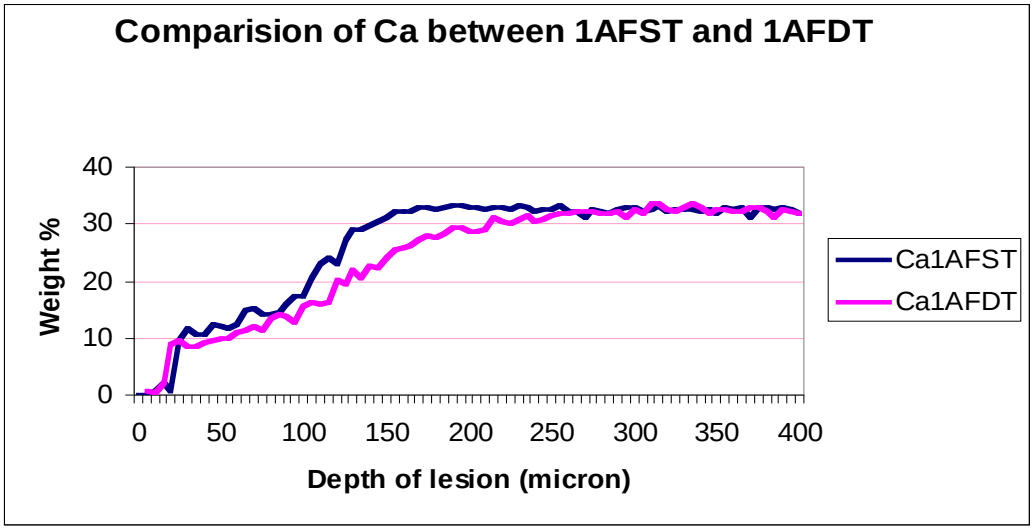
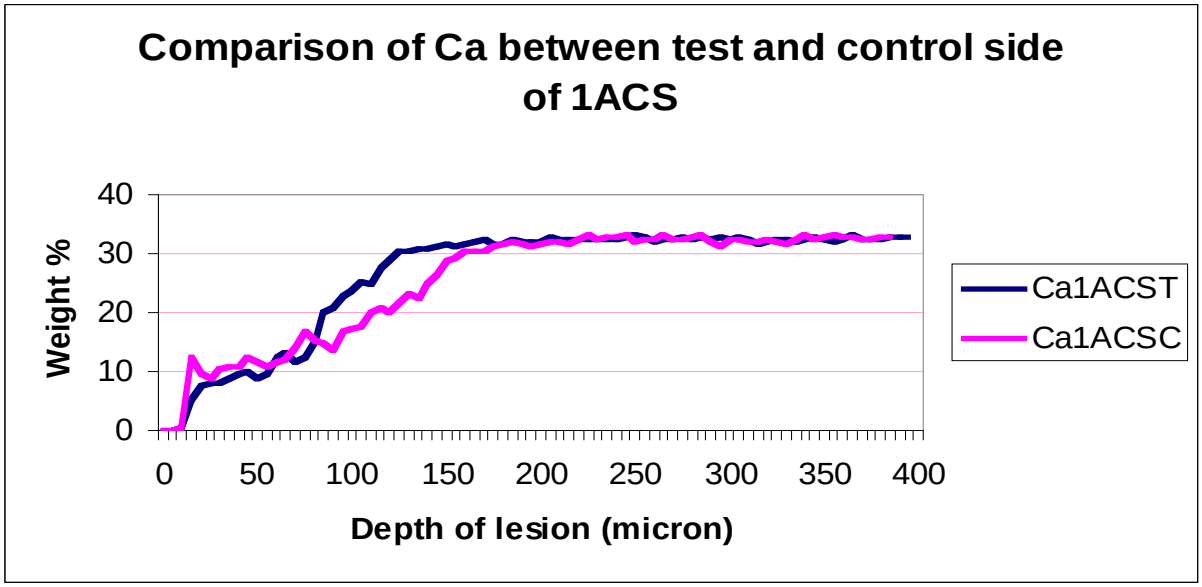


Figure 4: The test and control sides of representative samples supplied with SDTF/DDW



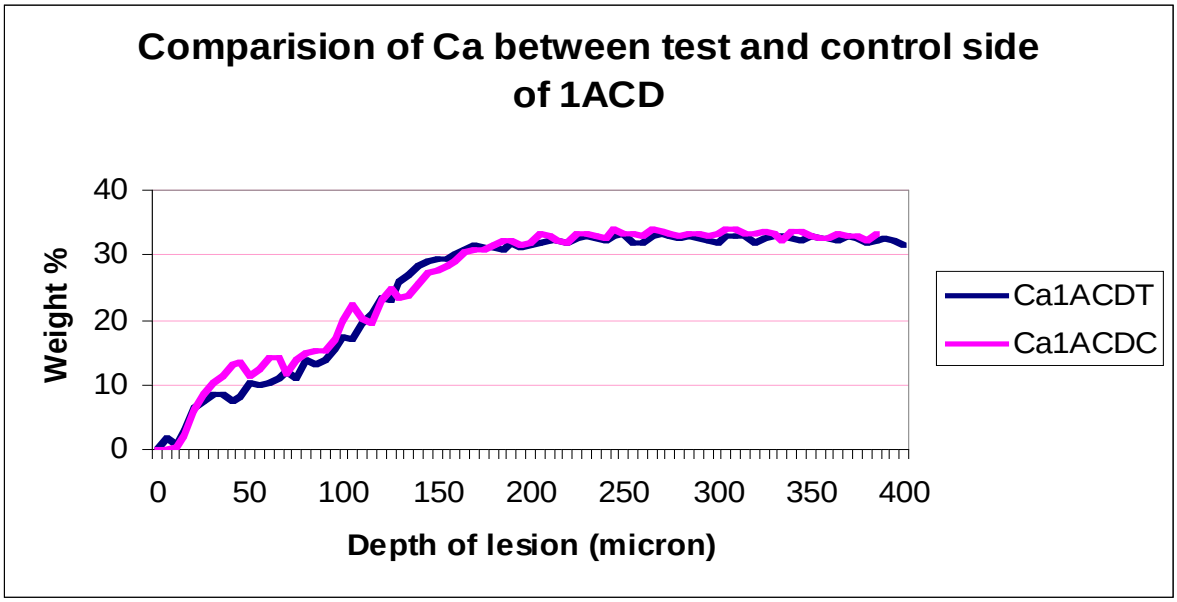
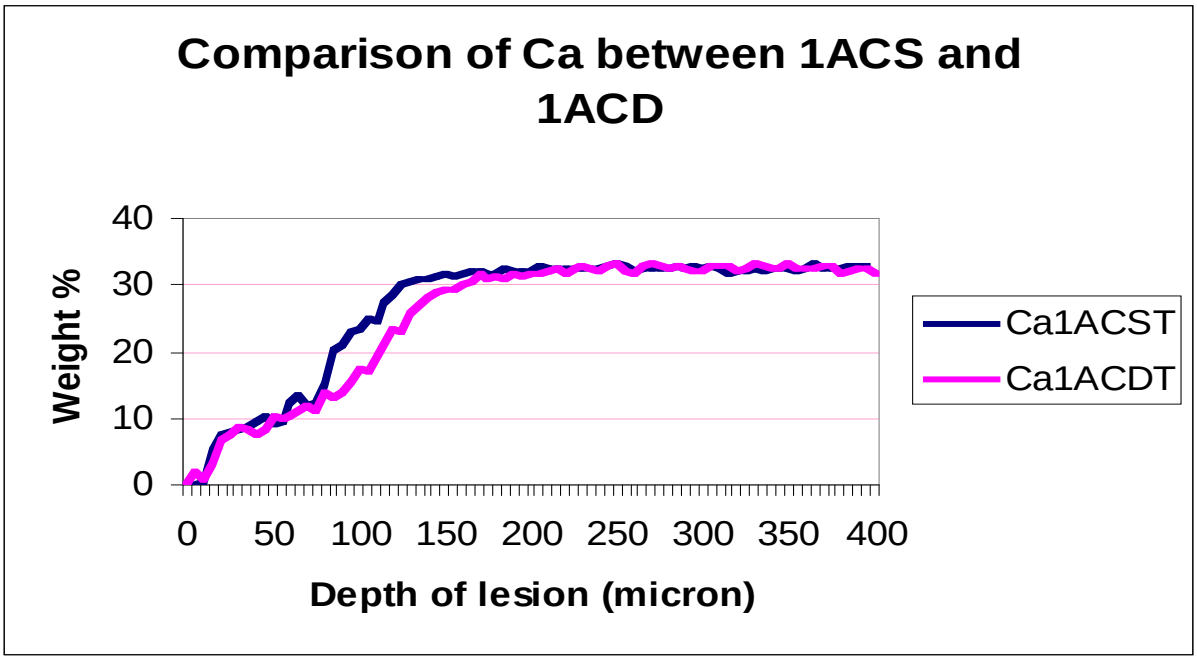


Figure 5: Comparison of calcium levels of samples restored in CR and supplied with SDTF/DDW



List of tables

Table 1: Illustration of the combination between restorative materials and diffusing solutions 151

Table 2: Sample codes for calcium baseline experiment 152

Table 3: AUC of calcium of samples restored in Fuji IX and supplied with SDTF..... 153

Table 4: Comparison of AUC calcium between the test and control sides of samples supplied with DDW..... 154

Table 5: Comparison of AUC of calcium between samples supplied with SDTF/DDW155

Table 6: Comparison of AUC calcium between the test and control side (CR/SDTF group)..... 156

Table 7: Comparison of AUC calcium between the test and control side (CR/DDW group 157

Table 8: Comparison of AUC calcium between the CR/SDTF and CR/DDW group... 158

Table 9: Calcium/phosphorus ratios of six representative samples..... 159

Table 1: Illustration of the combination between restorative materials and diffusing solutions

Restorative materials	DDW	SDTF
CR	CR/DDW	CR/SDTF
GIC	GIC/DDW	GIC/SDTF

Table 2: Sample codes for calcium baseline experiment

Pre-demineralization	Samples restored in GIC		Samples restored in CR	
	SDTF diffusion	DDW diffusion	SDTF diffusion	DDW diffusion
1 week	1AFS, 4AFS	1AFD, 4AFD	1ACS, 4ACS	1ACD, 4ACD
2 weeks	2AFS, 5AFS	2AFD, 5AFD	2ACS, 5ACS	2ACD, 5ACD
3 weeks	3AFS, 6AFS	3AFD, 6AFD	3ACS, 6ACS	3ACD, 6ACD

(NB. Number code 1,2,3,4,5,6 refers to 1 week (1 &4), 2 weeks (2&5) and 3 weeks (3&6) of demineralization respectively. Code "A" refers to the name of this experiment. Code "F" or "C" refers to restorative material which was placed into the cavity. Code "S" or "D" refers to SDTF or DDW was being supplied to the pulp chamber.)

Table 3: AUC of calcium of samples restored in Fuji IX and supplied with SDTF

Samples	Test	Control	Absolute diff
AUC of 1AFS	9269.2	9360.2	-90.9
AUC of 4AFS	10053.4	10093.7	-40.3
AUC of 2AFS	9414.1	9142.3	271.7
AUC of 5AFS	9185.4	9317.4	-132.0
AUC of 3AFS	8023.2	7332.3	690.9
AUC of 6AFS	8835.9	8582.7	253.3
Mean(SD)	9130(673)	8971(937)	158

By comparing the AUC data presented in Table 4, in the absence of SDTF (ie specimens perfused with DDW), the calcium levels on the control sides were significantly greater than those on the test sides which had been restored with Fuji IX.

Table 4: Comparison of AUC calcium between the test and control sides of samples supplied with DDW

Samples	Test	Control	Absolute diff
AUC of 1AFD	8592.9	9143.8	-551.0
AUC of 4AFD	8874.7	9576.0	-701.3
AUC of 2AFD	8770.3	9247.4	-477.2
AUC of 5AFD	9297.0	8987.0	310.0
AUC of 3AFD	6190.5	7065.4	-874.9
AUC of 6AFD	7592.9	7978.6	-385.8
Mean(SD)	8219(1143)	8666(952)	-446.7

P = 0.04

Table 5: Comparison of AUC of calcium between samples supplied with SDTF/DDW

Samples	SDTF	DDW	Absolute diff
AUC of 1AF	9269.2	8592.9	676.4
AUC of 4AF	10053.4	8874.7	1178.7
AUC of 2AF	9414.1	8770.3	643.8
AUC of 5AF	9185.4	9297.0	-111.5
AUC of 3AF	8023.2	6190.5	1832.7
AUC of 6AF	8835.9	7592.9	1243.1
Means(SD)	9130.2(973)	8219.7(1143)	910

P = 0.02

Table 6: Comparison of AUC calcium between the test and control side (CR/SDTF group)

Samples	Test	Control	Absolute diff
AUC of 1ACS	9556.2	9181.9	374.2
AUC of 4ACS	9922.4	9458.6	463.8
AUC of 2ACS	9131.5	8124.1	1007.3
AUC of 5ACS	9445.9	8927.5	518.4
AUC of 3ACS	9092.1	7334.9	1757.1
AUC of 6ACS	8167.1	8594.8	-427.7
Means(SD)	9219.2	8603.6	615.5

P = 0.09

Table 7: Comparison of AUC calcium between the test and control side (CR/DDW group)

Samples	Test	Control	Absolute diff
AUC of 1ACD	9071.8	9317.2	-245.4
AUC of 4ACD	8971.2	9913.9	-942.6
AUC of 2ACD	9598.0	8785.8	812.3
AUC of 5ACD	9405.9	8508.8	897.2
AUC of 3ACD	8294.1	7781.1	513.1
AUC of 6ACD	8167.1	8272.9	-105.8
Means(SD)	8226.9	8114.8	154.7

P = 0.2

Table 8: Comparison of AUC calcium between the CR/SDTF and CR/DDW group

Samples	SDTF	DDW	Absolute diff
AUC of 1AC	9556.2	9071.8	484.5
AUC of 4AC	9922.4	8971.2	951.2
AUC of 2AC	9131.5	9598.0	-466.5
AUC of 5AC	9445.9	9405.9	39.9
AUC of 3AC	9092.1	8294.2	797.9
AUC of 6AC	8167.1	8733.3	-566.2
Means(SD)	9219.2	9012.4	206.8

P = 0.82

Table 9: Calcium/phosphorus ratios of six representative samples

Depth (μm)	1AFS	2AFS	3AFS	1ACS	2ACS	3ACS
0	4.9	5.8	2.0	4.9	3.1	4.2
5	2.0	1.9	2.0	2.0	2.0	2.1
10	2.0	1.7	2.0	2.0	1.9	2.0
15	2.0	1.9	2.0	2.0	2.0	2.0
20	2.0	2.0	2.1	2.0	2.0	2.0
25	2.0	2.0	2.0	2.0	2.0	2.0
30	2.0	2.1	2.0	2.0	2.0	2.0
35	2.1	2.0	2.0	2.1	2.0	2.1
40	2.1	2.0	2.0	2.1	2.0	2.0
45	2.0	2.0	2.1	2.0	2.0	2.1
50	2.0	2.1	2.1	2.0	2.0	2.1

Author Covering letter to the Australian Dental Journal (6)

AUSTRALIAN DENTAL JOURNAL

In submitting the manuscript titled

COMPARISON OF REMINERALIZATION EFFECT BETWEEN

FUJI IX AND KETACT MOLAR

to the *Australian Dental Journal* I/we declare that:

1. The results are original, not falsified or plagiarised form any source.

2. All people involved with this report and all grants and scholarships which supported this work are duly acknowledged.

3. Credit to authorship is only to those who have participated substantially in the research work and preparation of this manuscript.

4. This paper is not currently under consideration for publication elsewhere.

5. All financial and personal relationships which might bias the interpretation of the work described in this manuscript have been fully disclosed

My Anh Vu Thanh

Date 16/07/2011

MANUSCRIPT 6

Comparison of remineralization effect between Fuji IX and Ketac Molar

My Anh Vu Thanh

John McIntyre

Lindsay Richards

School of Dentistry, The University of Adelaide, Adelaide, SA 5005 Australia

(Manuscript of a Review to be submitted to the Australian Dental Journal)

Abstract

Objective: To compare the effect of Fuji IX and Ketac Molar on remineralization of the demineralized dentine. Method: Class one cavities of similar dimension were cut into the occlusal surfaces of 30 extracted, intact third molar teeth. These teeth were painted with varnish leaving the cavity floors exposed for generation of artificial caries (4 teeth each for 7-14 and 21 days of demineralization). Teeth were assigned to two groups: 18 teeth using Fuji IX and 12 teeth using Ketact Molar. One half of the demineralized cavity floor was protected with nail varnish (control side) and Fuji IX or Ketac Molar restorations placed (test side). A reservoir was connected to each tooth to supply Simulated Dentine Tubular Fluid under hydrostatic pressure to each pulp chamber for 21 days during which ion exchange between GICs and demineralized dentine was proceeding. After three weeks, teeth were detached from the system, sectioned and prepared for Electron Probe Analysis to determine profiles of Ca, P, F and Sr.

Results: In the Fuji IX group, relative weight percents of calcium on the test sides tended to be greater than those on the control sides. The Ketac Molar group exhibited significantly higher calcium level on the control sides. Comparing calcium levels in demineralized dentine between the Fuji IX and Ketac Molar groups revealed no significant differences. The Ca/P ratios in demineralized dentine of both groups increased toward the ratio of apatite from the superficial lesion to the body lesion. Conclusion: The results indicated that the Fuji IX restorative material was associated with increased calcium levels on the test side and therefore enhanced the reformation of lost apatite. However, when Ketac Molar (a calcium-rich material) was placed, there

was an increase in calcium level on the control side. The effect of Ketac Molar on remineralization needs to be investigated further.

Introduction

The influence of SDTF (1) on remineralization was demonstrated in previous stages of this study (2).

The objectives of these experiments were to investigate:

- The interaction between SDTF and other calcium rich materials
- The effect of exposure time of demineralized dentine to SDTF on remineralization in the presence of Fuji IX.

Twelve teeth with pre-demineralized dentine (generated as describe in methodology of Manuscript 3) were used and the basic *in vitro* model was applied in this experiment.

The objective of this experiment was to investigate the interaction between a calcium-based GIC (Ketac Molar) and SDTF in comparison to that from the strontium-based Fuji IX.

Material and methods

One capsule of Ketac Molar (3M ESPE) was mixed for 10 seconds using the mixing machine then was inserted into a gun. The first 5mm of the Ketac Molar was discarded, and the rest was placed into the cavity as described for Fuji IX. The restored crowns were then sealed onto a Perspex base using sticky wax as described previously. The system was supplied with the SDTF under the prescribed hydrostatic pressure which was maintained through experimental time and was placed in an incubator at

37^0C for 21 days. In order to simulate the oral environment more closely, an "artificial saliva" solution was placed in the chamber surrounding the restored cavities.

Following this time, the samples were prepared for EPMA as described above. Samples were coded as shown in Table 1.

Results

Comparison of calcium levels between the test and control sides of all samples

Twelve samples were included in this experiment and eleven samples were analysed (one sample (K6b) was excluded because of a damaged surface). AUC values of calcium, phosphorus, fluoride and strontium were calculated and compared between the test and the control sides and between samples. Table 2 presents that data for this experiment. Generally, calcium levels on the control sides were significantly greater than those of the test sides.

Figure 1 illustrates the comparison of calcium level between the test and control sides of two representative samples. Sample K4a was subjected one week of demineralization and sample K5a was subjected two weeks of demineralization. Both of them were remineralized under Ketac Molar restorations and supplied with SDTF. The second chart shows much greater increase in the calcium on the control side compared to that of the first chart.

Comparison of aggregate mineral uptake on the test and control sides

When the aggregate element uptake was considered (compared with data for calcium alone), AUC values of all elements on the control sides were significant greater than those on the test sides (Table 3).

Figure 2 illustrates the mineral profiles of the test and control sides in a representative sample from this experiment. In the first chart, the calcium and fluorine levels are

higher in the restoration. Fluoride level was also high in the demineralized dentine while on the control side, the fluoride level was almost nil.

Comparison of calcium plus fluorine levels between the test and control sides

Phosphorus levels on the control side were also greater than those of the test side ($p=0.003$). There is no difference in strontium levels between the test and control sides ($p=0.2$). The fluorine levels were found greater on the test than the control sides ($p=0.0006$).

Table 4 shows the aggregate Ca+F on the test and control sides. As shown in the table, there is no statistical difference in aggregate Ca+F between the test and control sides.

Comparison of AUC between Fuji IX and Ketac Molar groups

Table 5 includes the mean AUC of calcium profiles of the test and control sides of the samples restored in Fuji IX and Ketac Molar by different levels of pre-demineralization (one, two and three weeks).

Table 6 shows the comparison of mean AUC of calcium profiles between Fuji IX samples to those of Ketac Molar samples (test side only). The mean AUC of calcium profiles in samples restored in Fuji IX tended to be greater than those of the samples restored in Ketac Molar as shown in the fourth column. However, the difference is not significant with p values 0.25; 0.79 and 0.15 for one, two, three weeks of pre-demineralization, respectively.

Calcium /Phosphorus ratios

Table 7 shows the calcium phosphorus ratios of representative samples by depth of lesion. In most samples, the Ca/P ratio was from 1.9 to 2 from the lesion front through the whole depth of the lesion. Sample 3a (on the test side) showed low calcium phosphorus ratios. This will be discussed later.

Discussion and conclusion

Three experiments were carried out to answer the question arose from previous stages of experiments. This experiment addressed the interaction of SDTF with other calcium rich materials.

In the presence of Ketac Molar and SDTF, there was a decrease in calcium levels on the test side in comparison to those on the control sides. However, the calcium phosphorus ratios increased towards the ratio of calcium apatite. The results indicated that the Fuji IX restorative material was associated with increased calcium levels on the test side and therefore enhanced the reformation of lost apatite. However, when Ketac Molar (a calcium-rich material) was placed, there was an increase in calcium level on the control side. The effect of Ketac Molar on remineralization needs to be investigated further.

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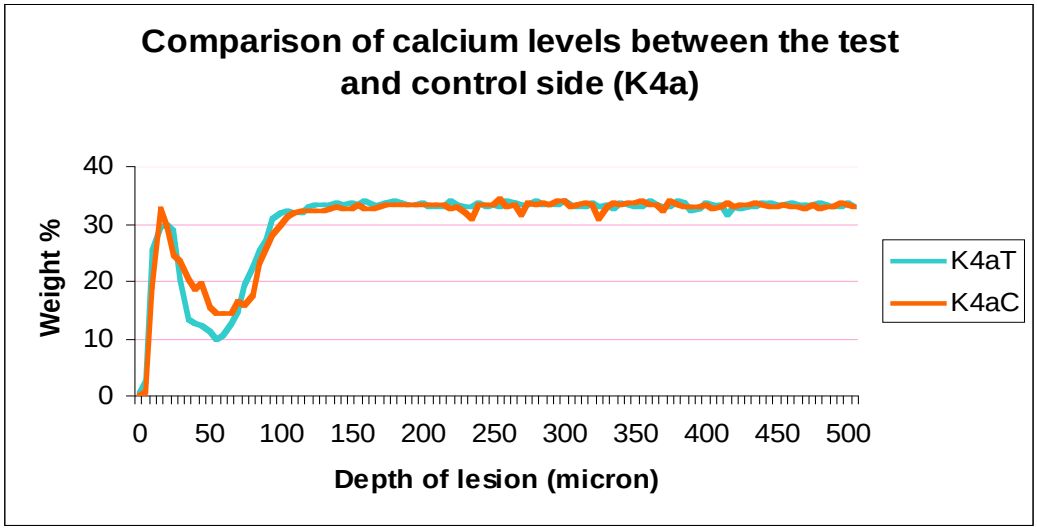
Legends

Figure 1: Comparison of calcium profiles between the test and control sides 169

Figure 2: Mineral profiles on the test and control sides of a representative sample170

Figure 1:Comparison of calcium profiles between the test and control sides



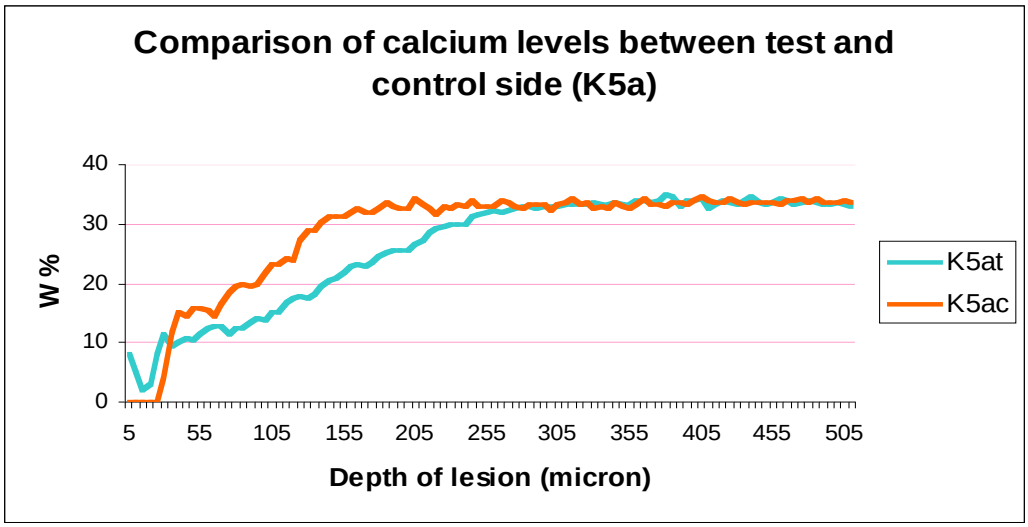
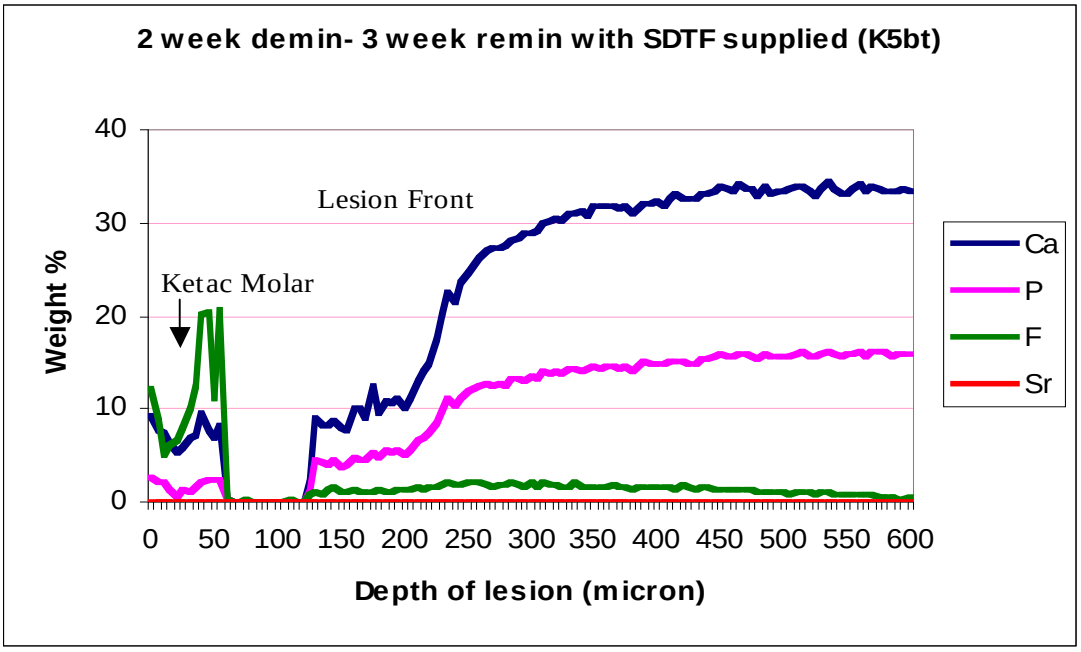
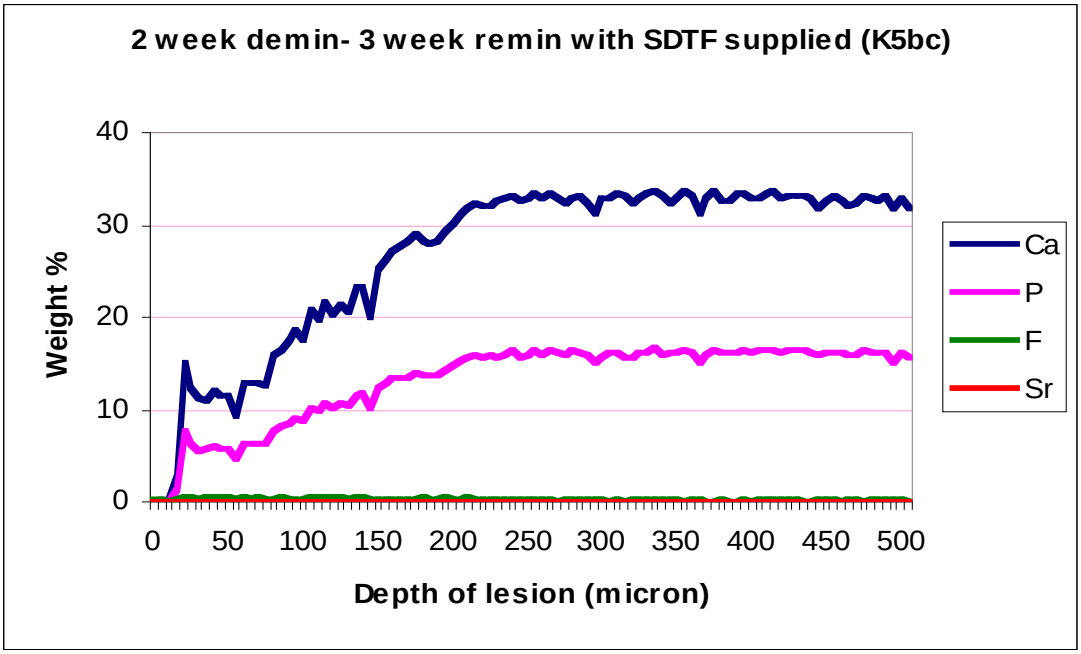


Figure 2:Mineral profiles on the test and control sides of a representative sample





List of tables

Table 1: Sample codes for the experiment using Ketac Molar as a restorative material 172

Table 2: Comparison of calcium profiles between the test and control sides 173

Table 3: Aggregate mineral uptake on the test and control sides of all samples..... 174

Table 4: Comparison of AUC calcium and fluorine between the test and control side175

Table 5: Comparison of AUC Ca between Fuji IX and Ketac Molar samples 176

Table 6: Comparison of AUC Ca between the Fuji IX and Ketac Molar samples (test sides) 177

Table 7: Calcium/phosphorus ratios of some representative samples 178

Table 1: Sample codes for the experiment using Ketac Molar as a restorative material

Demineralization time	Sample codes
1 week	K1a, K1b, K4a, K4b
2 weeks	K2a, K2b, K5a, K5b
3 weeks	K3a, K3b, K6a, K6b

(NB. K means the restorative material in this experiment was Ketac Molar. 1&4 refers to one week, 2&5 refers to two weeks and 3&6 refers to 3 weeks of demineralization. “a” and “b” are codes for samples in the same category (same demineralization, restorative material and diffusing solution).

Table 2: Comparison of calcium profiles between the test and control sides

Sample's name	Test	Control	Absolute diff
AUC of K1a	8476.3	8772.0	-295.7
AUC of K1b	8392.9	9034.7	-641.8
AUC of K4a	10280.8	10263.7	17.0
AUC of K4b	6758.1	7057.1	-299.0
AUC of K2a	9205.6	9668.0	-462.4
AUC of K2b	6849.5	7032.4	-182.9
AUC of K5a	8320.1	9834.5	-1514.4
AUC of K5b	8283.8	8737.8	-454.0
AUC of K3a	8654.6	8972.1	-317.5
AUC of K3b	6849.5	7032.4	-182.9
AUC of K6a	7710.8	8644.2	-933.4
Means(SD)	8161.9(1078)	8640.8(1144)	-478

P=0.04

Table 3: Aggregate mineral uptake on the test and control sides of all samples

Ca+P+F+Sr	Test	Control	Absolute diff
AUC of K1a	12688.5	13100.3	-411.8
AUC of K1b	12562.6	13454.8	-892.2
AUC of K4a	15575.0	15474.8	100.3
AUC of K4b	10670.5	10682.5	-12.0
AUC of K2a	14010.4	14498.6	-488.2
AUC of K2b	10466.6	10489.9	-23.3
AUC of K5a	12783.1	14597.9	-1814.7
AUC of K5b	12687.8	13131.5	-443.7
AUC of K3a	13046.0	13428.9	-383.0
AUC of K3b	10466.6	10489.9	-23.3
AUC of K6a	12021.4	12986.3	-964.9
Means(SD)	12452.6(1549)	12939.5(1710)	-486.9 (563)

P=0.016

Table 4: Comparison of AUC calcium and fluorine between the test and control side

Ca+F	Test	Control	Absolute diff
AUC of K1a	8631.5	8841.5	-210.1
AUC of K1b	8731.3	9122.7	-391.4
AUC of K4a	10580.1	10561.3	18.8
AUC of K4b	7460.0	7198.9	261.1
AUC of K5a	8937.6	9922.8	-985.2
AUC of K5b	8821.4	8848.5	-27.1
AUC of K2a	9474.1	9755.5	-281.4
AUC of K2b	7133.1	7096.6	36.5
AUC of K3a	8884.4	9047.7	-163.3
AUC of K3b	7133.1	7096.6	36.5
AUC of K6a	8392.3	8763.7	-371.4
Means (SD)	8561.7(1028)	8750.5(1174)	-188.8

P=0.08

Table 5: Comparison of AUC Ca between Fuji IX and Ketac Molar samples

Mean Ca	Fuji IX			Ketac Molar		
	Test	Control	Absolute diff	Test	Control	Absolute diff
1week	9228.3	9625.0	-396.7	8477.0	8781.9	-304.9
2weeks	8363.8	7529.7	834.1	8164.7	8818.2	-653.4
3weeks	9047.5	8840.0	207.5	7738.3	8216.2	-477.9

There was no statistical analysis for this table.

Table 6: Comparison of AUC Ca between the Fuji IX and Ketac Molar samples (test sides)

Mean Ca	Test Fuji IX	Test Ketac Molar	Absolute diff
1week	9228.3(922)	8477.0(1078)	751.3
2weeks	8363.8(1187)	8164.7(974)	199.1
3weeks	9047.5 (901)	7738.3(902)	1309.2

Table 7: Calcium/phosphorus ratios of some representative samples

Depth (μ)	K1aT	K1aC	K2aT	K2aC	K3aT	K3aC	K4aT	K4aC	K5aT	K5aC	K6aT	K6aC
0	12.8	1.8	3.5	1.8	0.9	7.6	3.5	7.2	1.9	3.9	6.9	1.8
5	2.4	1.8	2.1	1.9	21.7	4.2	4.1	3.6	2.5	5.6	3.4	2.0
10	2.1	1.9	1.8	1.9	6.8	2.0	1.9	2.0	2.3	2.1	2.0	3.4
15	2.3	2.0	1.8	2.0	1.2	2.0	2.0	2.0	2.0	2.1	2.0	2.1
20	2.2	1.9	1.8	2.0	1.1	2.0	2.0	2.0	2.1	2.1	2.0	1.9
25	2.1	2.0	1.9	2.1	1.1	2.0	2.0	2.0	2.1	2.0	2.0	1.9
30	2.1	2.0	1.9	2.0	1.1	2.0	1.9	2.0	2.1	2.1	2.0	2.0
35	2.1	2.0	1.9	2.1	1.1	2.0	1.9	2.0	2.1	2.1	2.0	2.0
40	2.0	1.9	2.0	2.0	1.1	2.0	2.0	2.0	2.1	2.1	2.0	2.0
45	2.0	2.1	2.0	2.0	1.0	2.0	2.0	2.1	2.1	2.1	2.1	2.0
50	2.1	2.0	2.0	2.1	1.0	2.0	2.0	2.1	2.1	2.1	2.0	2.1